Abstracts from the World Federation of Neuro-Oncology Second Quadrennial Meeting and the Sixth Meeting of the European Association for Neuro-Oncology

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Abstracts*

1. EXTENDED ABSTRACT: ADVANCES IN DIAGNOSIS OF SUPRATENTORIAL LOW-GRADE GLIOMAS IN ADULTS

J.-Y. Delattre; Hôpital de la Salpêtrière, Paris, France

In more than 80% of cases, the first manifestation of low-grade glioma (LGG) is a partial or (secondarily) generalized seizure in a "young" adult (median age, 35–39 years) who is otherwise in good general health with a normal neurological examination. Much more rarely, progressive deficit, cognitive dysfunction, or intracranial hypertension will reveal the disease. In this setting, MRI of the brain with and without gadolinium infusion is mandatory. The MRI aspect is not specific, but the diagnosis is strongly suspected when a nonenhancing mass (hypointense on T1-weighted images and hyperintense on T2-weighted images or FLAIR sequences) is discovered in the "frontal-temporal-insular" region or within the "parasagittal" frontal-parietal regions, the most frequent and characteristic locations of LGG (Duffau and Capelle, 2004). Occasionally, contrast enhancement is seen, or the tumor widely infiltrates the brain at the onset (gliomatosis cerebri). MR spectroscopy and PET scan (fluorodeoxyglucose and methionin) are useful to guide a biopsy and for differential diagnosis. The most frequently considered differential diagnosis includes encephalitis in young patients and stroke in older patients (Calli et al., 2002). Definite diagnosis eventually relies on microscopic and genetic examination of a tumor sample. Microscopic examination is crucial to exclude tumors that can be cured by surgery alone, such as dysembryoplasic neuroepithelial tumor, ganglioglioma, or even pilocytic astrocytomas, which are not considered here, and to detect features of anaplasia, a common finding in apparently "benign" tumors on MRI (Scott et al., 2002). The value of pathological examination to precisely identify the tumor cell type is more debatable. Indeed, the WHO classification of LGGs (Kleihues, 2000) relies on morphological criteria to define astrocytomas, oligodendrogliomas, or mixed gliomas. This classification remains imperfect because of incomplete reproducibility, even for a single observer, and lack of specific marker of tumor subtype (Mokhtari et al., 2005). This fact is illustrated by a dramatic rise in the incidence of oligodendrogliomas in many centers over the last decade associated with a concomitant reduction of astrocytomas, even if the diagnostic criteria did not change significantly during this period (Burger, 2002, and data not shown). As a consequence, the respective frequency of tumor subtypes varies considerably among institutions, a feature with practical implications because evidence suggests that the management of these tumors should be tailored according to the main tumor cell type (Hoang-Xuan et al., 2004).

Molecular classification is an important adjunct to the classification of LGG. Up to 75% to 80% of LGGs have one of the two key "early" genetic alterations reported in gliomas, including chromosome 1p loss or P53 expression (which is linked to P53 mutation), and 20% to 25% have both alterations or neither. Overall, these two molecular alterations are strongly mutually exclusive. Tumors with 1p loss almost always have a morphological pattern of oligodendrogliomas ("honeycomb" appearance) or rarely a mixed glioma pattern (Reifenberger et al., 1994). On the other hand, tumors with P53 expression are more heterogeneous: 50% of them are fibrillary astrocytomas, but the other half have either an oligodendroglioma or a mixed glioma pattern. Thus, an astrocytoma pattern is practically never associated with 1p loss alone, but an oligodendroglioma or mixed glioma pattern may be associated with either 1p loss or P53 expression.

There is a correlation between the profile of molecular alterations and tumor location (Laigle-Donadey et al., 2004; Zlatescu et al., 2001). Chromosome 1p–deleted tumors are preferentially located in the frontal regions and frequently infiltrate widely the parenchyma, and P53-expressing tumors are mainly located in the parietal-temporal-occipital regions and are more circumscribed on MRI. Interestingly, a correlation between P53 mutation and preferential topography of the tumor has also been noted in other tumors, such as gastric or colon cancers.

Knowledge of the genetic status of LGGs has important consequences because chromosome 1p loss seems to be one of the most important favorable prognostic factors in LGG, possibly surpassing classic factors such as age, performance status, histological subtype, or tumor size at diagnosis. Furthermore, 1p-deleted LGGs are chemosensitive in half of the cases, but 1p-intact tumors much more rarely respond to chemotherapy (Hoang-Xuan et al., 2004).

Since a biopsy does not necessarily encompass the most aggressive part of a tumor, microscopic examination of a small specimen taken at the periphery of the lesion may be falsely reassuring when it detects only features of LGG. In these circumstances, detection of the so-called late genetic alterations of gliomas, particularly the ominous combination of EGFR amplification with chromosome 10q loss, may alert the clinician that the underlying tumor is much more malignant than expected by histology.

The natural history of LGG is changing, with a striking "increase of survival" over the last two decades. This feature reflects the fact that the diagnosis is made much earlier than before and underlines the need for a careful evaluation of the risk/benefit ratio before administration of potentially toxic treatment. An important observation is that LGGs grow inexorably over time during the period, which often lasts many years, preceding the malignant transformation and terminal phase of an LGG course (Mandonnet et al., 2003), a feature pleading for early surgery when it is possible.

*Authors whose names appear in italic are the presenting authors.

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2. EXTENDED ABSTRACT: PROSPECTIVE CLINICAL TRIALS OF ADULT-SUPRATENTORIAL LOW-GRADE GLIOMA

E.G. Shaw; Department of Radiation Oncology, Comprehensive Cancer Center, Brain Tumor Center of Excellence, Wake Forest University School of Medicine, Wake Forest, NC, USA

There are three current controversies in the radiotherapeutic management of adults with supratentorial low-grade glioma (LGG) based on numerous retrospective studies that have been published in the medical literature. The first is the optimum timing of radiation therapy (RT). Following maximum surgical resection, should RT be given immediately postoperatively, or is surgery alone adequate treatment, with RT added only if imaging progression occurs? Second, if RT is to be given, is there an advantage in terms of local control and survival for higher rather than moderate to lower doses of RT? Third, given the modest cure rates of surgery with or

without RT, will disease-free and overall survival be improved by adding chemotherapy to RT? Four prospective randomized clinical trials in adults with supratentorial LGG were conducted in the United States, Canada, and Europe during the 1980s and 1990s that addressed these questions (Eyre et al., 1993; Karim et al., 1996, 2002; Shaw et al., 2002; van den Bent et al., 2004). The data from the nearly 1000 patients treated in these studies are summarized in this abstract. In addition, the schemata from recently completed and planned phase 2 and phase 3 clinical trials in the United States and Europe will be reviewed.

Summary of Published Clinical Trials: The five-year overall survival and progression-free survival rates in these four published studies ranged from 58% to 72% and from 37% to 55%, respectively. European Organization for Research and Treatment of Cancer (EORTC) Study 22845 randomized 311 adults to postoperative observation or RT. There was no difference in the five-year survival rate between the two arms. Irradiated patients had a significantly improved five-year progression-free survival rate. EORTC Study 22844 randomized 379 adults to low- versus high-dose RT. Similarly, an intergroup study conducted by the North Central Cancer Treatment Group, the Radiation Therapy Oncology Group (RTOG), and the Eastern Cooperative Group randomized 211 adults to low- versus high-dose RT. There was no difference in the five-year overall or progression-free survival rates between the two dose groups in either study. A Southwest Oncology Group study randomized 60 adults with incompletely resected LGG to RT alone or RT plus lomustine (CCNU) chemotherapy. There was no difference in outcome between the two treatment arms. Important prognostic factors for overall survival in these trials include extent of surgical resection, histology, tumor size, and age.

Summary of Recently Completed and Planned Clinical Trials: The RTOG recently completed Study 9802, in which adults with supratentorial LGG were placed into risk groups and treated accordingly. Low-risk patients, defined as those younger than 40 years *and* who underwent gross total resection were observed postoperatively. The preliminary data from 111 low-risk patients will be presented at this meeting (see Shaw et al., 2002). High-risk patients in RTOG 9802, defined as those aged 40 years or older *or* who underwent subtotal resection or biopsy, were randomized to RT alone (54Gy to tumor with a 2cm margin) or RT followed by six cycles of PCV chemotherapy (procarbazine, CCNU, and vincristine). It will be several years before data from the high-risk group will be available. The RTOG has just opened a randomized phase 2 study of RT (54Gy) followed by temozolomide chemotherapy, or temozolomide both during and following RT. The Eastern Cooperative Oncology Group has just submitted a concept to the National Cancer Institute for a phase 3 clinical trial in "high-risk" adults with supratentorial LGG, randomizing them to RT alone (50.4Gy to tumor with a 2cm margin) or RT followed by six cycles of temozolomide chemotherapy. The EORTC is planning a phase 3 clinical trial in adults with supratentorial LGG, randomizing them to RT (50.4Gy) or temozolomide (no RT), stratifying patients by chromosome 1p deletion status (present versus absent) (Dr. Martin van den Bent, personal communication, November 30, 2004).

Conclusions: Based on the information presented, the following conclusions can be made: In adults with LGG, there is no difference in overall survival whether RT is given postoperatively or delayed to the time of recurrence. However, about two-thirds of adults with LGG will develop tumor progression by five years following surgery alone. When RT is administered, lower doses produce a similar survival outcome as higher doses with less neurotoxicity. Data on whether chemotherapy (PCV or temozolomide) either alone or with RT improves outcome is forthcoming from recently completed or ongoing prospective clinical trials.

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4. EXTENDED ABSTRACT: MOLECULAR CYTOGENETIC TESTING IN NEUROONCOLOGY

R. Jenkins; Mayo Clinic and Foundation, Rochester, MN, USA

Testing for genetics abnormalities in gliomas is rapidly becoming a part of clinical neuro-oncology. Combined 1p/19q deletion; EGFR amplification, mutation, or vIII overexpression; and MGMT methylation or underexpression are just the first of many genetic markers that have or will be part of routine clinical practice. This presentation will focus on the discovery, validation (clinical and analytic), development, and implementation of molecular cytogenetic tests in gliomas, with emphasis on 1p and 19q deletion testing.

Combined 1p and 19q deletion is associated with gliomas of oligodendroglial lineage. In addition, several retrospective studies suggest that patients whose glioma has 1p and 19q deletion have a better survival and a better response to chemo- and/or radiation therapy. Recently, Cairncross and colleagues reported preliminary analyses of RTOG 94–02 (Cairncross et al., 2004). RTOG 94–02 was a randomized trial to determine if dose-intense treatment with PCV (procarbazine, lomustine, and vincristine) before radiation therapy prolongs overall survival (primary endpoint) or progression-free survival (secondary endpoint) versus radiation therapy alone in anaplastic oligodendrogliomas and mixed oligoastrocytomas. Patients whose tumor had 1p and 19q deletion had a significantly better overall survival and a significantly longer time to progression when treated with PCV. These retrospective and prospective studies have spurred the development of clinical 1p and 19q deletion testing. Fluorescence in situ hybridization (FISH) 1p and 19q deletion testing was formally offered for Mayo Clinic patients (and for other patients through the Mayo Medical Laboratories) during the fall of 2002. Since the introduction of the clinical test, we have performed approximately 300 tests per year. The presentation will describe the implementation of the FISH 1p and 19q deletion test and our current experience using this test.

Because of the clinical significance of 1p and 19q deletions in gliomas, there is strong interest in the identification of the relevant gene (or genes) on these chromosome arms. We are following multiple strategies to identify the target genes, including deletion mapping in primary tumors and cell lines, somatic cell hybrid analyses, comprehensive expression analysis, and genetic association studies. The presentation will review the current status of these 1p and 19q gene identification experiments.

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5. EXTENDED ABSTRACT: OVERVIEW AND CURRENT STATUS OF BRAIN TUMOR EPIDEMIOLOGY

M. Wrensch; Department of Neurological Surgery, University of California, San Francisco, CA, USA

Epidemiology aims to describe the occurrence of a disease in place and time, understand and explain the distribution and causes of the disease, and address factors that influence survival after diagnosis. Major challenges in conducting epidemiologic studies of brain tumors include (1) substantial histologic and molecular heterogeneity of tumors between and within histologic groups, (2) the relative rarity of any given subtype, (3) geographic and temporal heterogeneity in reporting requirements and classification systems, (4) a paucity of well-established risk factors, and (5) the rapid fatality associated with the most common and lethal form of primary malignant brain tumors, glioblastoma multiforme, necessitating studies that often involve the use of proxy respondents for gathering potentially relevant life-history information. To meet these challenges, recent descriptive epidemiologic studies of primary brain tumors highlight strengths and weaknesses in population data to encourage consistent and reliable classification of primary brain tumors to enable meaningful tracking of temporal and geographic variation. For example, the Central Brain Tumor Registry of the United States (CBTRUS, www.cbtrus.org) provides "a resource for gathering and disseminating current epidemiologic data on all primary brain tumors, for the purposes of accurately describing their incidence and survival patterns, evaluating diagnosis and treatment, facilitating etiologic studies, establishing awareness of the disease, and ultimately, for the prevention of all brain tumors." Etiologic and prognostic studies of brain tumors increasingly require multidisciplinary collaboration between surgeons, neuro-oncologists, geneticists, toxicologists, epidemiologists, and molecular and environmental scientists. Molecular tumor markers are being identified that predict survival and treatment response with hope of even greater gains in this area with emerging array technologies. Regarding risk factors, studies of inherited susceptibility and constitutive polymorphisms in genes pertinent to carcinogenesis (e.g., DNA repair, detoxification, and immune function genes and mutagen sensitivity) have revealed provocative findings. Consistent inverse associations of history of

allergies with glioma risk observed in several large studies suggest a possible role of immune factors in gliomagenesis or progression. Studies also continue to suggest that brain tumors might result from workplace, dietary, and other personal and residential exposures. Recent studies of cell phone use have suggested that acoustic neuromas, but not other primary brain tumors, might be influenced by cell phone use. Only a small proportion of primary brain tumors are attributable to proven and widely accepted causes of brain tumors (i.e., rare hereditary syndromes, therapeutic radiation, and immune suppression giving rise to brain lymphomas), suggesting the need for further discovery. Progress in understanding primary brain tumors will require large studies of well-defined histologic and molecular tumor types incorporating assessment of potentially relevant information on subject susceptibility and environmental and noninherited endogenous factors (viruses, radiation, and carcinogenic or protective chemical exposures through diet, workplace, oxidative metabolism, or other sources). To facilitate this progress, in February 2003, researchers and advocates formed the Brain Tumor Epidemiology Consortium (BTEC) (2004–2006 European Co-Chairs, Beatrice Malmer, Sweden, and Siegal Sadetzki, Israel, and United States Co-Chairs, Faith Davis, Illinois, and Melissa Bondy, Texas). The National Cancer Institute provided support for the initial meeting, and the National Brain Tumor Foundation, Pediatric Brain Tumor Foundation, Preuss Foundation, and CBTRUS have supported additional meetings. BTEC is a self-directing consortium committed to developing multicenter interdisciplinary collaborations that will lead to a better understanding of etiology, outcomes, and prevention of brain tumors. Current initiatives include family studies and additional epidemiologic studies of childhood brain tumors, adult glioma, and meningioma.

6. EXTENDED ABSTRACT: QUALITY OF LIFE IN BRAIN TUMOR PATIENTS: MEASUREMENT AND SUPPORT

M.J.B. Taphoorn; Medical Centre Haaglanden, Department of Neurology/Neuro-Oncology, The Hague, The Netherlands

The objective of any anticancer therapy extends well beyond prolongation of survival. Palliation of symptoms and maintenance or improvement of quality of life are important goals of therapy. Thus, the benefits of existing or new cancer treatments that maintain or extend survival time need to be weighed against side effects and a possible decrease in the patient's health-related quality of life (HRQOL). Health-related quality of life has become an increasingly important endpoint in cancer studies, next to outcome measures such as overall and disease-free survival, and is most relevant in patients who cannot be cured of their disease (Efficace and Bottomley, 2003).

In brain tumor patients, palliating symptoms and maintaining or improving HRQOL is particularly relevant for several reasons. First, patients with primary brain tumors (mainly gliomas) or metastatic tumors in the central nervous system have a dismal prognosis and cannot be cured of their disease. Second, brain tumor patients not only have to cope with clinical symptoms, such as motor deficit and epilepsy, but they are usually also confronted with a decline in cognitive and emotional functioning as a result of cerebral disease. Third, side effects of treatment for brain tumors may have an even further negative impact on cerebral functioning (Meyers, 1997).

Measurement of HRQOL: Despite the specific relevance for measuring HRQOL in brain tumor patients, the interest in HRQOL emerged relatively late in this patient group compared with more common cancers such as breast or lung cancer. This has to do with the low incidence of primary brain tumors, a former therapeutic nihilism toward brain cancer, and the fact that the subjective nature of HRQOL assessment may be problematic in brain tumor patients with mental impairments.

Health-related quality of life is defined as a multidimensional concept consisting of at least physical, psychological, and social phenomena. Measuring outcome in terms of tumor size, time to tumor progression, and overall survival is relatively simple compared with outcome measures such as impairment, disability, or handicap, which require (symptom) scales (Heimans and Taphoorn, 2002). These objective scales, however, do not adequately measure the patient's HRQOL. Health-related quality of life is an even more complex outcome measure, demanding a multidimensional instrument that should be filled out by the patient (self-report questionnaire). Both generic and disease-specific questionnaires have been developed and validated to assess HRQOL in cancer patients. To measure HRQOL in brain tumor patients, the generic European Organization for Treatment and Research of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) is used in combination with the Brain Cancer Module (BN 20) or the Functional Assessment of Cancer Therapy (FACT) generic questionnaire together with the FACT brain module (Osaba et al., 1996; Weitzner et al., 1995).

Health-Related Quality of Life in Brain Tumor Patients: HRQOL is increasingly being measured as a secondary outcome in brain tumor trials in which the efficacy of a (new) therapy is being evaluated. This holds true for both low-grade and high-grade glioma patients.

Compliance with serial assessments of HRQOL is one of the major problems in randomized trials. A low compliance with HRQOL assessment is related to performance status and outcome and may cause a serious bias in results (Roa et al., 2004; Walker et al., 2003).

Health-related quality of life in newly diagnosed high-grade glioma patients appeared to be comparable to that in lung cancer patients and was significantly worse than that of healthy controls (Klein et al., 2001). In contrast, cognitive deficit was far more pronounced in glioma patients than in lung cancer patients, reflecting the specific neurological deficit. From studies in both newly diagnosed and recurrent high-grade glioma it is known that HRQOL is related to disease burden and neurological deficit (Osoba et al., 1997).

In a comparison between low-dose and high-dose radiation schedules in low-grade glioma patients, there appeared to be no difference in survival, but HRQOL was more seriously impaired in the high-dose group than in the low-dose group (Kiebert et al., 1998).

In two randomized trials the impact of temozolomide on HRQOL in glioblastoma multiforme patients was investigated. No negative impact of temozolomide on the patients' HRQOL was observed, in contrast to the toxic effect of procarbazine (Osoba et al., 2000a; Taphoorn et al., 2004). Temozolomide even improved HRQOL in some of these patients, and this was also observed in patients with recurrent anaplastic astrocytoma (Osoba et al., 2000b).

Supportive Treatment: The brain tumor and its treatment may have significant physical, cognitive, emotional, and social effects on the patient. The patient's partner and family may also experience a negative emotional and social impact. Apart from treatment of the tumor itself, supportive treatment of the patient and the patient's family may include medication (antiepileptic drugs, steroids, antidepressants) and/or psychological and/or cognitive support. As there is a steady increase in effective treatments for brain tumor patients, the number of long-term survivors will grow. Long-term negative effects of the tumor and its treatment demand an increasing effort of doctors, nurses, and psychologists for supportive care (Remer and Murphy, 2004).

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7. PRELIMINARY RESULTS OF RTOG PROTOCOL 9802: A PHASE II STUDY OF OBSERVATION IN COMPLETELY RESECTED ADULT LOW-GRADE GLIOMA

E.G. Shaw,[1] Minhee Won,[2] David G. Brachman,[3] Christopher J. Schultz,[4] John H. Suh,[5] Jan C. Buckner,[6] Geoffrey R. Barger,[7] Stephen W. Coons,[8] Dennis E. Bullard,[9] and Minesh P. Mehta[10]; [1]Radiation Oncology, Winston-Salem, North Carolina; [2]MA, RTOG, Philadelphia, Pennsylvania; [3]Radiation Oncology, Phoenix, Arizona; [4]Radiation Oncology, Milwaukee, Wisconsin; [5]Radiation Oncology, Cleveland, Ohio; [6]Medical Oncology, Rochester, Minnesota; [7]Neuro-Oncology, Detroit, Michigan; [8]Neuropathology, Phoenix, Arizona; [9]Neurosurgery, Raleigh, North Carolina; [10]Human Oncology, Madison, Wisconsin; USA

In 1998, the RTOG initiated Protocol 9802 for adults with supratentorial low-grade glioma. Patients were divided into two groups based on risk. Low risk was defined as age. These patients were observed postoperatively with serial magnetic resonance imaging (MRI) scans and form the basis of this report. Eligibility criteria included histologically proven WHO grade II A, O, or OA based on central pathology review, age ⩾18 and ⩾60, Neurologic Function Score (NFS) ⩽3, supratentorial tumor location, GTR, pre- and postoperative MRI scans available, and signed consent form. MRI scans were obtained every 6 months. Prognostic factors analyzed for their effect on overall survival (OS), progression-free survival (PFS), and tumor recurrence included age (⩾30 years), gender (male vs. female), KPS (⩽90 vs. 100), NFS (0 vs. 1–3), histology (A = astrocytoma or astrocytoma-dominant OA vs. O = oligodendroglioma or oligodendroglioma-dominant OA), contrast enhancement on pre-operative MRI scan (present vs. absent), pre-operative tumor diameter (⩾4cm), and baseline mini-mental status exam score. Between 1998 and 2002, 116 patients were entered, 111 of whom were analyzable. OS and PFS at 3 years for all patients was 97% and 73%, respectively. The only two prognostic factors predicting for significantly poorer PFS in uni- and multivariate analyses were histology (univariate $P = 0.02$, multivariate $P = 0.03$, hazard ratio = 2.33) and pre-operative tumor diameter (univariate $P = 0.002$, multivariate $P = 0.006$, hazard ratio = 2.90). The crude incidence of tumor recurrence was 38% for A vs. 20% for O and 43% for tumors ⩾4 cm vs. 18% for tumors <4 cm. The 3-year PFS was 89% for O tumors ⩾4 cm. An assessment of extent of surgical resection based on the pre- vs. postoperative MRI scans is ongoing and will also be analyzed as a possible prognostic factor. These data can be used to identify low-risk low-grade glioma patients who may be candidates for postoperative adjuvant treatment.

8. STUDY OF THE INDIVIDUAL CORTICAL ORGANIZATION, CONNECTIVITY, AND PLASTICITY APPLIED TO THE SURGERY OF LOW-GRADE GLIOMAS: FUNCTIONAL AND ONCOLOGICAL RESULTS

H. Duffau,[1] L. Taillandier,[2] P. Gatignol,[1] M. Leroy,[1] E. Mandonnet,[1] D. Denvil,[1] M. Lopes,[1] M.C. Mitchell,[1] and L. Capelle[1]; [1]Hôpital Salpêtrière, Neurosurgery, Paris; [2]C.H.U. Nancy, Neurology, Nancy; France

While considered by more and more authors, surgery of low-grade gliomas (LGG) remains matter of debate, because of (1) the risk for generating sequelae (2) the impact, still controversial, of resection on the natural history of LGG. Taking account of the dynamic interactions between the brain and LGG, we used functional mapping methods in order to study the individual cortical organization, connectivity, and plasticity for each patient harboring an LGG, and to apply these data to increase the benefit/risk ratio of surgery. From 1996 to 2004, 152 patients with no or only a slight deficit were operated on for a supratentorial LGG located near (57 cases) or within (95 cases) eloquent areas. All the procedures were performed by using intraoperative cortico-subcortical electrical stimulations, which allowed tailoring of the resection according to functional boundaries. The 152 patients had neurological and neuropsychological assessment before and after surgery. The quality of resection was systematically evaluated on repeated post-operative MRI. Pre- and post-operative functional MRI was performed in a subgroup of patients. Intraoperative study of the dynamic organization of the functional networks was possible in all cases. Despite a frequent immediate post-operative worsening, 94% of patients recovered their preoperative status (or even improved) and returned to a normal socio-professional life within 3 months after surgery. The resection was total or subtotal in 88% of cases. With a median follow-up of 50 months, a significant statistical relationship was observed between the survival and the quality of resection ($P = 0.02$). Moreover, by comparing these results with a similar surgical series of 100 LGGs operated on in the same institution without intraoperative functional mapping, we showed that electrical stimulation allowed a significant decrease of postoperative sequelae (5% vs. 17%, $P = 0.002$) and a significant increase of the quality of resection (51% vs. 25.4% of subtotal and 37% vs. 6% of total resections, $P < 0.001$). These findings suggest a dynamic spatio-temporal functional organization, with (a) before surgery, the recruitment of compensatory areas, explaining the lack of deficit despite the tumor growth in eloquent regions, (b) immediately after surgery, the occurrence of a deficit, likely due to the resection of invaded areas participating (but not essential) to the function, and (c) 3 months after surgery, a recovery due to long-term functional reshaping. The application of this brain dynamic potential can be considered to significantly extend the limits of surgery in eloquent areas while minimizing the risk of sequelae.

9. INTRAOPERATIVE LANGUAGE MAPPING IN MULTILINGUAL PATIENTS WITH GLIOMAS

L. Bello, C. Giussani, F. Acerbi, M. Fava, V. Songa, P. Baratta, R.M. Villani, and S.M. Gaini; Neurosurgery, University of Milano, Milano, Italy

Surgical removal of lesions located close to or in areas of the brains mediating speech requires the use of intraoperative techniques to localize which cortical areas and subcortical tracts have those functions. Localization of speech is particularly problematic in patients that are usually fluent with different languages. It has been reported that in bilingual patients multiple and separate areas of the cortex mediate the different languages. Other investigations pointed out that in bilingual patients common areas of the brain are responsible for those functions. We report here five cases of patients harboring a left frontal glioma who were proficient with three to five different languages in which a multiple language brain mapping was undertaken during awake craniotomies for tumor removal. They were 3 males and 2 females, age ranging from 34 to 61 years. Language proficiency was tested by submitting patients to confrontation tests for each language during the pre-operative examination. Each language was tested serially starting from the first acquired language. Language mapping was undertaken during asleep-awake craniotomy, by the use of an Ojemann cortical stimulator and by using the largest current that did not produce afterdischarge (from 3.5 to 6 mA) during counting and confrontation naming tests. Subcortical stimulation by using the same current threshold was also applied during tumor resection, in a back and forth fashion. Our data showed that each language has separate and distinct cortical centers. Cortical areas for first acquired language had a larger cortical representation, whereas those for the secondly acquired languages were localized in more distinct cortical sites. Subcortical stimulations found tracts for the first acquired language in 4 patients, whereas those for the other languages in 3 patients. Subcortical tracts for the first language had a larger representation. Three patients experienced a decrease in fluency immediately after surgery, mainly affecting the first acquired language, which fully recovered in two patients in two months and partially in one. These findings support the existence of language-specific cortical sites and the concept that intraoperative mapping should be performed for all the languages the patient is fluent for, to maximally preserve functional language integrity. In addition, confrontation naming test is more accurate than counting for localization of functional areas.

10. 5-AMINOLEVULINIC ACID-BASED PHOTODYNAMIC DETECTION OF VARIOUS BRAIN TUMORS

K. Mishima, R. Nishikawa, and M. Matsutani; Saitama Medical School, Neurosurgery, Saitama-ken, Japan

It has been established that 5-aminolevulinic acid (ALA) induces the accumulation of fluorescent porphyrins in malignant gliomas, a phenomenon potentially exploitable to guide tumor resection. However, the usefulness of fluorescence-guided resections of other brain tumors, especially low-grade tumors, has not been studied. Here, we examined the value of ALA-induced fluorescence for detecting various brain tumors including low-grade tumors. Forty-seven patients underwent ALA-induced protoporphyrin fluorescence detection. Three hours before the induction of anesthesia, 1 g 5-ALA/body was administered orally. Intraoperatively, red porphyrin fluorescence was observed with a 455-nm long-pass filter after excitation with violet-blue (405 nm) light. Fluorescing and nonfluorescing

samples taken from the tumor tissues were examined histologically. Bright red fluorescing tumor tissues were observed in 100% (9 of 9) of glioblastomas, 66% (2 of 3) of anaplastic astrocytomas, 100% (3 of 3) of anaplastic oligodendrogliomas, 0% (0 of 2) of diffuse astrocytoma and oligodendroglioma, 100% (3 of 3) of pilocytic astrocytomas, 72% (8 of 11) of meningiomas (WHO grade I), 33% (1 of 3) of atypical meningiomas, 62% (5 of 8) of germ cell tumors, 66% (2 of 3) of malignant lymphoma, and 50% (1 of 2) of hemangioblastomas. The observations in this study indicate the usefulness of 5-ALA-induced tumor fluorescence not only for guiding malignant glioma resection but also for guiding resection of benign tumors such as pilocytic astrocytoma or meningioma. ALA-mediated fluorescence detection may enhance the completeness of benign tumor removal and increase the diagnostic accuracy of intraoperative biopsies.

11. VOLUMETRIC EXTENT OF RESECTION AND RESIDUAL CONTRAST ENHANCEMENT AT INITIAL SURGERY AS PREDICTORS OF OUTCOME IN ADULT PATIENTS WITH HEMISPHERIC ANAPLASTIC ASTROCYTOMA

G.E. Keles, E.F. Chang, K.R. Lamborn, T. Tihan, C.-J. Chang, S.M. Chang, and M.S. Berger; University of California San Francisco, Department of Neurological Surgery, San Francisco, California, USA.

The available literature evaluating the role of extent of resection for anaplastic astrocytomas, as a distinct histological group, is limited, and assessment of residual disease has not been quantitative. To investigate the prognostic significance of volumetrically assessed extent of resection on time to tumor progression (TTP), overall survival (OS), and tumor recurrence patterns, we retrospectively analyzed preoperative and postoperative tumor volumes on 102 adult patients from the time of the initial resection for a hemispheric anaplastic astrocytoma. Histological diagnosis of anaplastic astrocytoma was confirmed based on pathology re-review for all patients using the current World Health Organization criteria. Patients with recurrent anaplastic astrocytoma were not included in this study. Quantification of tumor volumes was based on a previously described method involving computerized image analysis of magnetic resonance imaging scans. Volumetric analysis was conducted on contrast-enhancing tumor volumes on T1-weighted MR images for 67 patients who had contrast-enhancing tumors, in addition to measurements of T2 hyperintensity for all 102 patients. The variables analyzed included age, Karnofsky Performance Status (KPS), preoperative tumor volume (T1 enhancement and T2 hyperintensity), percent of resection (POR), and volume of residual disease (VRD) (T1 enhancement and T2 hyperintensity). All patients had postoperative radiotherapy, and 94% (96/102) of the patients received chemotherapy. Presence or absence of preresection enhancement, actual volume of this enhancement, and the percentage of preoperative enhancement as it relates to the total T2 tumor volume did not have a statistically significant impact on TTP or OS. In addition to age, VRD measured on T2-weighted MR scans was the most significant predictor of TTP. (Unlike low-grade gliomas, there was no statistically significant relationship between extent of resection, i.e., POR and VRD, and histology at the time of recurrence, i.e., grade 3 vs. grade 4.) This retrospective analysis of a histologically uniform group of hemispheric anaplastic astrocytomas treated in the MR era suggests that residual tumor volumes, as documented on postoperative imaging studies, may be a prognostic factor for time to progression and survival for this patient population.

12. POST-OPERATIVE OUTCOME OF ANTERIOR SKULL BASE MENINGIOMAS

M. Kirsch, A. Knüppel, and G. Schackert; Carl Gustav Carus University Hospital, Neurosurgery, Dresden, Germany

The purpose of this retrospective study was to analyze the outcome and recurrence of anterior skull base meningiomas. A total of 571 meningiomas were operated upon at the Neurosurgical Department, Technical University of Dresden, between January 1994 and December 2002. Of these 571 meningiomas, 151 were located within the anterior skull base, namely, the frontal base, sphenoid wing, tuberculum sellae, cavernous sinus, and olfactory groove. One hundred forty-one patients were amendable for follow-up including regular ambulatory visits and a questionnaire. The median follow-up including MR imaging was 2.5 years (0-7.6 years). The median age was 62.5 years, and the male:female ratio was 1:2.5. The patients most commonly presented with visual deficits (37%), headaches (25%), dizziness (16%), seizures (12%), or symptoms of organic psychosis (11%). Median tumor volume was 9.7 cm^3, mean volume was 26.1 $\pm$ 35.6 cm^3. Most frequent histology subtypes were meningothelial (67%) and transitional (18%) meningiomas, whereas WHO grade II meningiomas appeared only twice (1.4%). Seizures were most frequently associated with lateral sphenoid wing and frontal base tumors, whereas visual acuity changes, dizziness, and double vision were associated with medial sphenoid wing, olfactory groove, and tuberculum sellae tumors. Tumor size correlated with appearance of symptoms. Intraoperative radicality was in 16% Simpson grade 1, 59% grade 2, 13% grade 3, and 15% grade 4. Biopsies (grade 5) were not done. Operative radicality was associated with location, with medial sphenoid wing and cavernous sinus meningiomas being the most difficult to resect completely. Pre-operative angiography was completed in 30 patients, of which 12 were embolized. Upon initial post-operative MR imaging, residual or recurrent tumor was diagnosed in 33% of cases, which was corrected on repeated follow-up imaging to 12.7%. Simpson grading correlated and angiomatous or fibrous histological subtype correlated with frequency of recurrence. Glasgow outcome scale rating demonstrated that five patients (3.6%) died within six months post-op (grade 1), one patient remained in grade 2, and 80.7% recovered completely and improved to grade 5. Simpson grade 1 and 2 resected tumors were more likely to show a good outcome (GOS 5 in 97% and 82%, respectively), compared to incompletely resected tumors. Age but not tumor volume or gender correlated with outcome. Of pre-operatively full-time-employed patients, 64% were able to return to their previous workplace, 27% had to start a gradual re-integration program, and 9% were not able to return to work. Anterior skull base meningiomas can be resected dependent on their location and involvement of other structures, such as vessels and cranial nerves. The most important predictors of post-operative outcome were Simpson grade, age, and location. Repeated MR is required for reliable assessment of recurrent tumor growth.

13. TAILORING OF ANTI ANGIOGENIC THERAPIES TO TUMOR STAGE IMPROVES THERAPEUTIC EFFICACY IN MOUSE MODELS OF HUMAN GLIOMAS

C. Giussani,[1] F. Acerbi,[1] S. Sangiorgi,[2] F. Colleoni,[1] G. Carrabba,[1] V. Lucini,[1] M. Pannacci,[1] G. Tomei,[2] S.M. Gaini,[1] A. Bikfalvi,[3] and *L. Bello*[1]; [1]University of Milano, Neurosurgery, Department of Neurological Sciences, Milano, Italy; [2]Neurosurgery, University of Insubria, Varese, Italy; [3]INSERM Unit EPI 0113, University of Bordeaux I, Talence, France

Systemic administration of inhibitors of angiogenesis, migration, and proliferation successfully inhibits the growth of experimental malignant gliomas. Nevertheless, after some time, tumor escapes treatment. In this work we investigated the role of changes in tumor vasculature during glioma development on the significance of the therapeutic effect. We initially studied the changes in tumor vasculature occurring in the well-established and surgical resection glioma models in nude mice. Brains from animals sacrificed at various time points from tumor cell injection or tumor removal were studied by immunofluorescence, immunohistochemical, and vessel casting techniques. Early tumors were composed of irregular highly angiogenic vessels, uncovered from pericytes. Tumor vasculature in late tumors showed a complex regional heterogeneity, with highly angiogenic areas and areas composed of more regular vessels covered by pericytes. We then investigated the effect of different inhibitors on various stage of vasculature development. Inhibitors that exhibit various activities on tumor or endothelial cells and that act by different mechanisms were administered to early, mean, and late tumors in both glioma models. Efficacy was evaluated by survival in long-term experiments and by evaluating changes in tumor vasculature by immunofluorescence, immunohistochemical, and vessel casting techniques. PF-4/CTF, a pure anti angiogenic drug that acts mainly by inhibiting FGF, was more effective on early tumors; PF-4/DLR, which inhibits FGF and VEGF and acts on both tumor and endothelial cells, was effective on both early and late tumors. A low-dose chemotherapy regimen based on carboplatin and etoposide was more active on late tumors. Based on these findings, we designed a scheme of treatment characterized by the sequential administration of these agents, alone or in combination. PF-4/CTF followed by PF-4/DLR, initially alone and then in combination with low-dose chemotherapy, was administered to early tumors. A combination of PF-4/DLR and PF-4/CTF followed by PF-4/DLR and low-dose chemotherapy was given to late tumors. Both schemes afforded a prolonged inhibition of glioma growth, which was more effective than the single administration of each inhibitor alone or in combination. Taken together, these findings indicate that tailoring the scheme of administration to the stage of vasculature development optimizes efficacy and further postpones the appearance of tumor escape.

14. VEGF COOPERATES WITH SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE (SPARC) TO ACTIVATE INTRACELLULAR GLIOMA SURVIVAL PATHWAYS

Q. Shi,[1] S. Bao,[1] D. Bigner,[2] and *J. Rich*; Departments of [1]Surgery, [2]Pathology, and [3]Medicine, Duke University Medical Center, Durham, North Carolina, USA

Secreted protein acidic, rich in cysteine (SPARC) is an extracellular matrix protein expressed in many advanced cancers, including malignant gliomas. We and others have previously shown that human glioma cell lines engineered to overexpress SPARC adopt an invasive phenotype. We now show that SPARC expression increases cell survival under stress initiated by serum withdrawal through a decrease in apoptosis. Phosphatidylinositol 3-OH kinase (PI3K)/AKT is a potent pro-survival pathway that contributes to the malignancy of gliomas. Cells expressing SPARC display increased AKT activation with decreased caspase 3/7 activity. Exogenous SPARC rapidly induces AKT phosphorylation, an effect that is blocked by a neutralizing SPARC antibody. Further, AKT activation is essential for the anti-apoptotic effects of SPARC as the decreased apoptosis and caspase activity associated with SPARC expression can be blocked with dominant-negative AKT or a specific AKT inhibitor. As tumor cells face stressful microenvironments particularly during the process of invasion, these results suggest that SPARC functions, in part, to promote tumor progression by enabling tumor cells to survive under stressful conditions. We have now probed the signaling events upstream from AKT activation by SPARC. SPARC binds and regulates growth factor presentation to cells. We examined the impact of SPARC and several growth factors on activation of intracellular pathways, including AKT. While IGF1 induced AKT phosphorylation, the addition of SPARC did not alter this activity. In contrast, VEGF and SPARC each modestly increased AKT phosphorylation the combination more significantly induced AKT phosphorylation. Additionally, focal adhesion kinase (FAK) appears upstream of AKT as both SPARC alone and in combination with VEGF induced FAK phosphorylation before AKT phosphorylation. Dominant negative FAK (FRNK) induced cell death suggesting – like AKT in these cells – FAK is essential to cell survival. In summary, we have now directly linked SPARC to essential tumor processes – including invasion, survival, and angiogenesis. SPARC warrants further investigation as a contributor to glioma malignancy and as a potential therapeutic target. (J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar. This work was also supported by NIH grant NS047409 to J.N.R.).

15. INHIBITION OF INTRACEREBRAL GLIOBLASTOMA GROWTH BY TREATMENT WITH A NOVEL ONE-ARMED ANTI-MET ANTIBODY

T. Martens,[1] N.O. Schmidt,[1] C. Eckerich,[1] R. Fillbrandt,[1] R. Schwall,[2] M. Westphal,[1] and *K. Lamszus*[1]; [1]University Hospital Hamburg-Eppendorf, Department of Neurosurgery, Hamburg, Germany; [2]Genentech, Inc., South San Francisco, California, USA

The proto-oncogene encoded tyrosine kinase receptor MET and its ligand scatter factor/hepatocyte growth factor (SF/HGF) are strongly upregulated in malignant gliomas. The SF/HGF-MET system is important for glioma cell migration, invasion, proliferation, and angiogenesis. We used a novel single-chain anti-MET antibody to inhibit glioblastoma growth in an orthotopic model. U87 glioblastoma cells were xenografted into the brains of nude mice. On day 1 or day 7 after tumor cell injection, osmotic minipumps with intratumoral catheters were implanted. The one-armed anti-MET antibody (40 mg/day) was infused intratumorally until 3 weeks after tumor cell injection. Tumor size, proliferation, apoptosis, microvessel density, and expression of extracellular matrix (ECM) molecules were analyzed by immunohistochemistry. cDNA arrays were performed to determine the effect of the anti-MET antibody on the expression of invasion-related genes in vitro. Functional effects of the anti-MET antibody were analyzed in vitro. The effects of the anti-MET treatment on tumor size and morphology were very similar, regardless whether treatment was initiated on day 1 or day 7. Tumor volumes were reduced by >95% ($P < 0.001$) in animals treated with the anti-MET antibody compared with controls. Tumor cell proliferation was reduced by >75% ($P < 0.001$) in treated tumors; microvessel density was reduced by >90% ($P < 0.001$); apoptosis was increased by >60% ($P < 0.05$). Interestingly, the tumor cell density was >2-fold higher in controls than in treated tumors, in which a striking increase in ECM deposition between tumor cells was apparent. Immunohistochemically, strong increases in staining intensities for laminin, fibronectin, and tenascin were found in tumors treated with the anti-MET antibody. cDNA arrays revealed downregulation of uPA, tPA, MMP7, MMP15, and MMP16 and upregulation of PAI-1 in U87 cells treated with the anti-MET antibody, which may explain the increase in ECM proteins in vivo. Proliferation and migration of U87 cells in vitro were inhibited by the anti-MET antibody. Local treatment with the one-armed anti-MET antibody can inhibit intracerebral glioblastoma growth almost completely. The responsible mechanisms appear to include anti-proliferative, anti-angiogenic, anti-migratory, and pro-apoptotic effects as well as enhancement of ECM deposition, presumably caused by decreased matrix degradation.

16. ANGIOPOIETIN-2 INDUCES GLIOMA CELL INVASION BY STIMULATING MMP-2, MT1-MMP AND LN 5 GAMMA 2 EXPRESSION THROUGH TIE2-INDEPENDENT PATHWAYS

B. Hu,[1] P. Guo,[2] M.J. Jarzynka,[2] R. Nishikawa,[3] T. Hirose,[4] and *S.-Y. Cheng*[2]; Cancer Institute and Departments of [1]Medicine and [2]Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; Departments of [3]Neurosurgery and [4]Pathology, Saitama Medical School, Saitama, Japan

A hallmark of malignant human gliomas is their ability to diffusely invade into surrounding brain tissues. We have previously reported that angiopoietin-2 (Ang2), a known angiogenic factor, induces glioma cell invasion through the activation of matrix metalloprotease-2 (MMP-2). In this study, we analyzed 57 human glioma biopsies (WHO grade I to IV) displaying a distinct invasive edge and 39 glioma specimens that only contain the central region of the tumors and found that Ang2, MMP-2, MT1-MMP, and LN 5 gamma 2 were co-overexpressed in the invasive areas, but not in the central regions of the glioma tissues. Statistical analyses revealed a significant link between the preferential expression of these molecules and invasiveness. Western blot analyses of total protein extracted from the microdissected primary glioma specimens showed an upregulation and activation of MT1-MMP and LN 5 gamma 2 at the invasive edge of the tumors versus the tumor center, supporting this observation. Analysis of engineered U87MG glioma xenografts which express Ang2, revealed an increased expression of MT1-MMP and LN 5 gamma 2 in actively invading glioma cells, along with MMP-2 upregulation. Stimulation of glioma cells by overexpressing Ang2 or exposure to exogenous Ang2 promoted the expression and activation of MMP-2, MT1-MMP, and LN 5 gamma 2. Furthermore, Ang2 directly binds to beta 1 integrin in Tie2-deficient U87MG cells inducing the activation of FAK, p130Cas, ERK1/2, and JNK. Ang2-stimulated MMP-2 expression and secretion was attenuated by a functional neutralizing anti-beta 1 antibody, by an ERK1/2 inhibitor, and by a JNK inhibitor. Stable expression of a specific negative regulator of FAK, FAK related non-kinase (FRNK) but mutant FRNK-S1034, inhibited Ang2-stimulated phosphorylation of FAK and p130Cas, blocked the activation of ERK and JNK, and decreased Ang2-stimulated MMP-2 expression and secretion. Inhibition of beta 1 integrin, FAK, p130Cas, ERK1/2, and JNK also attenuated Ang2-stimulated glioma cell invasion. Lastly, expression of FRNK, but not FRNK-S1034, by glioma xenografts in the brain derived from U87MG/Ang2 cells suppressed Ang2-induced glioma cell infiltration and MMP-2 expression. These data suggest that upregulation of Ang2, MMP-2, MT1-MMP and LN 5 gamma 2 is associated with glioma invasiveness and demonstrate a mechanism whereby binding of Ang2 to glioma cells regulates MMP-2 expression and secretion through the integrin and FAK signaling pathways.

17. PROMOTION OF MALIGNANT GLIAL CELL POLARITY AND INVASION BY DRR-1

R. Waldkircher de Oliveira, K. Petrecca, A. Angers-Loustau, M. Seyed-Sadr, and R. Del Maestro; Montreal Neurological Institute, Neurology/Neurosurgery, Montreal, Canada

Invasion is a major factor responsible for the failure of brain tumor treatment. There are currently no chemotherapeutics directed at controlling the infiltration of malignant glial cells into normal brain. In order to identify invasion-associated genes we designed an unbiased, genome-wide functional screen based on tumor cell invasion. A normal adult human brain cDNA library was stably infected into a glioma cell line using retroviral transduction. Tumor spheroids generated from these cells were implanted into a 3D collagen matrix. Hyperinvasive cells were isolated from the matrix and cDNA inserts identified. *Downregulated in renal cell carcinoma* (Drr1) was identified as a potent mediator of glial cell hyperinvasion in this screen. Time-lapse video microscopy reveals that Drr1 overexpression promotes invasion, whereas RNAi-mediated expression reduction inhibits invasion in a 3D tumor model. Importantly, human glioma sampling reveals that Drr1 is highly expressed in invasive gliomas, whereas expression is not detectable in noninvasive gliomas. Further, we have identified Drr1 as a novel actin-microtubule polylinker that promotes invasion by enhancing cell polarity using a pericentrin localization assay. These findings uncover a novel glioma invasion gene and its mechanism of action, which provides an explanation for the highly invasive nature of Drr1-expressing human gliomas.

18. PHARMACOLOGIC INHIBITION OF MTOR ACTIVITY ATTENUATES GLIOMA CELLS INVASION BY DOWNREGULATING NEUROPILIN-1 EXPRESSION

T.J. Liu,[1] T. LaFortune,[1] J. de Groot,[1] H. Lane,[2] and W. Yung[1]; [1]The University of Texas, M.D. Anderson Cancer Center, Neuro-Oncology, Houston, Texas, USA; [2]Novartis Pharma AG, Basel, Switzerland

The mammalian target of rapamycin mTOR controls a spectrum of cellular events such as initiation of translation, ribosome biogenesis, and cell growth and proliferation. Pharmacologic inhibition of mTOR activity by rapamycin has been shown to elicit antitumor activity possibly through G_1 cell cycle arrest and inhibition of VEGF expression. Currently, an analogue of rapamycin, RAD001, is being used in clinical trial for different cancers including gliomas. Glioblastoma multiforme is a malignant tumor that is extremely refractory to therapy because of rapid growth and local invasive potential of these tumors. In this study, we sought to examine the effect of antagonizing mTOR activity by RAD001 on glioma tumor invasion in vitro. Four different glioma cells were treated with RAD001 for 72 h before harvesting for Western blotting, RT-PCR, microarray, and in vitro Matrigel gel invasion analyses. Glioma cells were stably transfected with NRP-1 expression and control vector followed by G418 selection. The resulting G418-resistant cells were examined for their NRP-1 expression and in vitro invasion propensity. Glioma cell lines treated with RAD001 resulted in a reduction of mTOR downstream target genes expression such as S6K1 and the eukaryotic initiation factor 4E-binding protein 1. Inhibition of mTOR activity with RAD001 in tumor cells also leads to G_1 cell cycle arrest and reduction in VEGF secretion consistent with previously documented studies. Importantly, invasion propensity of tumor cells is greatly inhibited by RAD001 as assessed in an *in vitro* Matrigel invasion assay. Interestingly, RAD001 treatment does not affect either the expression or the activity of MMP2 in tumor cells. Using Affymetric microarray analyses we discovered the expression of neuropilin-1 (NRP-1) is decreased by RAD001. NRP-1, initially found to be involved in axon growth during neuronal development, is expressed in both tumor cells and endothelial cells. It has been shown that NRP-1 is a co-receptor for $VEGF_{165}$ and controls cell motility. Semiquantitative PCR further confirmed the microarray results, which suggests that RAD001 affects NRP-1 mRNA expression. Exogenous NRP-1 expression significantly increases glioma cells invasion and promotes anchorage-independent growth. Our results demonstrate that anticancer activity of RAD001 may be a combination of growth arrest, antiangiogenesis, and anti-invasion effects. Furthermore, the potential anti-invasion activity of RAD001 is likely through controlling cell motility by inhibiting NRP-1 expression.

19. POLYMORPHISMS IN DNA REPAIR GENES AND SUSCEPTIBILITY TO PRIMARY INTRACRANIAL BRAIN GLIOMAS

M. Butler,[1] A. Ruder,[1] T. Carreon,[1] M. Waters,[1] M. Yeager,[2] R. Welch,[2] S. Chanock,[2] and P. Schulte[1]; [1]National Institute for Occupational Safety and Health, Cincinnati, Ohio; [2]National Cancer Institute, Gaithersburg, Maryland; USA

Enzymes in base excision (BER), nucleotide excision (NER), double strand break/recombination (DSB/RR), mismatch (MMR), and direct-damage DNA repair pathways are important in the repair of diverse types of DNA damage. Polymorphisms in many of the genes encoding these enzymes have been identified as risk factors for environmentally and occupationally caused cancers. We evaluated the associations of polymorphisms in BER (*ADPRT* V762A, *APEX* D148E, *MUTYH* Ex1+8A>C>G/T, *OGG1* S326C, *POLB* IVS11-235A>G, *XRCC1* R399Q, R280H, R194W, *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), NER (*ERCC2* D312N, K751Q, *ERCC4* R415Q, *ERCC5* H1104D, *RAD23B* A249V, *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), DSB/RR (*NBS1* Q185E, *RAD52* Y415stop, *XRCC2* R186H, *XRCC3* T241M, *XRCC4* N298S), MMR (*MLH1* I219V, *MSH2* G322D), and direct-damage repair (*MGMT* I143V, R178K, L84F) as risk factors for primary intracranial gliomas in the Upper Midwest Health Study, a population-based case-control study including rural residents of four states with high glioma incidence. Glioma cases (N = 798) were identified from hospitals, private physicians, and registries. Control participants (N = 1175) were stratified samples of licensed drivers and HCFA enrollees. Questionnaires elicited occupational and environmental exposures. DNA was obtained from 451 controls with no self-reported cancer and from 316 cases. TaqMan and MGB Eclipse methodology were used to characterize genotypes. In unadjusted analyses, a polymorphism in *ADPRT* (V/V 67% of controls, 75% of cases, odds ratio [OR] 1.48, 95% confidence interval [CI] 1.07–2.04) had a statistically significant association with glioma, and polymorphisms in three other genes showed associations with glioma of borderline statistical significance: (1) *RAD23B* A/V + V/V, 32% of controls, 38% of cases, OR 1.32, CI 0.97–1.78; (2) *ERCC5* H/H, 61% of controls, 68% of cases, OR 1.33, CI 0.98–1.78; and (3) *XRCC4* N/N 74% of controls, 80% of cases, OR 1.37, CI 0.97–1.94. For each DNA repair pathway, multivariate logistic analyses included all polymorphisms in the pathway plus ever/never living on a farm and ever/never smoking, as surrogates for occupational and environmental exposure. Adjusting for these factors did not change odds ratios substantially. Our results should be confirmed in additional glioma case-control studies. Future analyses of our data will include assessing the risk of DNA repair polymorphisms under specific exposure conditions, such as exposures to pesticides, solvents, and UV light.

20. A GLTSCR1 HAPLOTYPE IS ASSOCIATED WITH OLIGODENDROGLIOMA DEVELOPMENT

R.B. Jenkins,[1] T. Kollmeyer,[1] K. Buckner,[1] W. Bamlet,[2] K.V. Ballman,[3] and P. Yang[2]; [1]Mayo Clinic, Division of Laboratory Genetics; [2]Mayo Clinic, Department of Health Sciences Research; [3]Mayo Clinic, Cancer Statistics Unit, Rochester, Minnesota, USA

Deletions of 19q have been associated with gliomas, especially oligodendrogliomas. In addition, oligodendrogliomas with 19q deletion have a better survival. We have previously described a 150-kb minimal deletion region in gliomas that maps to 19q13.33 that contains three novel candidate genes (*GLTSCR1*, *EHD2*, and *GLTSCR2*). Polymorphisms in loci near this deletion region (ERCC1, ERCC2, RAI, ASE-1, and D19S246) have been associated with basal cell, breast and lung carcinoma, and mixed oligoastrocytomas. A polymorphism within GLTSCR1 has recently been associated with prostate cancer aggressiveness. We have recently shown that a polymorphism in GLTSCR1 (SNP rs1035938) is associated with oligodendroglioma development. We have now evaluated five additional SNPs within GLTSCR1 using the same cohort of glioma cases and general controls. One of the polymorphisms (Novel 4 – A to G at position 304 from the putative transcription start site) is a novel SNP discovered during GLTSCR1 mutation screening studies of sporadic glioma specimens. Of these five SNPs, two (Novel 4 and rs1005911) were found to have borderline association with oligodendroglioma development by allele-based analysis (P for both = 0.052). However, the prevalence of the at-risk allele was significantly increased in patients whose oligodendroglioma have 19q deletion (P for Novel 4 = 0.025; P for rs1005911 = 0.014). By genotyping 10 CEPH families we were able to determine the most prevalent haplotypes for the six total GLTSCR1 SNPs we tested. We then assessed the prevalence of these haplotypes in the cases with glioma and the normal controls. One haplotype (haplotype 1, ACTCGG) was more prevalent in gliomas than in controls (27.5% vs. 20.4%, P = 0.067). The prevalence increased to 36.7% in gliomas with 19q deletion (P vs. controls = 0.009; P vs. gliomas without 19q deletion = 0.02). The increased prevalence of this haplotype in gliomas was primarily due to its prevalence in oligodendrogliomas (34%, P vs. controls = 0.010) and among the oligodendrogliomas, those with 19q deletion (45%, P vs. controls = 0.001; P vs. oligodendrogliomas without 19q deletion = 0.002). Interestingly, this haplotype is retained in 8 of 10 oligodendrogliomas with 19q deletion. Even though it had a low overall prevalence, another haplotype (ACCCGG) was significantly more prevalent in the controls than the cases (2% versus 0%; P = 0.035). The high-risk and low-risk haplotypes only differ by the presence of the GLTSCR1-exon-1 T allele (rs1035938). These preliminary data strongly suggest that a polymorphism (mutation) is in linkage disequilibrium with the GLTSCR1 ACTCGG haplotype and is associated with oligodendroglioma development, especially those with 19q deletion.

21. NON-ENZYME-INDUCING ANTIEPILEPTIC DRUG USE IN BRAIN TUMOR PATIENTS: EXPERIENCE WITH LEVETIRACETAM

G.J. Stevens, G.H. Barnett, M.A. Vogelbaum, and D.M. Peereboom; Cleveland Clinic Foundation, Brain Tumor Institute, Cleveland, Ohio, USA

It has been estimated that 20% to 40% of brain tumor patients experience a seizure by the time of diagnosis, accounting for 60,000 patients annually. The hepatic enzyme-inducing medications such as phenytoin and carbamazepine have been the drugs of choice for treatment. There may be advantages to using non-enzyme-inducing drugs such as levetiracetam (LEV) to help decrease medication interactions. A retrospective analysis was completed using our brain tumor database that identified all brain tumor patients treated over the past three years with LEV. Patients were evaluated for tumor type, seizure type and frequency, and medication side effects. At the time of the abstract, 278 brain tumor patients were identified. The breakdown included 91 glioblastomas, 24 anaplastic astrocytomas, 15 anaplastic oligodendrogliomas, 13 mixed anaplastic gliomas, 24 low-grade astrocytomas, 46 low-grade oligodendrogliomas, 15 mixed low-grade gliomas, 19 meningiomas, 18 metastases, and 13 tumors defined as "other". Of the 278 patients, 10 (3.5%) stopped or dose reduced the LEV for behavioral

reasons. LEV produced a >50% reduction in seizure activity in 60% of patients, and 70% of patients were maintained on LEV as monotherapy. Brain tumor patients are often on multiple medications, which can increase the possibility of drug interactions. For the past 3 years we have been using the non-hepatic enzyme-inducing drug LEV for brain tumor patients requiring seizure medication. LEV appears well tolerated and an effective drug to control seizures in brain tumor patients. A detailed breakdown of LEV use in brain tumor patients will be presented.

22. PROSPECTIVE QUALITY OF LIFE ASSESSMENT USING EORTC QLQ 30 AND BRAIN CANCER MODULE (BN 20) IN 257 ADULT PATIENTS WITH PRIMARY BRAIN TUMORS SEEN CONSECUTIVELY IN A TYPICAL NEUROONCOLOGY CLINIC

R. Jalali,[1] A. Budrukkar,[1] R. Kamble,[2] R. Sarin,[1] and S. Parab[2]; [1]Tata Memorial Hospital, 113, Radiation Oncology, and [2]Brain Tumor Foundation, Tata Memorial Hospital, Mumbai, India

A majority of quality of life (QOL) data for patients with brain tumors is available as a part of some other study, and there is a relative lack of information in patients seen routinely in clinical practice. The purpose of this study was to evaluate QOL in patients with primary brain tumors as seen in a typical neurooncology clinic, in our setup using validated local language questionnaires. Two hundred and fifty-seven adult patients presenting *consecutively* in our neurooncology clinic from 1 January 2003 to 31 December 2003 were prospectively accrued in the study. A majority of the patients had some sort of surgical intervention before the accrual. All patients underwent a detailed neurological assessment, evaluation of QOL using EORTC questionnaire (QLQ-30), specific Brain Cancer module (BN 20), and daily activities by modified Barthel's index. Assessments were done before starting treatment (typically radiation therapy [RT]) or chemotherapy [CT] in some patients), at completion of RT/CT, and at each follow-up (at least two). The questionnaires were administered in English, Hindi, and Marathi according to the patients' needs using EORTC-ratified versions of Hindi and Marathi (local Indian languages). Mean post-treatment follow-up QOL was compared with the pre RT values. Initial evaluation was done for patients accrued in the first 6 months (N = 137), including 48% below the age of 40 years, 45% between 41 and 60 years, and 6% above 60 years with male-to-female ratio of 2.5:1. Eighty-five percent of the patients had at least primary education, and KPS was above 90 in 68% of the patients. Ninety-seven patients received RT up front and form the analyzed patient population. Sixty-one percent of the patients completed the questionnaire by themselves, 16% required assistance because of poor neurological condition, 11.5% because of illiteracy, and 11.5% because of other causes. At the end of RT, there was a statistically significant improvement in the overall global QOL score, the benefit of which continued at 3 months. Significant improvement was seen in functional scores of physical ($P = 0.000$) and role domain ($P = 0.007$), while the difference in the emotional, cognition, and social scores were not significant. There was deterioration in the symptom scale with respect to nausea and vomiting ($P = 0.04$). In the BN 20 module there was a significant deterioration in scores of hair loss ($P = 0.001$) and local itching ($P = 0.01$). QOL assessment using EORTC QOL 30 and BN20 in validated local language is simple to administer, is generally well understood, and can be used even in routine patients in a busy neurooncology clinic. Patients receiving RT as an up-front modality showed a significant improvement in global, physical, and role functioning and transient worsening in scores of hair loss and itching of skin. An update of all 257 patients at an extended follow up will be presented in the meeting.

23. HUMAN TELOMERASE GENETIC VARIATION PREDICTS SURVIVAL OF PATIENTS WITH GLIOBLASTOMA MULTIFORME

L. Wang, R. El-Zein, K. Aldape, M. Gilbert, Q. Wei, and *M. Bondy*; M.D. Anderson Cancer Center, Neuro-Oncology, Houston, Texas, USA

Glioblastoma multiforme (GBM) is the most common glioma with the poorest survival. Although extent of surgery or a combination of radiotherapy or chemotherapy may improve the outcome, it is important to identify and evaluate biomarkers that might be useful for screening patients and possibly modifying treatment. Level of human telomerase (*hTERT*) mRNA or protein expression has been comprehensively evaluated in most primary tumors for therapeutic purpose. A functional variant of *hTERT* MNS16A-short tandem repeats (S allele) is known to be associated with higher expression levels of *hTERT* mRNA compared with the MNS16A-long (L) allele. In this study, we investigated whether the *hTERT* MNS16A variant genotype predicted survival benefit for 362 patients with GBM. All patients were surgically resected, with some having more complete resection, received radiotherapy, chemotherapy, or a combination. We found a significantly different survival outcome among patients with different *hTERT* MNS16A genotypes, with the median survival of 24.7 months (95% CI = 14.7–29.7) for the SS-genotype, 14.0 months (95%CI=13.2–15.4) for the SL-genotype, and 13.1 months (95% CI = 12.2–15.7) for the LL-genotype ($P = 0.0131$ by Log-Rank testing). These data suggest a dominant effect of the L allele. Compared with the SS-genotype, the hazard ratio (HR) of the combined SL and LL-genotype was 1.83 (95% CI = 1.18–2.83, $P = 0.007$) after adjustment for age, sex, and extent of surgery, and 2.00 (95% CI = 1.29–3.09, $P = 0.002$) after adjustment for age, sex, combined surgery, chemotherapy, and radiotherapy. On the basis of these observations, we conclude that the functional MNS16A-genotype of *hTERT* may modify survival of GBM and serve as a potential biomarker to assess treatment outcomes. However, larger studies are needed to verify our findings.

24. VALIDATION OF BLINDED EVENTS REVIEW COMMITTEE (ERC)–DETERMINED TIME TO NEUROLOGIC PROGRESSION (TTNP) DEMONSTRATES CORRELATION WITH SURVIVAL, RADIOLOGIC PROGRESSION, AND FUNCTIONAL INDEPENDENCE END POINTS

M.F. Renschler,[1] J.A. Smith,[1] W.R. Shapiro,[2] R.A. Patchell,[3] M.J. Glantz,[4] L.D. Recht,[5] M.P. Mehta,[6] and S.C. Phan[1]; [1]Pharmacyclics, Sunnyvale, California; [2]Barrow Neurological Institute, Phoenix, Arizona; [3]University of Kentucky, Lexington, Kentucky; [4]University of Massachusetts, Worcester, Massachusetts; [5]Stanford University, Palo Alto, California; [6]University of Wisconsin, Madison, Wisconsin; USA

Survival is a standard end point for brain metastasis trials, but it inadequately measures treatment benefit because of competing risks for death from systemic disease, and it does not account for neurologic function, an important quality of life consideration. There is no standardized and validated tool to measure TTNP. The purpose of this study was to test the validity of ERC-determined TTNP. In a prospective, randomized phase 3 trial of whole brain radiation ± motexafin gadolinium for patients (pts) with brain metastases, 401 pts underwent standardized neurologic exam, neurocognitive tests (NCT), evaluation of symptoms, functional independence (Barthel Index), and MRI. Using a prespecified algorithm, the ERC reviewed blinded clinical data, excluding MRI, to score progression if 2 of 3 functional domains (NCT, neuro exam, neuro symptoms) showed progression on consecutive visits (Mehta, J. Clin. Oncol. 21, 2529, 2003). TTNP was compared with survival, MRI progression, and time to loss of functional independence by using log-rank tests and Kaplan-Meier plots. Patients with TTNP = 1, 2, 3, or 4 months had a shorter median survival (by 4.4, 3.9, 3, and 2.4 months, respectively), compared with pts who did not have neurologic progression at those times. The validity of a blinded ERC-determined TTNP end point was demonstrated by high correlations with survival, radiologic progression, and loss of functional independence end points. We conclude that ERC-determined TTNP measures clinical benefit and is sensitive to change.

25. ADVANCES IN DRUG DELIVERY

M. Westphal; Department of Neurosurgery, University Hospital Hamburg Eppendorf, Germany

Drug therapy for gliomas has been consistently hampered by lack of specificity, systemic toxicity, lack of penetration, limitations to size of the molecules, and poor diffusion into normal brain. Therefore, methods have been developed for local delivery. Intracavitary delivery has been used mostly in the context of slow-release biodegradable polymers, which has resulted in high local drug concentration but variable depth of diffusion into the tissue. Releasing BCNU, this technique has gone through successful phase 3 trials. Under investigation for the same technique are different compounds, modified release kinetics, higher doses, and drug combinations. Pericavitary injection of slow-release polymer with 5-FU after resection of glioblastoma is used as a radiation sensitizer, and a phase 2 study is just published and phase 3 in preparation. A major advance is the direct, slow infusion of therapeutic compounds into tissue, either the tumor or the surrounding "normal" brain. This convection-enhanced delivery (CED) allows for any substance to be delivered on the "other side" of the blood-brain barrier. Large and complex molecules can be used, with their distribution properties depending on their physicochemical characteristics (charge, solubility, size). Presently, large fusion molecules of ligands for cell surface receptors with toxins are under evaluation in phase 2 and 3. Being directed at cell surface molecules expressed only on tumor cells and not on normal brain cells, this therapy is aimed to be in the crosshairs of compartmental delivery and a compartmental specificity. Many more molecules can be designed, depending only on compartmental specificity, especially antibodies, either naked or coupled to effector molecules. Distribution will be different for every new compound and will also depend on heterogenous tissue factors like scars, cysts, and hemorrhages in a tumor but also favorable white matter tracts and possibly less permissive areas like basal ganglia or areas of

prior injury in "normal" brain. Further development requires cooperation with neuroradiologists to study fluid movements with dti, which does not really prove where the compounds go. Also, computer modeling is currently being explored for its predictive value to create "isodistribution curves". In addition to the large protein-based therapeutics, classical compounds like taxol are under investigation for CED. Prodrug therapy is another way to reduce systemic toxicity but requires the specific transduction of glioma cells with a converting enzyme. This is achieved by viral or liposomal vectors, and a new phase 3 trial based on a TK transducing adenovirus will start in 2005. Research into the biology of the specialized endothelial cells of the blood-brain barrier has revealed transporter molecules which when employed experimentally may afford specific delivery of conjugated therapeutics across the blood-brain barrier.

26. ADVANCES IN MOLECULAR DIAGNOSIS

A. Perry; Washington University, Neuropathology, St. Louis, Missouri, USA

Diffuse gliomas are clinically, pathologically, and genetically heterogeneous malignancies. Patient age and histology are the two most powerful prognostic parameters, though there remains significant clinical variability, both in terms of overall survival and response to therapies. Our understanding of glioma tumorigenesis and progression has advanced greatly over the last 15 years, though few observations have successfully translated into molecular diagnostic assays applied daily in neuropathology and neuro-oncology. The most common is chromosome 1p and 19q testing with combined 1p/19q deletions identifying "genetically favorable" oligodendroglial tumors with enhanced survival and responsiveness to both alkylating chemotherapeutic agents and radiation. It is predominantly, but not exclusively, associated with histologically classic grade II and III oligodendrogliomas (ODG), where codeletions are found in up to 80% to 90%, suggesting that this represents an early event. It is rare in pediatric ODGs, which suggests an alternate tumorigenic pathway in children. Mixed oligoastrocytomas are diagnostically challenging and are genetically heterogeneous. We recently found that survival was enhanced in those with 1p/19q codeletion, 19q deletion alone, or no detectable alterations, whereas it was decreased in those harboring 9p deletion, 10q deletion, and/or EGFR amplification. Small interstitial 1p and/or 19q deletions are also seen in some astrocytomas, though the whole arm 1p/19q codeletions are fairly specific to ODGs. We have not seen them in morphologic mimics, such as DNT, clear cell ependymoma, central neurocytoma, and small cell glioblastoma. The latter enters the differential diagnosis most often. However, survival is short and it is genetically characterized by 10q deletion (>95%), EGFR amplification (70%), and EGFR-vIII expression (50%). We have also seen rare "extraventricular neurocytomas" with 1p/19q codeletion, though there is evidence that some oligodendrogliomas undergo neuronal differentiation and these 2 entities are likely related. Lastly, high-throughput technologies, such as expression profiling and array CGH, provide new opportunities to identify candidate genes and groups of genes that may lead to molecular diagnostics applications in the future. The ultimate goal will be to provide accurate and cost-efficient glioma phenotyping that will enable targeted therapies with the highest likelihood of success for each individual patient.

27. MALIGNANT GLIOMA: ADVANCES IN RADIATION THERAPY

M. Mehta; University of Wisconsin, Human Oncology, Madison, Wisconsin, USA

Randomized clinical trials have established a survival benefit with adjuvant radiotherapy for malignant glioma, and indirect evidence supports a dose-survival relationship, between 0 and 60 Gy. Escalation beyond 60 Gy has been limited primarily by toxicities, but innovative approaches to achieve this aim have been developed, piloted, and after promising initial results, tested in phase 3 trials, only to result in negative findings. The best examples of these technologies include brachytherapy and radiosurgery, for both of which, promising phase 2 results were not corroborated in phase 3 trials. New technologies continue to be developed with the ultimate objective of achieving dose-escalation. Examples include 3-dimensional radiotherapy delivery techniques, with which doses up to 90 Gy have been explored (without improved local control), the balloon brachytherapy device known as Gliasite, intensity modulated radiotherapy (IMRT), etc. The purpose of IMRT is to produce exquisite shaping of the radiation dose-distribution to mimic the exact shape of the tumor, with a dramatic avoidance of nearby critical structures. The value and success of this modality is contingent on the hypothesis that prior dose-escalation efforts have failed as a consequence of inadequate target definition due to inherent limitations of current MRI methods. Perhaps with the advent of functional imaging with MR and PET, a more precise definition of the target might be achieved, and the irregularly shaped tumor could be accurately and conformally targeted by using IMRT for dose-escalation, ultimately resulting in improved local control. Other strategies have been used to achieve dose-escalation focus on molecular targeting. In the simplest of these approaches, a tumor-specific antigen is targeted with a locally instilled radiolabeled antibody to achieve a high target dose, and clinical trials evaluating this approach are under way. Other targeted approaches have attempted to identify molecularly targeted pathways that support proliferation and angiogenesis and circumvent apoptosis, with the demonstration of inhibitors of these pathways as potent radiosensitizers. Proof-of-principle of this concept comes from a recent phase 3 head and neck cancer trial. The EGFR and PI3 kinase pathways are two key examples of this, and initial clinical trials targeting one or both of these have been launched, and some preliminary data are available, which will be presented. Other radiosensitizers such as MGd and RSR-13, already in clinical testing, will be described. Historically, neutron irradiation has been explored as a way of increasing tumor dose-intensity, by exploiting the preferential dose-localization properties of neutron interaction with elements such as boron, which have a high cross-sectional area of intersection for neutrons. In order for such a strategy to be successful, tumor-specific preferential localization of boron needs to occur at an adequate concentration level. First- and second-generation boronated compounds have not met these criteria, and newer efforts are focused on two fronts: (1) developing tumor-targeted boron-containing compounds by tagging EFGR ligands and (2) evaluating tumor-specific gadolinium-containing agents, since gadolinium has a superior cross-sectional area of intersection with neutrons.

29. DEVELOPMENTAL ABNORMALITIES AND ONCOGENESIS IN THE BRAIN OF A TRANSGENIC E2F1 MOUSE MODEL

M.V. Olson, D.R. Medrano, H. Jiang, M.G. Lemoine, C. Gomez-Manzano, M.M. Alonso, K. Aldape, R. Price, W.K.A. Yung, and J. Fueyo; M.D. Anderson Cancer Center, Neuro-Oncology, Houston, Texas, USA

The E2F family of transcription factors are involved in tumor suppression and tumor generation. Although the Rb/E2F pathway is deregulated in most brain tumors, there is no direct evidence of the role of E2F1 in the generation or maintenance of brain tumors. To address this question, we generated a transgenic animal model driving expression of E2F1 through the GFAP promoter to glial cells and neuronal/glial precursors. Histological analysis of brain tissue revealed expression of the transgene in astrocytes, ependyma, and the Bergman glial cells of the cerebellum. Importantly, cells positive for E2F1 were also positive for proliferation markers such as PCNA and BRDU as well as apoptosis, detected through TUNEL assay. Overexpression of E2F1 led to the generation of a phenotype characterized by numerous neurological defects without a clear pathological frame. The early onset of these phenotypes suggests a developmental abnormality. When overexpression of E2F1 was introduced into an E2F4 null background, a new phenotype, not seen in either background alone, was induced and characterized by the development of a domed head, hypo- and hyperactivity, and seizures. MRI examination of these mice showed a dramatic hydrocephalus. Pathologic examination of the brains uncovered congenital triventricular hydrocephalus due to E2F1-induced hyperproliferation of the ependyma in the aqueduct, resulting in the death of the animal by 5 weeks of age. Parallel studies involving the overexpression of E2F1 and simultaneous inactivation of p53 resulted in neurological signs including tremors, ataxia, seizures, head tilt and paresia of the posterior limbs. Histological analyses of the brains from 3-month-old transgenic animals uncovered the production of neoplasms including a highly undifferentiated choroid plexus carcinoma with papillary and glandular features and an aggressive embryonal tumor of the cerebellum, expressing the pathologic features of medulloblastoma. Furthermore, immunohistochemical studies confirmed proliferation of cells expressing the hE2F1 transgene within the tumor tissue. This study is the first to provide direct evidence that E2F1 functions as an oncogene through the induction of brain cancer.

30. FOS-RELATED ANTIGEN 1 (FRA-1) MODULATES MALIGNANT FEATURES OF GLIOMA CELLS

W. Debinski and D.M. Gibo; Wake Forest University, Brain Tumor Center of Excellence, Winston-Salem, North Carolina, USA

We uncovered that a *c-fos* inducible vascular endothelial growth factor D (VEGF-D) is ubiquitously upregulated in high-grade gliomas (HGG). However, HGG overexpress a Fos-related antigen 1 (Fra-1) rather than c-Fos among the activating protein 1 (AP-1) transcription factors. Therefore, we have examined an effect of ectopic Fra-1 or a knockdown of this transcription factor in H4 (low Fra-1, non-tumorigenic), U-87 MG (high Fra-1, highly tumorigenic), and A-172 MG (moderate Fra-1, non-tumorigenic) malignant glioma cells. We have transfected glioma cells with *fra-1*

in both sense (+) and anti-sense (-) orientation. The ectopic Fra-1 evoked prominent phenotypic changes in all cell lines studied: The cells became more polar with larger number of elongated processes. This was seen by standard microscopy and by changes in actin architecture using phalloidin staining. We noticed that *fra-1* siRNA, but not nonsense nucleotides, produced reversal of the morphological features associated with the ectopic Fra-1 (H4). The characteristic change in the phenotype seen in cell in vitro was carried over to tumors grown *in vivo* (U-87 MG). Of interest, completely non-tumorigenic H4 cells started to form tumors when transfected with *fra-1* transgene, at an 80% of tumor take. Moreover, the genotype of H4[*fra-1*](+) cells changed significantly, since 18 different genes became overexpressed, at least fourfold vs. controls, with a targeted cDNA microarray analysis (1056 genes). Conversely, *fra-1*(-) altered prominently the morphology (U-87 and A-172), anchorage-independent growth (U-87 and A-172), tumorigenic potential (U-87), and the expression of Fra-1 effectors, such as VEGF-D (U-87 and A-172). For example, *fra-1*(-) made cells more rounded, with fewer and shorter cellular processes (U-87 and A-172). Also, the U-87[*fra-1*](-) cells lost an ability to grow in agar, while U-87[*fra-1*](+) cells started to form multiple colonies; similar results were seen in A-172 cells. Furthermore, we found that by day 22, there were 80% U-87[*fra-1*](-) tumors formed (8 out of 10) of an average size 20 mm^3 while the size of U-87[*fra-1*](+) tumors (10 out of 10) was 135 mm^3. In addition, the U-87[*fra-1*](-) tumors were poorly vascularized, and the levels of VEGF-D in tumor cells were low compared to parental U-87 tumors. Thus, Fra-1 induces profound phenotypic changes in malignant glioma cells with associated significant changes in their genotype. Fra-1 engages mechanisms that promote tumorigenesis and anchorage-independent growth. Being that Fra-1 is frequently upregulated and also accumulates to high levels in response to AP-1 activation in malignant glioma cells, this transcription factor may likely play an important role in the maintenance or progression of malignant gliomas and potentially represents a new target for therapeutic interventions.

31. HUMAN BRAIN SLICES AS CONTROL ASSAY FOR TUMOR-TARGETED THERAPY IN VITRO

R.P. Weenink,[3] C.M.F. Dirven,[2] W. van Houdt,[2] D.P. Noske,[2] S. Idema,[2] M.L.M. Lamfers,[2] V.W. van Beusechem,[1] and R.W.H. Verwer[3]; [1]VU University Medical Center, Gene Therapy; [2]VU University Medical Center, Neurosurgery; [3]Netherlands Institute for Brain Research, Amsterdam, The Netherlands

To develop novel tumor-targeted anticancer drugs, such as oncolytic adenoviruses, early in vitro testing of efficacy is a prerequisite. True selectivity of the targeted drug toward tumor cells can be reliably assessed only when results with tumor tissue can be compared to those obtained with "normal tissue". Here we describe the use of cultured slices of normal human brain, obtained by epilepsy surgery, as a control assay for testing selective cytopathic activity of oncolytic adenoviruses. Methods to evaluate the cytopathic effect in relation to the viability of the brain slices are studied. Fresh surgical specimens of healthy cerebral cortex acquired during surgery for hippocampectomy were cut into slices of 200-μm thickness and kept in 24-well plates in culture medium (Verwer et al., FASEB J. 16 (2002) 54-60). Slices were infected with several different adenoviruses, i.e., wild-type Ad5 and replication-defective adenovirus vectors, Ad.CMV.Luc, and Ad.survivin.Luc (the latter only expressing the luciferase transgene in cells with active survivin transcription, i.e., tumor cells). Viability of cells was assessed before and after treatment by using the MTT-derived WST-1 assay (Roche), Live/Dead kit (L/D, Molecular Probes), and cytochrome oxidase activity histochemistry. Our results indicate that wild-type adenovirus can efficiently infect the brain slices. This resulted in a significant reduction of viability and energy metabolism, as measured by the L/D kit and cytochrome oxidase activity, respectively. The WST-1 assay appeared to be insufficiently sensitive. Experiments with Ad.CMV.Luc and Ad.survivin.Luc show that luciferase expression is significantly lower when using the virus with the tumor-specific promoter. The brain slice model is a valuable tool for assessment of the selectivity of tumor-targeted agents in neuro-oncology, such as modified adenoviruses. The brain slice model is particularly useful when the targeting strategy of the oncolytic drug is based on species-specific (human) proteins, causing xenografted animal tumor models to be of limited use for addressing the question of tumor selectivity. In the context of oncolytic agents, cytochrome oxidase activity appears to be the most favorable method to assess viability of brain slices.

32. NOVEL APPROACHES TO METASTATIC ANIMAL MODELS: A PILOT STUDY FOR BRAIN METASTASIS IN MICE, 3D TUMOR GROWTH IN CELLULOSE MATRIX, AND IN VIVO REAL-TIME IMAGING WITH BIOLUMINESCENCE

M. Mut,[1] J. Carpenter,[2] G. Redpath,[2] D. Mullins,[5] C. Slingluff,[4] B. Kundu,[3] E. Farace,[1] M. Lopes,[2] I. Hussaini,[2] and M. Shaffrey[1]; [1]Neurosurgery; [2]Pathology; [3]Radiology Research; [4]Surgery; and [5]Microbiology, University of Virginia, Charlottesville, Virginia, USA

Animal models for brain tumors have provided a significant insight in understanding molecular mechanisms of disease progression and development of new treatments. Despite the presence of many models available currently for glial tumors, there is no reproducible model to mimic human cancers that metastasize to brain in animals. This is a pilot study of a "brain melanoma metastasis model in mice" that may re-create the metastatic cascade of cancer. We have chosen melanoma, which is a tumor with high predisposition for metastasizing to brain. Human melanoma cell lines derived from patient's specimens with metastatic melanoma to the brain are harvested and cultured (VMM1 and 86). Cells are transfected with luciferase marker gene and after assessing its expression; cells are xenografted into two different groups of immunocompromised mice in this pilot study. In the first group made up of four animals, tumors embedded into cellulose matrix (Gelfoam) are injected stereotactically into brain to allow "activation" of melanoma cells in brain tissue milieu. In the second group of four animals, tumors are injected intradermally to re-create the metastatic cascade resulting in brain metastasis. Multiple transfers of cells from xenograft of the animal, whose tumor metastasizes to brain, into another animal will ensure continuity of the cycle. Both intradermal and intacranial injection groups are imaged for luciferase activity as reflecting tumor growth and patterns of metastasis without sacrificing the animals. All four animals in both groups reflected tumor growth in their original injection sites with luciferase activity detected within four weeks. Luciferase bioluminescence may be imaged with a simple, cooled, charged couple device (CCD) camera and is an inexpensive, non-invasive screening and sensitive way of assessing tumor growth even after intracranial injections. Application of the bioluminescence techniques provides in vivo, real-time imaging that is used for the first time in a brain metastasis model. This is a pilot study that utilizes novel approaches to metastatic animal models; namely, this is the first study to mimic the metastastic cascade to the central nervous system using cancer cell lines that are "predisposed" to brain metastasis and to evaluate in vivo, real-time imaging enabled by luciferase gene transfection into tumor cells.

33. LOCAL TREATMENT WITH AN IMMUNOSTIMULATORY CPG-OLIGONUCLEOTIDE IN PATIENTS WITH RECURRENT GLIOBLASTOMA: RESULTS OF A PHASE I TRIAL

A. Carpentier,[1,2] A. Tibi,[3] M. Richard,[4] L. Capelle,[1] F. Laigle-Donadey,[2] A. Behin,[2] M. Sanson,[2] R. Van Effenterre,[1] and J. Delattre[2]; [1]Neurosurgery, [2]Neurology, Hôpital de la Salpêtrière, Paris; [3]Agence Générale des Equipements et Produits de Santé (AGEPS), Paris; [4]Oligovax; Paris; France

Oligonucleotides containing one or several CpG motifs (CpG-ODN) display a strong immunostimulating activity, driving the immune response toward the Th1 phenotype. In preclinical studies, they have shown promising efficacy when injected locally in several cancer models, including gliomas. Limursen, a new phosphorothioate CpG-ODN, was administered intratumorally by convection-enhanced delivery in patients (pts) with recurrent glioblastomas (GBMs). Increasing doses were injected, starting at 0.5 mg and escalating to 1, 2, 5, 10 or 20 mg in cohorts of 3 to 6 patients. The primary objective was to determine the safety profile of intratumoral Limursen in patients with recurrent GBM. Twenty-four pts were enrolled in the study. All patients were previously treated with radiotherapy and, in most cases, with one or several lines of chemotherapy (1 chemotherapy in 10 pts, 2+ in 11 pts). At the time of inclusion, the median age was 56 years (range, 24–72 years), and median KPS was 80% (range, 60%–100%). Two adverse events were considered related to the procedure, a local hemorrhage along the catheter track and a pulmonary embolism secondary to the interruption of a long-term anticoagulant therapy. Adverse effects possibly/probably related to the studied drug were moderate. Spontaneously regressive grade 3 lymphopenia was reported in 6 patients. Grade 3 nonhematological toxicity consisted of reversible ALT elevation (2 pts at the highest dose). Six patients experienced fever above 38°C, mainly at higher doses. The fever peaked on day 3 and disappeared within a few days. Transient worsening of neurological conditions was observed at the highest dose in 3 patients. Preliminary evidence of antitumor activity was suggested with a local response at the site of injections in 2 patients. Three other patients had a stable disease for more than 3 months. Updated data for survival will be presented at the meeting. In conclusion, Limursen was well tolerated in patients with recurrent GBM, with sides effects mainly limited to transient worsening of neurological conditions and fever in a few patients. Limursen is now applied in a multicentric phase 2 study in recurrent glioblastomas.

34. EARLY PHYSIOLOGICAL AND METABOLIC EFFECTS OF INTRATUMORAL BCNU ON UNTREATED HUMAN GLIOMAS

M. Jenkinson,[1] E. Smith,[2] K. Kopitzki,[1] S. Vinjamuri,[3] C. Walker,[4] D. Pietronigro,[5] and P. Warnke[1]; [1]University of Liverpool, Neuroscience, Liverpool, UK; [2]The Walton Centre for Neurology and Neurosurgery, Neuro-radiology, Liverpool, UK; [3]Royal Liverpool University Hospital, Nuclear Medicine, Liverpool, UK; [4]Clatterbridge Cancer Research Trust, Bebington, UK; [5]Direct Therapeutics Incorporated, New York, USA

DTI-015 (BCNU dissolved in ethanol) utilizes solvent-facilitated perfusion for the intratumoral treatment of gliomas. We have investigated the early biological effects of intratumoral DTI-015 on newly diagnosed, circumscribed malignant gliomas. Magnetic resonance imaging (MRI) and spectroscopy (MRS), single photon emission computed tomography (SPECT), and computed tomography (CT) perfusion studies were used to assess the effect of DTI-015 on in vivo tumor physiology and metabolism. Nine patients (2 female, 7 male) with a median age of 58 years (range: 47–70 years) were enrolled into the study. Histological diagnosis was anaplastic astrocytoma (n = 3) and glioblastoma multiforme (n = 6). The median Karnofsky performance score was 90. Tumor volume was 13.6 ± 7.6 cm^3 (mean ± SD). The volume of DTI-015 injected was 4.3 ± 1.4 ml (mean ± SD) with a BCNU dose of 256 ± 82 mg (mean ± SD). Mean tumoral cerebral blood flow significantly reduced within 72 h of DTI-015 injection (paired *t*-test; mean reduction 17.3, $P = 0.001$, 95% CI, 10.0–24.6). Relative cerebral blood volume also reduced significantly (paired *t*-test; mean reduction 12.5, $P = 0.017$, 95% CI ,2.9–22.2). There was a significant reduction in FDG utilization (paired *t*-test; mean reduction 0.28, $P = 0.001$, 95% CI, 0.16–0.41) and thallium uptake (paired *t*-test; mean reduction 3.26, $P = 0.001$, 95% CI, 1.78–4.74), with an increase in the Lip1/Cr ratio (Wilcoxon signed ranks; $P = 0.034$) after DTI-015 injection. The data forms a biological basis for understanding the effects of high-dose BCNU on malignant gliomas. Early effects can be seen on the tumor vasculature and metabolism, resulting in a pattern of ischemic tumor necrosis.

35. IMATINIB MESYLATE (GLEEVEC) PLUS HYDROXYUREA: AN EFFECTIVE REGIMEN IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMA: PHASE 2 STUDY RESULTS

D.A. Reardon,[1] J. Quinn,[1] J. Rich,[1] J. Vredenburgh,[1] A. Desjardins,[1] S. Sathornsumetee,[1] A. Salvado,[2] Z. Nikolova,[2] D. Bigner,[1] and H. Friedman[1]; Department of Surgery, [1]Duke University Medical Center, Durham, North Carolina; [2]Novartis Pharmaceutical Corporation, East Hanover, New Jersey; USA

In this phase 2 study, we evaluated the activity of imatinib mesylate (Gleevec), an inhibitor of the PDGF receptor tyrosine kinase with anti-angiogenic activity and the ability to decrease tumor interstitial pressure, combined with hydroxyurea in the treatment of patients with recurrent malignant glioma. Eligibility criteria include the following: recurrent malignant glioma; age >18 years; KPS 60% or greater; less than grade 2 intratumoral hemorrhage; adequate hepatic, renal, and bone marrow function. Hydroxyurea is administered at 500 mg BID while Gleevec is administered at 500 mg BID for patients on enzyme-inducing anticonvulsants (EIAC; phenytoin, carbamazepine, and phenobarbital) and at 400 mg QD for those not on EIAC. Each treatment cycle is 28 days, and patients are evaluated for response every other cycle. Sixty-four patients have been enrolled to date, including 32 with recurrent GBM and 32 with recurrent AA/AO. The median age is 46 (range 21 to 68); 55% are male and 45% are on EIAC. All patients had prior XRT. The median number of prior chemotherapy agents was 3 (range, 1–5), and the median number of prior progressions was 2 (range, 1–7). Toxicity has been limited to grade 3 or 4 hematologic events in 20% and 5%, respectively, grade 3 edema in 8%, and grade 3 LFT abnormalities in 3%. Among GBM patients, radiographic responses have been observed in 9%, while 35% have achieved stable disease. Median progression-free survival (PFS) for patients with recurrent AA/AO and GBM are 10.9 and 14.4 weeks, respectively. At 6 months, 26.3% of GBM patients remain progression free. The rate of radiographic response, median PFS, and 6-mth PFS rate observed in this study among heavily pretreated patients with recurrent GBM compare favorably to results achieved with temozolomide in first relapse, indicating that a randomized trial of imatinib mesylate plus hydroxyurea versus temozolomide is warranted.

36. IMPROVED SURVIVAL OF HIGH-GRADE GLIOMA PATIENTS AFTER GENE THERAPY WITH AN ADENOVIRAL VECTOR CONTAINING THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE: A PHASE 2 STUDY

A. Sandmir,[1] M. Vapalahti,[1] A. Immonen,[1] K. Tynnela,[2] H. Hurskainen,[1] R. Vanninen,[3] G. Langford,[4] N. Murray,[4] S. Yla-Herttuala,[5, 6, 7] and M. Vapalahti[7]; Departments of [1]Neurosurgery, [2]Oncology, and [3]Radiology, [4]Department of Molecular Medicine A.I. Virtanen Institute, [5]Department of Medicine, University of Kuopio, Kuopio, Finland; [6]Ark Therapeutics, Research and Development, London, UK; [7]Gene Therapy Unit, Kuopio University Hospital, Kuopio, Finland

A phase 2 study was conducted to evaluate the efficacy and safety of using Herpes Simplex virus thymidine kinase in an adenoviral vector AdvHSV-tk (Cerepro, Ark Therapeutics Ltd) with intravenous ganciclovir in malignant glioma patients. This was a single center, randomized, controlled study involving 36 patients with operable primary or recurrent high-grade glioma. Seventeen patients were randomized to receive AdvHSV-tk gene therapy (3×10^{10} pfu) by local injection into the wound bed at the time of tumor resection, followed by intravenous ganciclovir, 5 mg/kg twice daily for 14 days. The control group of 19 patients received standard care consisting of radical excision. Patients in both groups with primary tumors received postoperative radiotherapy. AdvHSV-tk therapy increased mean survival from 39.0 ± 19.7 (SD) in control patients to 70.6 ± 52.9 weeks in patients in the active group (log-rank regression $P = 0.0095$). Median survival increased from 37.7 to 62.4 weeks. The percentage increase in mean survival was 81% and median survival was 65%. The therapy was well tolerated as assessed by adverse events, clinical chemistry, hematology, and immunology. There was no evidence of any deterioration in quality of life or increased use of concomitant medications. AdvHSV-tk gene therapy with ganciclovir is a new, well-tolerated, potentially effective therapy for operable high-grade glioma.

37. ROLES OF AURORA A MITOTIC KINASE IN THE DEVELOPMENT OF MALIGNANT GLIOMAS

H. Saya,[1] O. Kobayashi,[1] and J. Kuratsu[2]; [1]Tumor Genetics and Biology; [2]Neurosurgery, Kumamoto University, Kumamoto, Japan

Chromosomal instability and aneuploidy are remarkable hallmarks of human cancers. In most cancers, high rates of chromosome gains/losses leading to aneuploidy have been observed. The causes of aneuploidy involve failure in various critical mitotic events, including centrosome separation, chromosome alignment, chromosome segregation, and completion of cytokinesis. The error-free mitosis that is important to genomic integrity is regulated by phosphorylation reactions driven by several evolutionarily conserved serine/threonine kinases, known as mitotic kinases. Mitotic kinases include cyclin-dependent kinase 1 (Cdk1) and Polo-related, NimA-related, Aurora-related, and Warts-related kinases. In mammals, three members of this kinase family, Aurora-A, -B and –C, were identified. Recently, observations have revealed that Aurora-A kinase activity is required for various events during mitosis, such as G_2-M transition, centrosome separation, chromosome alignment and cytokinesis (Hirota et al., Cell 114, 585, 2003; Kunitoku et al., Dev. Cell 5, 853, 2003; Marumoto et al., J. Biol. Chem. 278, 51786, 2003). Given that not only elevated expression of Aurora-A but also depletion of Aurora-A leads to mitotic failure and multinucleation, it is speculated that the proper timing and amplitude of Aurora-A expression is important for accurate chromosome segregation and fidelity of chromosome transmission (Marumoto et al., Nat. Rev. Cancer, in press, 2005). Furthermore, we generated a transgenic mouse model to investigate the involvement of Aurora-A overexpression in the tumorigenesis and found that elevated Aurora-A expression induces malignant transformation in the mouse in the presence of p53 mutation/loss (Zhang et al., Oncogene 23, 8720, 2004). These findings indicate that mitotic aberrations induced by Aurora-A overexpression with p53-dependent checkpoint abnormality are critical factors for the cancer formation. We have analyzed Aurora-A expression in malignant gliomas and found that it is frequently overexpressed in anaplastic astrocytomas and glioblastomas. Especially, the Aurora-A overexpression is well correlated with giant cell formation in those tumors, which is consistent with data obtained in our transgenic mouse model. Our findings strongly suggest that aberrant expression of Aurora-A is a high risk factor for malignant progression of astrocytic tumors.

38. REGULATION OF PI3K SIGNALING AND TRANSFORMATION BY PTEN C-TERMINAL-INTERACTING PROTEINS

F. Furnari,[1] K. Okumura,[1] M. Mendoza,[2] M. Zhao,[3] R. Bachoo,[4] R. DePinho,[4] and W. Cavenee[1]; [1]Ludwig Institute-UCSD, La Jolla, California, USA; [2]UCSD, Biomedical Sciences Graduate Program, La Jolla, California, USA; [3]Shanghai Institutes for Biological Sciences, Institute of Biochemistry and Cell Biology, Shanghai, China; [4]Harvard Medical School, Boston, Massachusetts, USA

The *PTEN* tumor suppressor gene is frequently mutated in diverse tumor types, including those of endometrium, breast, prostate, lung, and high-grade glioma. The protein possesses an amino-terminal catalytic domain with lipid phosphatase activity which regulates the AKT pathway through modulation of the second messenger product of PI3K, PI(3,4,5)P3. While this catalytic region of the PTEN protein, which controls G_1 cell cycle progression and hence suppresses tumor formation, has been fundamentally defined, the function(s) of the carboxy-terminal region of the protein remain largely unknown. To address the issue we searched for PTEN-interacting proteins by yeast two-hybrid screening using the PTEN carboxy-terminal domain, which has been reported to contribute to membrane localization and protein stability as governed by casein kinase II-directed phosphorylation. Here we report the identification and characterization of two PTEN interacting proteins, the histone acetyltransferase, PCAF (p300/CBP associated factor), and the oncogenic, v-Jun transcriptional target, MSP58 (58-kDa microspherule protein). The association between PCAF and PTEN caused increased and direct acetylation of lysine residues (K125 and K128) within the catalytic cleft of PTEN, a structure required for PI(3,4,5)P3 selectivity. This PCAF-mediated acetylation of PTEN was dependent on the presence of growth factors. Reduction of endogenous PCAF activity using shRNA resulted in a loss of PTEN acetylation in response to growth factors and restored the ability of PTEN to downregulate PI3K signaling. The biological significance of these findings was evidenced by the capacity of PCAF to acetylate PTEN and to ablate PTEN-mediated suppression of PI3K/AKT signaling and G_1 cell cycle arrest. The retention of PI3K/AKT signaling and cell cycle regulatory activities of acetylation-resistant PTEN K125R and K128R mutants in the presence of enforced PCAF expression supports a causal relationship. The association of PTEN with MSP58 was mapped to the MSP58 FHA domain and required PTEN threonine 366, a site reported to be phosphorylated in vivo. Additionally, we showed that while MSP58 transformed $pten^{(-/-)}$ MEF cells, concurrent introduction of wild-type PTEN caused a dramatic reduction in the number of MSP58-induced colonies. This inhibition of cellular transformation required interaction with PTEN since a point mutant of its interaction domain (Thr366Ala) was without effect. Importantly, transformation inhibition did not, however, require PTEN to be catalytically active, as its G129R mutant could inhibit MSP58-driven transformation. Thus, the C-terminal region of PTEN provides novel biological functions to this tumor suppressor gene in its ability to regulate its lipid phosphatase activity through interaction with PCAF and in its ability to regulate cellular transformation through interaction with MSP58.

39. RAS/RAL-PATHWAY ACTIVATION SUPPRESSES CDC42 ACTIVATION/FLIPS EXPRESSION AND SENSITIZES GLIOMA CELLS TO TRAIL-INDUCED APOPTOSIS

R. Pieper, A. Panner, and M. Berger; UCSF, Neurosurgery, San Francisco, California, USA

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) protein is an attractive therapeutic molecule because it induces apoptosis in glioma cells, but not in normal astrocytes. The present study was initiated to better understand the basis for this tumor selectivity. Normal human astrocytes; astrocytes immortalized (but not transformed) by retroviral expression of E6, E7, and hTERT; and astrocytes transformed by expression of E6, E7, hTERT, and mutant V12 H-Ras were assessed by Western blot for expression of the caspase-8 inhibitor FLIPs, after which the cells were exposed to TRAIL (0–1000 ng/ml, 1 h) and the extent of TRAIL-induced apoptosis was determined by flow cytometry. Immortalized astrocytes were also retrovirally infected with constructs encoding mutant forms of Ras that selectively activated the Ras- PI3K (C40 Ras), Ras-Raf (S35 Ras), or Ras-Ral (G37 Ras) pathways, after which the effects of PI3K, Raf, or Ral activation on cellular transformation; the levels of the Ras/Ral target cdc42; FLIPs levels; and TRAIL sensitivity were monitored. While both normal and immortalized human astrocytes were resistant to TRAIL-induced apoptosis (up to 1000 ng/ml TRAIL) and expressed high levels of FLIPs, V12 H-Ras-transformed astrocytes exhibited low levels of FLIPs and underwent apoptosis following exposure to as little as 200 ng/ml TRAIL. Only expression of a retroviral construct encoding the Ras/Ral-selective G37 mutant (or introduction of activated Ral itself) transformed astrocytes, suppressed FLIPs levels, and sensitized the astrocytes to TRAIL-induced apoptosis in a manner comparable to mutant V12 H-Ras. In the cells expressing either V12 H-Ras or G37 Ras, suppression of FLIPs levels and enhanced TRAIL sensitivity was associated with suppression of phosphorylation/activation of the Ras/Ral target cdc42. Conversely, retroviral introduction of either cdc42 or FLIPs reversed V12 H-Ras/G37 Ras-mediated suppression of FLIPs levels and conferred TRAIL resistance. These results show that activation of the Ras/Ral pathway not only leads to cellular transformation, but also to inhibition of cdc42 activation/FLIPs expression and sensitization of glioma cells to TRAIL-induced apoptosis. The extent of Ras pathway activation (which has been shown to be proportional to glioma grade) may therefore be helpful in predicting the sensitivity of gliomas to TRAIL-induced apoptosis.

40. HISTONE DEACETYLASE INHIBITORS, N-BUTYRIC ACID AND TRICHOSTATIN A, INDUCE CASPASE-8-DEPENDENT BUT NOT CASPASE-9-DEPENDENT APOPTOSIS IN HUMAN MALIGNANT GLIOMA CELLS

T. Komata, T. Kanzawa, T. Nashimoto, H. Aoki, S. Endo, T. Kon, H. Takahashi, S. Kondo, and T. Ryuichi; Brain Research Institute, Niigata University, Neurosurgery, Niigata, Japan

Histone deacetylase (HDAC) inhibitors have both apoptotic and differentiating effects on various tumor cells. However, the mechanisms underlying the effect of HDAC inhibitors remain unclear. In this study, we investigated the function of antiproliferative effect of HDAC inhibitors, N-butyric acid and trichostatin A, on human malignant glioma cell lines, U251-MG and D54. MTT assay showed dose-dependent inhibition of cellular proliferation in both cell lines. Cell cycle analysis revealed increased sub-G_1 population in both lines, and G_1 arrest only in U251-MG cells. Induction of apoptosis was also supported by the occurrence of DNA fragmentation in tumor cells treated with HDAC inhibitors. Furthermore, caspase inhibition assay indicated that HDAC inhibitors-induced apoptosis was caspase dependent. Interestingly, neither mitochondrial membrane potential nor the expression of caspase-9 was changed by treatment with HDAC inhibitors, suggesting the possibility that HDAC inhibitors-induced apoptosis was not mediated by mitochondrial cell death pathway. On the other hand, immunoblotting assay confirmed increased expression of caspase-8 in both lines, and elevation of p21 but not of p27 protein in U251-MG cells following HDAC inhibitor treatment. Taken together, the HDAC inhibitors, N-butyric acid and trichostatin A, induce caspase-8-dependent but not caspase-9-dependent apoptosis with or without p21-mediated G1 arrest in human malignant glioma cells.

41. LOW-MOLECULAR-WEIGHT CALDESMON (L-CAD) AS A NEW SERUM MARKER FOR GLIOMA

P. Zheng,[1] W.C. Hop,[2] P.A.E. Sillevis Smitt,[1] M.J. van den Bent,[1] C.J.J. Avezaat,[1] T.M. Luider,[1] and *J.M. Kros*[1]; [1]Neuro-oncology and [2]Epidemiology & Biostatistics, [2]Erasmus M.C., Rotterdam, The Netherlands

Recent advances in the treatment of gliomas urge for a disease-specific serum marker more than ever. So far, no candidate glioma marker has reached the stage of clinical application. In a study using mass spectrometry we identified the specific presence of low-molecular-weight caldesmon (*l*-CaD) in cerebrospinal fluid (CSF) samples of glioma patients. We subsequently showed that *l*-CaD is specifically present and overexpressed in glioma vasculature but not in the blood vessels of normal brain. Additional RT-PCR experiments to microdissected components of glioma samples provided evidence that three out of four splice variants of the *CALD1* gene are specifically expressed in the glioma vasculature. Therefore, *l*-CaD may be regarded as a glioma-specific marker. Here we tested the feasibility of using *l*-CaD as a serum marker for glioma. A total of 230 serum samples, including sera of 57 patients with glioma, 107 patients with intracranial tumors other than glioma including metastases, 36 patients with various neurological diseases but no tumors, and 30 healthy subjects were tested for the *l*-CaD protein level by ELISA. The specificity of the assay was monitored by immunoprecipitation and immunoblotting (IP/IB). The serum level of appeared to be significantly higher in the group of glioma patients than in any of the control groups (p*l*-CaD levels between patients with low- or high-grade gliomas. The serum l-CaD level as determined by ELISA is a good discriminator between glioma patients versus patients with other intracranial tumors, other neurological diseases, and healthy people. It does not, however, provide information as to histological malignancy grade of the glioma. Prospective studies are initiated to test the contribution of the assay in making the diagnosis of glioma, or its feasibility for monitoring disease during treatment.

42. HIGH POSITIVE PREDICTIVE VALUE OF HEMIZYGOUS DELETIONS AT THE NOTCH2 LOCUS FOR SURVIVAL OF BRAIN TUMOR PATIENTS

B. Sivasankaran,[1] B. Jean-Louis,[1] I. Mihai CS,[1] T. Elisabeth,[1] D. Beatrice,[1] G. Anthony,[2] Z. Christian,[2] M. Adrian,[2] M. André R,[3] and R. Guido[4]; [1]Neuro Oncology and [2]Neurosurgical Clinic, University Hospital, Basel, Switzerland; [3]University Hospital, Department of Internal Medicine, Bruderholz, Switzerland; [4]Heinrich-Heine-University, Department of Neuropathology, Düsseldorf, Germany

Loss of heterozygosity (LOH) on chromosome 1p predicts responsiveness to chemotherapy in 70% of malignant oligodendrogliomas (OGs), pointing to a genetic factor for the response that distinguishes OGs from resistant glioblastomas (GBMs). We defined eight distinct haplotypes on a somatic deletion map on chromosome 1 of 26 OGs and 50 GBMs. In search for a correlation between survival and particular haplotypes, factor analysis, multivariate analysis, and non-parametric Kaplan-Meier curves were used. Test accuracy was determined by receiver operating characteristic (ROC) analyses. A consistent centromeric recombination breakpoint, clustered within 1 centiMorgan between markers D1S2696 and D1S2344, was prevalent in OGs, but not in GBMs ($P < 0.0001$). Hemizygous deletions at D1S2696, located within intron 12 of the Notch2 gene, correlated with better outcome ($P < 0.0001$). Interestingly, primary OGs and a subgroup of GBMs harbored overlapping single-copy microdeletions, defining a minimally lost region of 45 kb within the coding sequence of the Notch2 gene. LOH at the marker D1S2696 defines a new molecular classification that is independent of patient age and gender and equally well predicts survival time as histological and immunohistochemical classification ($P < 0.0001$). By ROC analysis, a cut-off of 24 months of survival time was defined resulting in a sensitivity and specificity for molecular classification of 80.8% and of 92.0%, respectively (positive and negative predictive value 84.0% and 90.2%). Simple, rapid, and highly reproducible molecular classification using marker D1S2696 at the Notch2 locus adds independent prognostic information to histological classification and identifies a subgroup of OG-like GBMs with long-term survival.

43. GENE EXPRESSION PROFILING LINKS INVASION-RELATED GENES TO POOR SURVIVAL IN OLDER GLIOBLASTOMA PATIENTS

J. Rich,[1] C. Hans,[2] B. Jones,[2] R. McLendon,[3] B. Rasheed,[3] A. Dobra,[2] H. Dressman,[4] D. Bigner,[3] J. Nevins,[4] and M. West[2]; [1]Department of Medicine [2]Institutes of Statistics and Decision Sciences, and Departments of [3]Pathology and [4]Molecular Genetics & Microbiology, Duke University Medical Center, Durham, North Carolina, USA

Despite the strikingly grave prognosis for older patients with glioblastomas, significant variability in patient outcome is experienced. To explore the potential for developing improved prognostic capabilities based on the elucidation of potential biological relationships, we performed analyses of genes commonly mutated, amplified, or deleted in glioblastomas and Affymetrix DNA microarray gene expression data from tumors of 43 glioblastoma patients of age greater than 50 for whom survival is known. No prognostic significance was associated with genetic changes in EGFR, TP53, $p16^{INK4A}$, or PTEN. Statistical analysis of the gene expression data in connection with survival involved exploration of regression models on small subsets of genes, based on computational search over multiple regression models with cross-validation to assess predictive validity. The analysis generated a set of regression models that, when weighted and combined according to posterior probabilities implied by the statistical analysis, identify patterns in expression of a small subset of genes that are associated with survival and have value in assessing survival risks. The dominant genes across multiple such regression models involve three key genes, secreted protein acidic and rich in cysteine (SPARC, osteonectin), Doublecortex and Semaphorin3B, which play roles in cellular migration processes. Additional analysis, based on statistical graphical association models constructed by using similar computational analysis methods, reveals others genes that support the view that multiple mediators of tumor invasion may be important prognostic factors in glioblastomas in older patients. No previous studies of which we are aware have elucidated conclusive links between expression of specific gene and survival of older glioblastoma patients. Our regression analyses using gene expression as explanatory of survival outcomes revealed that genes whose primary cellular effects may be the regulation of cellular migration appear as candidate markers of poor survival. Together these results suggest that tumor migration may represent an important effector of glioblastoma malignancy and may warrant accelerated development of specific therapies. Future studies will prospectively determine the link between the expression of SPARC, doublecortex, and SEMA3B in gliomas of all grades and patient outcome. This work was supported by a grant from the W.M. Keck Foundation. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar. This work was also supported by NIH grants NS047409 (J.N.R.).

44. CRITICAL ANALYSIS OF THE WHO HISTOPATHOLOGICAL GRADING FOR MENINGIOMAS. IMPACT ON POSTOPERATIVE RADIOTHERAPY AND FOLLOW UP IN GERMANY

M. Simon, J. Boström, P. Koch, and J. Schramm; University Hospital Bonn, Department of Neurosurgery, Bonn, Germany

We critically analyzed the clinical impact of the WHO histological grading for meningiomas including its role for postoperative radiotherapy/radiosurgery indications and MR follow-up protocols. The current (2000) and the 1993 WHO classifications were used to review the histological grade of 57 meningiomas operated at our institution. All German Neurosurgical Departments performing intracranial microsurgery were asked to detail their guidelines for radiation therapy and follow-up for meningiomas of different WHO grades. Comparing both WHO classifications, the current criteria downgrade 7/15 (47%) atypical (WHO grade II, MII) meningiomas to grade I (MI) and 4/6 (67%) anaplastic (WHO grade III, MIII) tumors to grade II. The use of specific criteria to diagnose atypia (MIB1 index >5%) and malignancy (brain invasion) only during the first review accounted for 3 grade II to I and all 4 grade III to II reclassifications, respectively. Indications for radiation therapy and MR follow-up protocols varied substantially with the histological grade and between institutions. After an incomplete resection, radiotherapy recommendations differed between MI and MII in 30/58 (52%), and between MII and MIII in 34/56 (61%) units. Our data document a considerable impact of the histological grading for meningiomas in clinical practice. However, the use of changing grading paradigms in recent years renders clinical decision making based on local and published experience difficult. The categories atypical and anaplastic meningioma, WHO grade II and III, respectively, have probably described quite different tumors in recent years. The clinical relevance of meningioma grading will only be properly recognized if diagnostic neuropathological labels are used consistently.

45. NOVEL STRATEGIES OF IMMUNOTHERAPY FOR MALIGNANT GLIOMA

M. Weller; University of Tubingen, Neurologische Klinik, Tubingen, Germany

The limited efficacy of surgery, radiotherapy, and chemotherapy in the treatment of malignant glioma calls for innovative treatment approaches targeting specific biological features of these tumors. Malignant glioma cells have long been known for the release of multiple established or putative immunosuppressive molecules, including transforming growth factor (TGF)-beta, prostaglandins, interleukin 10, CD95 ligand, or HLA-E/G. The very low frequency of systemic metastases in tumors which otherwise exhibit all features of malignancy has been attributed to an efficient glioma immune surveillance outside, but not inside, the central nervous system. Accordingly, promising strategies of immunotherapy based on these observations include (i) targeting the synthesis, release, or activity of glioma-derived immunosuppressive molecules and (ii) taking the presumably effective immune surveillance from the outside into the central nervous system. Paradigmatically, TGF-beta, the prime suspect for glioma-associated immunosuppression, may be antagonized by RNA interference, inhibition of proprotein processing or TGF-beta receptor antagonists, conferring resistance to TGF-beta-induced immune paralysis. In fact, while the biological neutralization of glioma-associated immunosuppressive molecules alone may not induce the immune rejection of these tumors, it may still be a precondition for active strategies of immunotherapy to be successful. Until specific tumor antigens for malignant glioma may be identified, the most promising strategies of immunotherapy include those based on the vaccination with autologous, genetically modified tumor cells in the context of a stimulatory immune environment, possibly employing dendritic cell immunity. Future experimental trials in glioma-bearing rodents and phase 1/2 clinical trials will have to demonstrate whether the initiation of an efficient efferent immune response against malignant glioma cells may be more easily triggered by peripheral vaccination or by efforts to create a strong immune stimulatory environment within a postsurgical tumor cavity.

46. PSEUDOTYPED, ONCOLYTIC ADENOVIRUSES USED TO TARGET GLIOMA STEM CELLS

J. Rebetz,[1,2,3] A. Edqvist,[1,2,3] L. Geironson,[1,2,3] H. Liu,[1,2,3] L.G. Salford,[1] X. Fan,[1,2,4] and B. Widegren[1,2,3]; [1]The Rausing Laboratory, Division of Neurosurgery, [2]Section of Immunology, [3]Division of Genetics, Department of Cell and Molecular Biology, and [4]Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Lund, Sweden

We have developed a number of oncolytic viruses based on a controlled gene expression by the human telomerase revere transcriptase promoter. We describe five different oncolytic viruses: (1) an adenovirus using the CMV promoter to express the herpes simplex virus thymidine kinase gene (HSV-TK) in combination with Gancyclivir, (2) an adenovirus using the hTERT promoter to express the HSV-TK gene in combination with Gancyclivir, (3) an adenovirus using the hTERT promoter to express the cytidine deaminase gene fused to a uracil kinase gene in combination with 5'-F-deoxycytidine, (4) an adenovirus using the hTERT promoter to express the adenoviral E1 proteins for replication in and lysis of hTERT positive cells, and (5) an adenovirus using the hTERT promoter to express a modified drosophila cytidine kinase, developed by ZGENE (Denmark) in combination with Gemcitibine. All of the hTERT promoter-containing viruses have an upstream chicken insulator element to prevent activation by cis-acting enhancer/promoters. This insulator element increases the specificity and integrity of the hTERT promoter. The proof of concept about this promoter construct is available (Edqvist et al. ibid). The adenovirus we use is a pseudotyped adenovirus that uses the CD46 molecule as the receptor instead of the coxsackie adenovirus receptor (CAR). The knob and the shaft of the fiber protein come from adenovirus type35. We will test these viruses on at least 20 different primary glioma cell cultures and xenografted subcutaneously growing gliomas. The frequency of hTERT positive cells before and after the treatment will be evaluated. Preliminary data will be given and discussed.

47. ANTISENSE-MEDIATED SUPPRESSION OF HEPARANASE GENE INHIBITS BRAIN METASTASIS OF MELANOMA CELLS

M. Roy,[1] J. Reiland,[1] B.P. Murry,[1] V. Chouljenko,[2] K.G. Kousoulas,[2] and *D. Marchetti*[1]; [1]Department of Comparative Biomedical Sciences and [2]Division of Biotechnology & Molecular Medicine, School of Veterinary Medicine, Louisiana State University at Baton Rouge, Baton Rouge, Louisiana, USA

Brain metastasis occurs in most of all human cancers and is a frequent manifestation of malignant melanoma progression. Successful invasion into brain by tumor cells must include attachment to microvessel brain endothelial cells, and, of relevance, degradation of surrounding extracellular matrix (ECM). Heparan sulfate proteoglycans (HSPG) are essential and ubiquitous macromolecules associated with the cell surface and ECM of a wide range of cells and tissues. Heparanase (HPSE-1) is an ECM degradative enzyme and a critical molecular determinant in metastatic events. The enzyme acts as an endo-beta-D-glucuronidase which degrades the heparan sulfate (HS) chains of HSPG at specific intrachain sites, resulting in bioactive HS fragments of discrete molecular weight size. To investigate effects of changes in heparanase gene expression in brain-metastatic melanoma (BMM) cells, we constructed adenoviral vectors containing the full-length human HPSE-1 cDNA in both sense (Ad-S/hep) and antisense orientation (Ad-AS/hep). We found increased HPSE-1 expression and activity in BMM following Ad-S/hep infection by Western blot analyses and specific HPSE-1 activity assays. Conversely, HPSE-1 content was significantly inhibited following infection with Ad-AS/Hep. Importantly, HPSE-1 modulation by these adenoviral constructs correlated with brain invasive cellular properties in vitro. Moreover, extensive brain metastasis formation was observed in athymic nu/nu mice injected with Ad-S/hep-infected BMM cells, while none of the mice injected with Ad-AS/hep showed any evidence of macroscopic malignancy. Our results suggest that HPSE-1 not only contributes to the brain-metastatic phenotype of melanoma cells, but also that the Ad-AS/hep-mediated inhibition of its enzymatic activity can be efficacious in the prevention and treatment of melanoma brain metastasis.

48. ADENOVIRAL VECTOR MEDIATED DETECTION OF TELOMERASE ACTIVITY AT SINGLE LIVING CELLS

A. Edqvist,[1] J. Rebetz,[1] M. Järås,[2] L. Salford,[3] X. Fan,[1,2] and B. Widegren[1,3]; [1]Section of Immunology, [2]Section of Molecular Medicine and Gene Therapy, and [3]The Department of Neurosurgery, The Rausing Laboratory, Lund University, Lund, Sweden

Telomerase activity is normally observed in highly proliferative tissues, stem cells, and most malignant cells and therefore offers an attractive target for therapeutic intervention and for diagnostic and prognostic purposes. The telomerase activity is predominantly controlled by the regulated expression of the catalytic subunit telomerase reverse transcriptase (hTERT) at the transcriptional level. Here, we developed adenoviral vectors for detecting telomerase activity in single living cells. In these vectors, the expression of destabilized enhanced green fluorescence protein with a half-life of 2 h (d2EGFP) is under the control of the hTERT promoter. Insulator DNA sequences were introduced to shield the hTERT promoter from cis-activating elements in the adenoviral vector backbone. Moreover, the vectors were retargeted to ubiquitously expressed CD46 as a cellular receptor. Following infection of telomerase positive (HeLa and A549 cells) or negative cells (fibroblast and WI-38 cells) with such vectors, the d2EGFP expression was detected in a telomerase activity–dependent manner, which correlated with the hTERT expression as assessed with real-time PCR. Furthermore, about 50% of the promyelocytic leukemic HL-60 cells were expressing d2EGFP following infection with telomerase reporting adenoviral vector, and the d2EGFP expression was significantly diminished in retinoic acid–induced differentiating HL-60 cells compared with nontreated control cells. Cell cycle analysis showed that the sorted d2EGFP+ HL-60 cells were mostly in the $S/G_2/M$ phase of cell cycle, whereas the d2EGFP- HL-60 cells mainly at the G_1 and early S phase. In addition, our telomerase reporting vectors allowed high levels of d2EGFP expression in early passage xenograft glioma cells and the percentage of d2EGFP expression glioma cells diminished in late passages. Thus, the dynamically regulated telomerase activity during cell proliferation and differentiation in single living cells can be identified following infection with our adenoviral reporting vectors. Our studies provide a powerful tool for identifying living cells with telomerase activity.

49. ROLE FOR C-JUN N-TERMINAL KINASE (JNK) IN RAS-MEDIATED NON-APOPTOTIC PROGRAMMED CELL DEATH

W. Mouri, T. Kayama, and C. Kitanaka; Yamagata University, Neurosurgery, Yamagata, Japan

Accumulating evidence now indicates that nonapoptotic, caspase-independent mechanisms play a critical role in oncogene- as well as anticancer-treatment-induced cellular suicide. We have found that the ras oncogene induces nonapoptotic programmed cell death in glioma and neuroblastoma cells, but the molecular mechanism involved therein remains undefined. Cell death was induced by transient cotransfection of active Ras (H-RasV12)- and green fluorescence protein (GFP)-expressing vectors and was assessed on the basis of morphology of GFP-positive cells. Ras and JNK protein expression levels were determined by immunoblotting with specific antibodies for each. Activation of JNK was monitored by immunoblot analysis using a JNK antibody that specifically recognizes JNK phosphorylated at Thr183/Tyr185. Blockade of JNK activity was achieved by the use of SP600125, a highly specific chemical inhibitor of JNK. Increase of phosphorylated (active) JNK was detected in the course of Ras-induced nonapoptotic cell death. Dose-dependence analysis showed that increased expression of Ras (via transfection of increasing amounts of Ras expression vector) is associated with increased JNK activity, yet an inactive Ras mutant (H-RasN17) failed to activate JNK as well as cell death itself. Time-course analysis demonstrated that JNK activation followed Ras expression and preceded cell death. SP600125 inhibited Ras-induced nonapoptotic cell death in a range of concentrations where it efficiently inhibited JNK activity. The results indicate that (i) Ras activates JNK during cell death induction, depending on its ability to transduce intracellular signals and that (ii) JNK likely has a causal role in triggering nonapoptotic cell death. Thus the data together suggest that JNK is involved in the death signal transduction of Ras-mediated nonapoptotic programmed cell death.

50. THE MOST CONSTITUTIVELY ACTIVE JNK ISOFORM, JNK2A2, IS PREFERENTIALLY EXPRESSED IN GLIOBLASTOMAS: IDENTIFICATION OF SPECIFIC SEQUENCES LEADING TO ITS ACTIVATION

J. Wong and J. Cui; Thomas Jefferson University, Kimmel Cancer Institute, Philadelphia, Pennsylvania, USA

C-jun N-terminal kinases (JNKs) are critical to regulating cell growth, proliferation, and apoptosis. Activation of the JNK pathway has been implicated in the formation of several human tumors, particularly gliomas. There are 10 different JNK isoforms. We previously showed that a 55KD JNK isoform is constitutively activated in 86% of human gliomas, which makes it the most frequent signaling alteration found in these tumors. We found that this isoform was specifically a JNK2 isoform and likely to be either JNK2a2 or JNK2β2. Notably, we showed that JNK2a2 possesses the strongest autophosphorylation activity among all isoforms. We now report our efforts to identify specific sequences that contribute to JNK2a2 activation and how this isoform contributes to glial tumorigenesis. We generated a series of chimeric cDNAs that join portions of JNK1a2, which lacks detectable autophosphorylation activity, with portions of JNK2a2, which has the strongest activity. Through in vivo and in vitro kinase assays, we defined a domain within JNK2a2 from amino acid 218 to 226 that is required for its autophosphorylation. Mutation of JNK2a2 to its counterpart of JNK1a2 in this region abrogated the autophosphorylation activity and c-jun substrate kinase activity in vivo and in vitro. The switching of JNK1a2 to JNK2a2 at this region enabled JNK1a2 to gain autophosphorylation activity. Next, we examined the expression of JNK1a2, JNK2a2, JNK1β2, and JNK2β2 in normal brain specimens and glioblastomas by RT-PCR. All four isoforms were expressed in normal brains (3/3). JNK1a2, JNK1β2, and JNK2β2 were found in only 18% (2/11) of these tumors. In contrast, JNK2a2 was found in 91% of glioblastomas (10/11). We then transfected U87-MG cells with GFPC1-JNK1a1, GFPC1-JNK2a2, and GFPC1- JNK2a2/APF (a dominant negative mutant form of JNK2a2) and obtained stable clones expressing similar levels of protein. We assessed the effects on parameters related to tumorigenesis including cell proliferation, soft agar colony formation, and tumor formation in athymic mice. JNK2a2 was consistently the most effective in promoting proliferation and tumor growth where the relative order was JNK2a2 > JNK1a1 > JNK2a2/APF. Since JNK activates transcription factors, we profiled gene expression using cDNA microarrays. There were 16 genes whose expression was upregulated by JNK2a2 but suppressed by JNK2a2/APF, including EIF-4E and TGF-a, which indicates that these genes may be critical for JNK2a2 effects on transformation. Our data indicate that glioblastomas specifically upregulate the most active JNK isoform which promotes tumorigenesis through upregulation of specific genes. The identification of specific sequences that lead to JNK2a2 activation will allow us to design specific inhibitors.

51. ESTABLISHMENT OF A CELL LINE DERIVED FROM HUMAN CENTRAL NERVOUS SYSTEM LYMPHOMA

S. Fujita, J. Ueda, H. Ikemoto, K. Mori, and N. Arita; Hyogo College of Medicine, Neurosurgery, Nishinomiya, Hyogo, Japan

Primary central nervous system lymphoma (PCNSL) exhibits several characters different from systemic lymphoma cells. PCNSL are usually confined to the central nervous system, vanish completely when treated with steroids, anticancer drugs or radiation, but relapse very rapidly. Little has been reported, however, on the genetic and biological nature of PCNSL, because of the scare tissue specimens derived from stereotactic biopsy. We established a cell line from human PCNSL and aimed to inquire its character of PCNSL. The cell line was established from the surgical specimen of PCNSL in the right putamen of a 68-year-old female, using primary explant technique. We investigated the character of lymphoma cells by immunocytochemistry, electron microscopic observation, and immunoblot analysis and at the same time, studied the response of cells against dexamethasone and methotrexate (MTX) in vitro. Apoptosis was analyzed by the Tunel method. To check the genetic changes, spectral karyotyping was performed. The population-doubling time of the cultured cell was 20 h. The cultured cell in RPMI1640 supplemented with 10% fetal bovine serum reacted with anti CD20, BCL2, and BCL6 antibodies. Electron microscopic observation revealed nuclear bleb, which was specific for lymphoma cell. Immunoblot analysis revealed BCL-2 and BCL-6 are expressed in the cultured cell. Representative karyotype was interpreted as 50,XX, +3(5), t(4;15)(q31;q15), del(6)(q21q25),+18(10). Inhibition dose (ID50) of MTX was 6 nM, and ID50 of dexamethasone was 2 nM. Apoptosis was detected after either MTX or dexamethasone treatment. Our results demonstrated that the established cell line (designated MCL2) could be the useful in vitro model of PCNSL.

52. THE MITOCHONDRIAL PATHWAY IS CENTRAL TO ERUCYLPHOSPHOCHOLINE-MEDIATED APOPTOSIS IN HUMAN GLIOMA CELL LINES

W. Kugler, F. Köhler, F. Buchholz, B. Erdlenbruch, and M. Lakomek; Universitäts-Kinderklinik, Pädiatrie I, Göttingen, Germany

Glioblastomas are highly chemo- and radioresistant tumors of the CNS. Therefore, new therapeutic approaches are urgently needed. Alkylphosphocholines (APCs) are membranophile agents not directly targeting cellular DNA. Erucylphosphocholine (ErPC) represents the prototype of a promising class of APC for parenteral administration. It has potent antineoplastic activity on various malignant tumors of different origin and accumulates within the brain. Of particular interest, ErPC induces apoptosis in glioma cells resistant to treatment with standard chemotherapeutics. Recently, we have shown that ErPC mediates apoptosis independent of p53 signaling and death receptor/ligand systems, whereas activation of caspases via mitochondria plays a major role. To analyze the contribution of the mitochondrial pathway in more detail, we investigated the cytotoxic effects of ErPC in tumor cells with defects in apoptosis. To this end, we silenced the expression of Apaf-1 and caspases-3 and -9 in human glioma cell lines by specific small interfering RNAs (siRNAs). The different siRNAs showed a variable degree of knockdown efficiency with respect to protein expression and induction of apoptosis arguing in favor of an essential role of the intrinsic pathway. As a further proof that the poor response to ErPC is due to the lack of an essential apoptosis gene, we used HeLa cells stably transfected with a dominant-negative caspase-9 construct, Apaf-1-negative melanoma cells, and MCF-7 cells harboring a deletion in the *Casp-3* gene. In these cell lines, cytotoxicity and apoptosis induction was drastically decreased compared to vector controls, to cells harboring a functional *Apaf-1* gene, and to cells stably transfected with the *Casp-3* cDNA, which thus corroborated our results in glioma cells. In particular, we provide evidence that caspase-3 is required for the activation of caspases-2, -6, and –8 and also participates in a feedback amplification loop. Together, our data suggest that components of the mitochondrial apoptosis pathway are essential for ErPC to effectively induce apoptosis, whereas elements of the death receptor pathway are dispensable. This work was supported by B. Braun-Stiftung.

53. TRAIL INDUCES PROLIFERATION OF MALIGNANT GLIOMA CELLS THROUGH C-FLIP-MEDIATED ERK1/2 PATHWAY

S. Vilimanovich,[1] J. Song,[2] L. Guilbert,[1] and *C. Hao*[2]; [1]University of Alberta, Laboratory Medicine & Pathology and Medical Microbiology & Immunology, Edmonton, Canada; [2]Emory University, Pathology & Laboratory Medicine, Atlanta, Georgia, USA

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induces apoptosis in TRAIL-sensitive human malignant glioma cells. Here we show that TRAIL stimulated cell growth in TRAIL-resistant glioma cells. TRAIL-induced cell growth in the resistant cells occurred through increased cell cycle progression as determined by flow cytometry analysis of propidium iodide–stained cells and Western blot analysis of retinoblastoma protein (pRb) phosphorylation. Western blot analysis of TRAIL-treated resistant cells revealed phosphorylation of ERK1/2 proteins, and in vitro kinase analysis confirmed the activation of the ERK1/2 kinases. ERK1/2 kinases are activated through dual phosphorylation by mitogen-activated protein kinase (MAPK)/ERK kinase (MEK). Treatment of the resistant cells with MEK1 inhibitor PD98059 eliminated TRAIL-induced ERK1/2 activation and cell proliferation. These results suggested that TRAIL-induced cell proliferation occurs through activation of the ERK1/2 pathway in TRAIL-resistant glioma cells. Inhibition of cellular Fas-associated death domain-like interleukin-1β-converting enzyme-inhibitory protein (c-FLIP) with small interfering RNA (siRNA) eliminated TRAIL-induced ERK1/2 activation, and proliferation as determined by cell viability assay, propidium iodide staining, and flow cytometry, and pRb phosphorylation. The results indicate that TRAIL-induced ERK1/2 activation and proliferation in TRAIL-resistant glioma cell lines is dependent upon the expression of the caspase-8 inhibitor c-FLIP. Furthermore, inhibition of c-FLIP expression sensitized the resistant cells to TRAIL-induced apoptosis as demonstrated by the cleavage of caspases. In conclusion, TRAIL triggers growth in TRAIL resistant malignant glioma cells through c-FLIP-mediated ERK1/2 pathway, and thus targeting c-FLIP-ERK1/2 pathway may overcome the resistance of malignant glioma cells to TRAIL treatment.

54. ROCK INHIBITION INDUCES ASTROCYTOMA MOTILITY IN A RAC1-DEPENDNET MANNER

B. Salhia, F. Rutten, and J. Rutka; The Hospital for Sick Children, Brain Tumour Research Center, Toronto, Canada

The intracellular mechanisms governing astrocytoma motility while poorly understood require modifications of the cytoskeleton. The Rho-GTPases (Rac, Cdc42 and Rho) are pivotal regulators of cytoskeletal organization and cell motility. We have shown that inhibition of ROCK, a serine/threonine effector kinase of Rho, with Y27632 or the dominant-negative mutant inhibits stress fibers and focal adhesions induced by LPA. In contrast to several studies demonstrating that inhibition of ROCK leads to decreased tumor cell invasiveness and motility, we found using a 2-dimensional radial migration assay that astrocytoma migration was significantly increased following treatment with Y27632. LPA also significantly stimulated the motility of astrocytoma cells. The observation that ROCK inhibition also led to increased membrane ruffling suggested that Rac activation was a possible mechanism in Y27632-induced motility. Rac-GTPases are thought to regulate membrane ruffling formation and cell migration in large part by stimulating actin polymerization. We demonstrated for the first time both directly and indirectly that Rac activation is an outcome of ROCK inhibition in astrocytoma cells. First, using a Rac-GTP pull-down assay, we show that U251 cells treated with Y27632 increased Rac activity compared to untreated controls. In addition, LPA alone or in combination with Y27632 also increased the levels of Rac-GTP. Next we show that Y27632 induces membrane ruffling in a Rac1-dependent manner since depletion of Rac1 strongly inhibits Y27632-induced membrane ruffles. These cells also reverse the stellate phenotype of Y27632 treatment without regaining actin stress fibers. In addition, Rac1-directed siRNA effectively overcame Y27632-induced motility by nearly 2-fold. Furthermore, Rac1 depletion alone inhibited the migration of U251 cells by about 50%. In summary, our data show that inhibition of ROCK plays a major role in regulating astrocytoma cell morphology, actin cytoskeleton, and migration through the activation of Rac1. In addition, astrocytoma migration seems to be occurring by two separate, Rho-independent, Rac-dependent mechanisms, ROCK inhibition and LPA stimulation. Our future studies will be directed toward determining the mechanisms that lead to Rac activation following ROCK inhibition or LPA stimulation in our cells. Increasing our understanding of the significant cross-talk that appears to be occurring between Rho-GTPase family members and their effector proteins will be important to identifying key elements within these pathways that can be exploited to inhibit the growth and invasiveness of human astrocytoma cells.

55. MEMBERS OF THE ETS FAMILY OF TRANSCRIPTION FACTORS BIND TO THE SITE INTRODUCED BY A SINGLE NUCLEOTIDE POLYMORPHISM IN THE MMP-1 PROMOTER

J. McCready,[1] V. Sykes,[2] W. Broaddus,[2] and H. Fillmore[2]; [1]Anatomy and Neurobiology; [2]Neurosurgery, Virginia Commonwealth University, Richmond, Virginia, USA

Matrix metalloproteinase-1 has been implicated in the metastasis and invasion of many types of tumors. A polymorphism exists in the proximal promoter region of MMP-1 that regulates MMP-1 transcription. This polymorphism consists of either the presence (2G allele) or absence (1G allele) of a guanine nucleotide adjacent to a pre-existing guanine nucleotide. This additional guanine nucleotide creates a binding site for the ETS family of transcription factors and leads to increases in MMP-1 transcription. In several aggressive and metastatic tumors studied, the incidence of the 2G allele is significantly higher. We found a significant difference in the distribution of the genotypes between healthy individuals and glioblastoma patients ($P = 0.031$) with an increase in the percentage of the 2G/2G genotype in the tumor population ($P = 0.018$). The aim of this study was to determine if the additional ETS binding site regulates the MMP-1 promoter in glioma cells. Transfection of three glioma cell lines with a 2G MMP-1 promoter reporter construct results in increased transcription when compared to transfection with the 1G reporter construct ($P = 0.02$). Identification of the proteins binding to the 2G promoter is the first step in understanding the regulatory effects this polymorphism has on MMP-1 transcription. ETS transcription factors are divided into subfamilies according to structural similarities. Results from RT-PCR and Western blot indicate that all members of the Ets and Pea3 subfamilies are present in seven glioma cell lines. To determine which members of these subfamilies bind to the 2G promoter, we performed DNA-protein pull-down assays. Our data indicates that Ets-1 binds to the 2G promoter, and we are currently evaluating the binding capability of the other members of these subfamilies. We are also conducting chromatin immunoprecipitation assays to determine which proteins bind to the 2G allele in the context of the cellular environment. Both hepatocyte growth factor and phorbol myristate acetate have been shown to increase binding of ETS members to the MMP-1 promoter leading to increases in MMP-1 promoter activity. We are assessing the ability of HGF and PMA to influence the binding of ETS proteins to the 2G promoter and subsequent MMP-1 transcriptional activity. Results from these studies will increase our knowledge of how MMP-1 is regulated in glioma cells. This information may lead to advances in therapies that artificially lower the levels of MMP-1 and indirectly control glioma invasion.

56. REGULATION OF UNCOUPLING PROTEIN-2 (UCP-2) EXPRESSION IN HUMAN GLIOMA CELLS BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) AGONISTS

J. Brown, J. Darling, S. Dunmore, and S. Bassey; University of Wolverhampton, RIHS, Wolverhampton, UK

Recent studies have suggested that the glitazones, a group of PPAR agonists commonly prescribed as therapy in type 2 diabetes, could have a role in regulation of cell viability in astrocytomas and glioma cell lines, possibly due to a modulation of reactive oxygen species (ROS) production. PPAR agonists are also known to regulate expression of the mitochondrial protein UCP-2, and UCP-2 has a purported role in ROS regulation amongst others. This study investigated the expression of UCP-2 in U251MG glioma cells and its regulation by PPAR agonists. U251MG glioma cells were cultured according to standard methods and treated with PPAR alpha, delta, and gamma agonists (10 μM WY14643, 10 nM PGI2, 10 μM rosiglitazone and 10 nM PGJ2, respectively) for 24 h. Total RNA was subsequently extracted and semiquantitative RT-PCR used to evaluate UCP-2 mRNA expression. Results showed that UCP-2 was expressed in all control and treatment samples and that its expression was regulated differentially by the various PPAR receptor subtype agonists tested. This novel finding that UCP-2 is expressed in glioma cells, and that its expression is regulated by PPAR agonists, suggests a potential mechanism for the cytotoxic effects of glitazones that have been previously reported, and describes a mechanism which could possibly be manipulated as a potential therapeutic avenue in the future.

57. GLYCOLYTIC GLIOMA CELLS SHOWED SENSITIVITY TO INHIBITION BY A MYRISTOYLATED PEPTIDE DERIVED FROM KEY PHOSPHORYLATION SITES ON GSK-3ALPHA/BETA

M.E. Beckner,[1] F. Burovic,[2] and I.F. Pollack[2]; [1]Pathology; [2]Neurological Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

U87 malignant glioma cells exhibit impressive migration in vitro. Previously, we demonstrated sensitivity of *phosphatase and tensin homolog (PTEN)*-mutated U87 cell migration to restoration of PTEN's constitutive negative regulation via a wild-type construct (kind gift of R. Abounader and J. Laterra, Johns Hopkins Univ.), and inhibition of phosphotidylinositol 3 kinase (PI3K) via wortmannin (Beckner et al., Proc. Am. Assoc. Cancer Res. 45, 694, 2004). In addition to regulatory effects of the upper activated PI3K/Akt pathway on the cytoskeleton, we propose that additional activation of migration occurs downstream via activated glycogen synthase (GS). Akt, Wnt, and other pathways converge at glycogen synthase kinase-3 (GSK-3). By phosphorylating and thus inactivating GSK-3, its constitutive inhibitory phosphorylations of GS are suppressed. Activation of GS was shown by decreased phosphorylation at S640 and increased phosphorylation of GSK-3alpha and Akt1 at S21 and T308, respectively, in hypoxia. Activation of GS also occurred in U87 pseudopodia. Activated GS aids in the removal of lactic acid in glycolytic, gluconeogenic astrocytic cells (Dringen et al., Brain Res. 623, 208; Biol. Chem. Hoppe Seyler 374, 343, 1993; Hevor, Biochemie 76, 111, 1994; Bernard-Helary K. et al., Glia 37, 379, 2002). U87 proteomics revealed a predominance of glycolytic enzymes with increased amounts in pseudopodia. HGF, Met, actin, and other proteins were also increased (M.E. Beckner et al., Lab Invest., in press). Intracellular lactate removal should aid migration of invasive glioma cells in vivo, especially hypoxic pseudopodial extension. To block phosphorylation of GSK-3, a myristoylated (Mys) peptide, Mys-CGPKGPGRRGRRRTSSFAEG, was generated to mimic GSK-3 alpha and beta's phosphorylation sites, S21 and S9, respectively, and a negative control, Mys-GRRGRRRPGCEKSPSGFTGA, was made. The mimic's sequence was published for GSK-3 Fusion Protein (Product #9278, Cell Signaling, Inc., Beverly, MA). The Mys-GSK-3 peptide induced a biphasic effect, including inhibition of normoxic and hypoxic U87 cell migration at concentrations 3-fold less than the control, with a differential effect on trypan blue exclusion also noted. Blocking phosphorylation of GSK-3 at cell membranes, especially pseudopodia, may suppress invasion. This research was supported by The Nick Eric Wichman Foundation and The Pittsburgh Foundation's Walter L. Copeland Fund for Cranial Research.

58. NOTCH2 INTRODUCES CBF1-INDEPENDENT PATHWAY IN GLIOBLASTOMA: EVIDENCE OF NOVEL NOTCH SIGNAL ELEMENT

M. Noha,[1] D. Yoshida,[2] D. Morimoto,[2] T. Asakura,[2] and A. Teramoto[2]; [1]Teikyo University School of Medicine, Department of Neurosurgery, Kawasaki; [2]Nippon Medical School, Department of Neurosurgery, Tokyo; JAPAN

Notch signaling is well known to play a crucial role in cell fate decisions during development. Furthermore, in humans, four different subtypes of Notch have already been recognized. In the past decade, many reports have implicated the notch protein in tumorigenesis, specifically because of overexpression of the Notch intracellular domains as the constitutive active form of Notch. However, the function of this truncated Notch is still not clear, and a few reports have described the correlation between each subtype of Notch and tumorigenesis. We previously reported that Notch signaling is associated with the evasion of apoptosis of glioblastoma. In this report, we focus on Notch1 and Notch2 expression in glioblastoma, and we discuss the function of Notch signaling in this tumor. Glioblastoma cell lines U373MG, U87MG, U251MG, A172, and T98G were investigated for expression of Notch1 and Notch2 protein and mRNA by using Western blot and RT-PCR, respectively. The intracellular distribution of these two Notch proteins was then revealed individually by immunocytochemistry using specific antibody to Notch1 and Notch2. Downstream of Notch signaling, truncated Notch protein binds CBF1 and expresses HES1. Such protein-to-protein interaction was also confirmed in glioblastoma cell lines by immunoprecipitation. Finally, by using specific siRNA, Notch1 and Notch2 expression was silenced, and subsequently, mRNA expression of HES1, as the target gene of Notch signaling, was measured by real-time PCR. All cell lines expressed Notch1 and Notch2 and showed a tendency to express notch2 dominantly. In addition, intracellular Notch expressed strongly, which indicates that Notch signaling was well activated in glioblastoma as well as in carcinoma of other organs. Immunocytochemistry, immunoprecipitate, and gene silencing by siRNA more clearly differentiated between Notch1 and Notch2 attributes in glioblastoma. A number of Notch2 proteins distributed in nuclei and Notch1 in the perinuclei area of cytoplasm. Furthermore, Notch2 no longer bound CBF1 and expression of HES1 was not affected, which suggests that Notch2 might introduce novel CBF1-independent pathway in glioblastoma cell lines. Notch2 signaling might play an unexpected role in glioblastoma, through novel unidentified Notch pathway components.

59. CYTOPLASMIC TRANSLOCATION OF PTEN TUMOR SUPPRESSOR IS MEDIATED BY PI3K/AKT/MTOR/P70S6K SIGNALING CASCADES

J. Liu[1], K. Aldape[2], Z. Mao[1], and W. Yung[1]; [1]Neuro-Oncology and [2]Pathology, UT M.D. Anderson Cancer Center, Houston, Texas, USA

The *PTEN* tumor suppressor gene is commonly deleted or mutated in a large number of advanced tumors including GBMs. PTEN functions primarily as a phosphoinositide phosphatase that specifically antagonizes PI3K-mediated signaling pathways. PTEN is preferentially expressed in the nucleus of differentiated or resting cells, and increased nuclear PTEN expression is associated with G_0/G_1. However, the regulation of PTEN's nuclear-cytoplasmic shuttling remains poorly understood. In this report, we used mouse astrocytes and NIH3T3 cells to study the molecular mechanisms involved in regulation of PTEN nuclear export. In agreement with other reports, we show that PTEN is preferentially localized in the nucleus during G_0/G_1 phase and is exported into the cytoplasm during G_1/S transition. We further demonstrate that dominant-negative mutants for Akt (Akt-AAA) and for p70S6K (K113R) as well as inhibitors for PI3K (LY294002), PDK1 (Staurosporine), mTOR (RAD-001), and sodium salicylate (for p70S6K), but not for MEK (PD98059), suppress the nuclear export of PTEN protein. Conversely, constitutively active AKT mutant (AKT-DD) promotes PTEN's cytoplasmic translocation. In addition, we also demonstrate that PTEN interacts with p70S6K in vivo and in vitro. Taken together, these findings strongly suggest that PI3K/AKT/mTOR/p70S6K signaling cascades, p70S6K in particular, are pivotal in regulating PTEN's subcellular localization. This scenario is reminiscent of "yin and yang" reciprocal regulation between PI3K and PTEN during the course of cell cycle progression. Interestingly, our immunohistochemistry results show that PTEN is predominantly expressed in the cytoplasm of GBM tumors that positively correlates with the phosphorylation of S6. Furthermore, we also observe preferentially cytoplasmic localization of PTEN in a GBM cell line, LN229, which is presumably due to the constitutive activation of p70S6K that can be blocked by sodium salicylate but not by RAD-001. Our long-term goal is to establish the relationship between PTEN's subcellular localization and the status of activation of PI3K downstream effectors, such as AKT, mTOR, or p70S6K in order to improve molecular diagnosis, prognosis, and individualized therapy for GBMs.

60. THE NOTCH SIGNALING PATHWAY AND GROUCHO/TLE CO-REPRESSORS IN MENINGIOMA PATHOGENESIS

I. Cuevas, G. Baia, A. Jun, P. Costello, J. Bollen, A. McDermott, and *A. Lal*; Brain Tumor Research Center, Neurological Surgery, University of California San Francisco, San Francisco, California, USA

Meningiomas constitute the second most common central nervous system tumor. Atypical and malignant meningiomas are associated with a poor clinical outcome and higher rates of recurrence when compared to benign meningiomas. Relatively little is known about the molecular events important in the pathogenesis and malignant progression of meningiomas. To determine the molecular changes associated with meningiomas, we used serial analysis of gene expression (SAGE). We focused our initial analysis on the 165 genes that are induced in high-grade meningiomas because this population is expected to contain components of activated signal transduction pathways. A novel finding from this screen is the induction of three downstream components of the Notch signaling pathway: the transcription factor, Hairy and Enhancer of Split 1 (*HES1*), and two members of the Groucho/Transducin-like enhancer of split (Gro/TLE) family of co-repressors, *TLE2* and *TLE3*. Gro/TLE co-repressors interact and modulate the activity of a wide range of transcriptional regulatory systems, one of which is HES1. The SAGE results were validated by performing quantitative PCR on a larger, independent set of meningiomas. We confirm that *HES1* transcript levels are induced in meningiomas of all three grades while induction of *TLE2* and *TLE3* is specific to high-grade meningiomas. In particular, overexpression of *TLE3* occurs in 50% of malignant meningiomas, with the amplitude of induction ranging from 4-fold to 65-fold. Immunohistochemistry revealed that TLE3 is correctly localized to the nucleus, where it is supposed to function as a transcriptional co-repressor. We also find induction of other components of the Notch signaling pathway like NOTCH2 and JAGGED1 in a subset of meningiomas. The above results lead us to hypothesize that Gro/TLEs are important for the malignant progression of meningiomas and that one of the mechanisms by which this occurs is by modulation of the Notch signaling pathway. We are currently overexpressing and silencing components of the Notch signaling pathway and TLE repressors to delineate their relevance in meningioma growth and tumorigenesis.

61. THE IN VITRO ANTICANCER ACTIVITY OF GLIVEC AGAINST GLIOBLASTOMA MULTIFORME IS NOT RELATED TO PDGFRAND KIT EXPRESSION

S. Spiegl-Kreinecker,[1] C. Pirker,[2] R. Silye,[3] J. Buchroithner,[1] J. Pichler,[4] J. Fischer,[1] M. Micksche,[2] and W. Berger[2]; [1]Department of Neurosurgery, Wagner Jauregg Hospital, Linz; [2]Clinics of Internal Medicine I, Institute of Cancer Research, Vienna; [3]Institute of Pathology, Wagner Jauregg Hospital, Linz; [4]Department of Internal Medicine, Wagner Jauregg Hospital, Linz; Austria

Overexpression of the tyrosin kinase receptors (RTKs) PDGFRa/b and c-Kit is a common event in glioblastomas, therefore representing obvious targets for the small molecule inhibitor Glivec. Moreover, the PDGFRa gene is amplified in a small subgroup of glioblastoma patients. The aim of our study was to compare the expression of PDGFR isoforms a and b as well as c-Kit with the therapeutic efficacy of Glivec in glioblastoma primary cell cultures. Western blot was performed to detect respective protein levels of PDGFRa/b and c-Kit in 43 primary cell cultures from astrocytic brain tumor surgery specimens. Tumor samples were obtained during surgery and histologically verified according to WHO criteria as glioblastoma multiforme. Immunohistochemical expression of PDGFRa and c-Kit was determined in selected paraffin sections (N = 5). Sensitivity against Glivec (10–50 μM) was analyzed by MTT tests. Chromosomal aberrations were studied by means of comparative genomic hybridization (CGH) using DNA isolated from cell cultures as well as corresponding tumor sections. Of 43 primary cell cultures, 30 (70%) expressed detectable levels of PDGFRa; 34/43 (79%) displayed PDGFRb protein expression, whereas 25/43 (58%) showed expression of both receptor isoforms. Expression of c-Kit was detectable in 17/43 (40%) of the analyzed cell cultures. In one highly PDGFRa-overexpressing cell culture, high-level amplification of the respective gene at chromosome 4q12 was detected by CGH. This expression level was well in accordance with response to Glivec. Four of five tumor sections displayed heterogenous PDGFRa immunostaining. Staining intensity differed between and within samples and was high in giant tumor cells and endothelial cells. Widespread but weak staining could be observed in fibrillary tumor cells. In one case, no immunoreactivity was detectable. PDGFRa immunostaining of tumor sections corresponded well with respective cell cultures. This was not the case for c-Kit. Sensitivity against Glivec could be observed in 19/21 investigated cell cultures, with IC_{50} ranging between 19 and 42 μM. Two of 21 were resistant against Glivec despite high expression level of PDGFRa and c-Kit, respectively. Sensitivity against Glivec did not correlate with expression of any of the investigated RTKs. Summing up, our data demonstrate frequent expression of PDGFRa/b and/or c-Kit in glioblastoma cells. However, the in vitro chemosensitivity against Glivec

is not related to expression levels of these targeted RTKs. The relationship of PDGFRa gene amplification in a small subgroup of glioma patients and Glivec sensitivity should be further investigated, since a glioblastoma cell culture with PDGFRa gene amplification was sensitive against Glivec in our in vitro experiments.

62. THE PTEN TUMOR SUPPRESSOR GENE SHIFTS CELLULAR RESPONSES TO TRANSFORMING GROWTH FACTOR-BETA TOWARDS TUMOR SUPPRESSION IN MALIGNANT GLIOMAS

A. Hjelmeland,[1] M. Hjelmeland,[1] C. Kontos,[2] D. Bigner,[3] X. Wang,[1] and *J. Rich*[2]; [1]Surgery; [2]Medicine; [3]Pathology; [4]Pharmacology & Cancer Biology, Duke University, Durham, North Carolina, USA

Transforming growth factor-β (TGFβ) is a multifunctional cytokine commonly expressed by malignant gliomas that regulates a diverse set of biological activities, including proliferation, apoptosis, differentiation, motility, extracellular matrix deposition, and angiogenesis. Although glial cells are growth inhibited by TGFβ, glioma cell lines are resistant to TGFβ-mediated growth inhibition yet retain responsiveness to the effects of TGFβ on the neoplastic phenotype—secretion of angiogenic factors, induction of invasion, and immune escape. The molecular mechanisms through which TGFβ shifts from a tumor suppressor to a tumor enhancer in advanced cancers are poorly understood. Recent work suggests that the phosphatidyl 3-OH inositol kinase (PI3K) pathway interacts with TGFβ signaling at multiple levels. We therefore sought to determine the functional significance of PTEN expression on TGFβ-mediated transcription. Restoration of wild-type PTEN into a PTEN null glioma cell line inhibited TGFβ-induced transcription in a PTEN concentration-dependent fashion, whereas mutant PTEN lacking both protein and lipid phosphatase activity did not have a similar effect. In a cell line with wild-type PTEN expression, stable knockdown of PTEN expression with short hairpin RNA (shRNA) increased TGFβ transcriptional activation or inhibition in multiple TGFβ responsive promoters. Results with these luciferase reporters suggest that PTEN functions through a mechanism distinct from other PI3K pathway components previously shown to interact with SMADs. To elucidate the mechanism by which PTEN inhibits TGFβ transcription, we examined TGFβ-induced phosphorylation of key intracellular mediators (the SMADs) with PTEN expression. C-terminal SMAD phosphorylation was moderately decreased with wild-type PTEN expression but not mutant PTEN, including a mutant that retains protein phosphatase activity. Reconstitution of wild type but not phosphatase dead mutant PTEN into PTEN null glioma cell lines re-established growth inhibition in response to TGFβ. In keeping with the biphasic nature of TGFβ signaling, PTEN reconstitution blocked invasion through an artificial matrix induced by TGFβ. Reciprocally, inhibiting PTEN expression in a PTEN wild type cell line through expression of shRNA directed against PTEN inhibited TGFβ-induced motility. Thus, loss of PTEN expression may promote cellular responses to TGFβ involved in tumor progression. Reintroduction of PTEN may shift TGFβ cellular responses towards a tumor suppressive phenotype by restoring sensitivity of glioma cell lines to TGFβ-mediated growth inhibition while blocking TGFβ-mediated invasion. This work was also supported by NIH grants NS047409. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

63. RNAi-MEDIATED SIMULTANEOUS DOWNREGULATION OFUPAR AND uPA RESULTS IN THE DOWNREGULATION OF THE REGULATORY ASSOCIATED PROTEIN OF mTOR (RAPTOR), A COLLAPSE IN MITOCHONDRIAL DY, AND THE INDUCTION OF PRO-APOPTOTIC GENES IN SNB19 HUMAN GLIOMA CELLS

C. Gondi, S. Kondraganti, W.C. Olivero, D. Dinh, M. Gujrati, and *J.S. Rao*; Department of Biomedical and Therapeutic Sciences, UIC College of Medicine at Peoria, Peoria, Illinois, USA

Our previous results have demonstrated the ability of a plasmid-based RNAi system to silence uPAR and uPA using a bicistronic construct. Urokinase plasminogen activator (uPA) and its receptor (uPAR) are overexpressed during glioma cell invasion and progression. Pro-uPA, when bound to its receptor uPAR, is activated to uPA, which in turn activates plasminogen to plasmin. The binding of uPA to uPAR initiates a cascade of signaling events involving integrins mediated by MEK and PI3-K. SNB19 cells were transfected with plasmid-expressing RNAi targeting uPAR (pUR), uPA (pUP), uPAR-uPA simultaneously (pU2), empty vector (EV), or scrambled vector (SV). Western blot analysis of RAPTOR (regulatory associated protein of mTOR) showed a marked decrease in expression levels when transfected with plasmids expressing either pUP or pU2. In contrast, cells transfected with pUR did not exhibit a decrease in RAPTOR levels, which indicates that uPA levels are integral in maintaining the activity of mTOR. In addition, Ki67 levels decreased in pUP- and pU2-transfected cells. However, downregulation of uPAR alone did not result in a decrease of Ki67 levels, further indicating the involvement of uPA with proteins other than its traditional receptor. Mitochondrial transition event was measured by using fluorescent cation dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzamidazolocarbocyanin iodide, where red fluorescence indicates DY collapse. The simultaneous downregulation of uPAR and uPA resulted in a decrease of red florescence, whereas the downregulation of either uPAR or uPA alone did not. These data strongly suggest the involvement of the uPA uPAR complex in mitochondrial DY maintenance and collapse. The simultaneous downregulation of uPAR and uPA also induced caspase 8 activation accompanied by cytochrome c release, thereby indicating the initiation of apoptosis. Of further interest is that, in pU2-transfected cells, PARP levels were elevated and cleaved PARP levels were decreased 72 h after transfection, but 120 h after transfection, PARP cleavage, an indicator of apoptotic progression, was observed. In conclusion, the simultaneous downregulation of uPAR and uPA inhibited cap-dependent mRNA translation via downregulation of RAPTOR, initiated apoptosis by mediating the collapse of mitochondrial DY, and induced caspase 8 and PARP cleavage. These results clearly suggest that the simultaneous targeting of uPAR and uPA has potential for cancer gene therapy.

64. OVEREXPRESSION OF MARCKS IN EGFRvIII-EXPRESSING GLIOBLASTOMA MULTIFORME

J. Micallef,[1] J. Caldwell,[1] M. Moran,[1] and *A. Guha*[1,2]; [1]Neurosurgery Division, Toronto Western Hospital, Toronto, Ontario; [2]HSC, Neurosurgery Division, Labatts Brain Tumor Centre, Toronto, Ontario; Canada

The most common gain of function mutation in malignant astrocytomas (GBMs) is amplification or overexpression of the wild-type (wt) and mutated epidermal growth factor receptors (EGFRs), the most common of which is EGFRvIII. The differential signaling pathways utilized by EGFRvIII vs. wt-EGFR, contributing to its more aggressive behavior, is not known. ICAT, a mass spec–based analytical technique to evaluate differential protein profiles, demonstrated that GBM explant xenografts harboring EGFRvIII expressed much higher levels of MARCKS, as compared to GBMs, which express only wt-EGFR. MARCKS has been previously implicated in tumor invasion and breast cancers, but not in gliomas or EGFR signaling. MARCKS overexpression in EGFRvIII GBMs was verified by Western immunoblot analysis on a larger panel of GBM cell lines with Tet-Off expression of EGFRvIII or wtEGFR, GBM xenografts with ± EGFRvIII and GBM operative specimens with ± EGFRvIII. Differences at the level of the proteome, evaluated by proteomic-based techniques such as ICAT, allow us to understand biological and clinical similarities or differences between subtypes of human diseases, such as GBMs. Current work involves assessing the functional role of MARCKS overexpression in GBMs. We will discuss our experiments with siRNA downregulation of MARCKS in GBMs expressing EGFRvIII and MARCKS overexpression in GBMs expressing only wt-EGFR. If MARCKS contributes to the aggressive biology of EGFRvIII expressing GBMs, it may be an additional biological target.

65. OVEREXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND MAINTENANCE OF MALIGNANT PHENOTYPE IN MENINGIOMAS

I. McCutcheon,[1] K. Bian,[2] J. Li,[1] and F. Murad[2]; [1]M.D. Anderson Cancer Center, Neurosurgery, and [2]Integrative Biology and Pharmacology, Houston Medical School, The University of Texas, Houston, Texas, USA

Because metastatic activity, host defense mechanisms, and level of differentiation seem to be correlated to iNOS expression, a role for iNOS has been proposed in the onset and progression of malignant diseases. Under physiological condition, NO acts as an intracellular secondary messenger and provides an efficient system for cellular regulation, interaction, and defense. The expression of 3 isoforms of NOS in human gliomas and in peritumoral areas has been analyzed by several groups. Although the induction of iNOS has been noted in human meningioma (Bakshi et al., 1998; Broholm et al., 2003; Ellie et al., 1995; Hara et al., 1996), a clear correlation between meningiomas and iNOS expression has not been established. Human meningiomas of benign, atypical, and anaplastic/malignant grade (n = 10) were excised and primary explant cultures obtained. Histopathological confirmation of grade was obtained. By using Western blot, expression of inducible nitric oxide synthase (iNOS) was demonstrated for cultured cells and intact tissue fragments. Integrity of downstream NO signaling pathways was tested by exposing cultured cells at early passage to NO donor compounds. The downstream actions of NO take two forms, (1) cGMP-dependent; and (2) cGMP-independent, which are mediated by reactive nitrogen species produced by the interaction of

NO with O_2 or with superoxide radicals ($O_2^{\cdot-}$). The first was tested by measuring levels of guanyl cyclase and of cGMP, and the second by assay of nitrotyrosine formation, in NO-donor-stimulated cells of each grade. A clear correlation was shown between iNOS expression and the degree of tumor malignancy, with lowest expression seen in benign tumors and highest seen in those of anaplastic grade. In malignant meningioma cells both membrane-bound and soluble guanylyl cyclase (sGC) were un-regulatable, and the cGMP levels were very low despite significantly high levels of iNOS expression. In contrast, in benign meningioma cells, sGC responded to NO normally, and a marked increase of cGMP was observed upon NO donor stimulation. Thus, the downstream pathways of NO signaling in malignant meningioma appear to be shut down. In additional experiments, a significant increase in nitrotyrosine formation (a biomarker for cGMP-independent NO action) was detected in malignant cells. We conclude that iNOS expression correlates with degree of malignancy in meningiomas. However, in anaplastic meningiomas, cGMP-dependent signaling pathways are inactive, but cGMP-independent pathways are not. These data suggest that in malignant meningioma cells, iNOS overexpression may contribute to a survival advantage mediated by protein tyrosine nitration.

66. MOLECULAR GENETIC STUDY FOR HEMANGIOBLASTOMAS AND FUNCTIONS OF VHL GENE

H. Kanno,[1] A. Kubo,[1] T. Mimura,[1] Y. Tanaka,[1] and I. Yamamoto[1];
[1]Yokohama City University Graduate School of Medicine, Neurosurgery, Yokohama, Japan

Molecular genetic analysis for germline or somatic mutation of von Hippel-Lindau (VHL) gene in central nervous system (CNS) hemangioblastomas (HBs) with and without VHL disease contributes to surgical treatment and follow-up. Forty-nine patients bearing HBs (sporadic 33, VHL 16) underwent surgery and genetic diagnosis for germline and somatic VHL mutations. Locations of the tumors were cerebellum, 39; brain stem, 3; and spinal cord, 10. Fourteen of 16 VHL cases had multiple tumors while 3 of 32 sporadic cases had one tumor. Twelve sporadic HBs showed somatic mutations (missense 6, truncation-type 6) but not germline mutations. In addition, 24 sporadic HBs showed loss of heterozygosity (LOH) on 3p, in which the VHL gene is located. These results suggested that the inactivation of VHL genes on both alleles was a cause of genesis in the majority of sporadic hemangioblastomas and that the VHL gene functioned as a tumor suppressor gene. Eleven of 15 VHL cases showed VHL germline mutations (missense 8, truncation-type 4). Patients with truncation-type VHL germline mutation were more frequently associated with renal cell cancer (RCC). Causes of death were postoperative complications in 2 sporadic patients and tumor development in 2 VHL patients. Clinically ambiguous cases, whether sporadic or VHL, should be analyzed for VHL germline mutation. It might be recommended that HBs with VHL should be surgically treated if symptomatic, while asymptomatic small ones should be observed or treated with radiosurgery. Functions of the VHL gene include not only tumor suppression in HB as the above but also neuronal differentiation, which we demonstrated. Herein we show neuronal regeneration with donor of VHL-gene or peptide transferred stem cells (neural stem cell, bone marrow stromal cell, skin stem cell, ES cell). Transplantation with VHL-gene transferred stem cells dramatically improved symptoms of neuronal disease model rats (Parkinson, cerebral infarction, spinal injury), and they functioned as neurons in the brain. It was suggested that neuronal differentiation by VHL protein was related to ubiquitination and resolution of Notch under normoxia but not under hypoxia. In addition, synthetic VHL oligopeptide (elongin-binding site at a-domain) showed induction potential for neuronal differentiation equal to transduction with viral vector. In the future, neuronal regeneration with VHL gene or peptide would be useful for the clinical level.

67. LIGAND-INDEPENDENT ACTIVATION OF THE EGFRvIII: A NATURALLY OCCURRING MUTATION OF THE EGFR COMMONLY EXPRESSED IN GLIOMA

T. Johns, A.A. Vitali, R.M. Perera, S. Vernes, and A.M. Scott; Ludwig Institute for Cancer Research, Oncogenic Signalling, Heidelberg, Australia

Mutations of the epidermal growth factor receptor (EGFR) gene are found at a relatively high frequency in glioma, with the most common being the de2-7 EGFR (or EGFRvIII). This mutation arises from an in-frame deletion of exons 2–7, which removes 267 amino acids from the extracellular domain of the receptor. Despite being unable to bind ligand, the de2-7 EGFR is constitutively active at a low level. Transfection of human glioma cells with the de2-7 EGFR has little effect in vitro, but when grown as tumor xenografts this mutated receptor imparts a dramatic growth advantage. We have now mapped the phosphorylation pattern of de2-7 EGFR, both in vivo and in vitro, using a panel of antibodies unique to the different phosphorylated tyrosine residues. Phosphorylation of de2-7 EGFR was detected constitutively at all tyrosine sites surveyed both in vitro and in vivo, including tyrosine 845, a known target in the wild-type EGFR for src kinase. There was a substantial upregulation of phosphorylation at every tyrosine residue of the de2-7 EGFR when cells were grown in vivo compared to the receptor isolated from cells cultured in vitro. Upregulation of phosphorylation could be mimicked in vitro by the addition of specific components of the ECM such as collagen via an integrin-dependent mechanism. Since this increase in in vivo phosphorylation enhances de2-7 EGFR signaling, this observation explains why the growth enhancement mediated by de2-7 EGFR is largely restricted to the in vivo environment. In a second set of experiments we analyzed the interaction between EGFRvIII and ErbB2. Co-expression of these proteins in NR6 cells, a mouse fibroblast line devoid of ErbB family members, dramatically enhanced in vivo tumorigenicity of these cells compared to cells expressing either protein alone. Detailed analysis of these xenografts demonstrated that EGFRvIII could heterodimerize and transphosphorylate the ErbB2. Since both EGFRvIII and ErbB2 are commonly expressed at gliomas, this data suggests that the co-expression of these two proteins may enhance glioma tumorigenicity.

68. LONG-TERM SURVIVAL IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED IN PHASE 2 STUDIES WITH ANP

R.A. Weaver, S.R. Burzynski, T.J. Janicki, B. Burzynski, G. Jurida, and B. Szymkowski; Burzynski Clinic, Houston, Texas, USA

The purpose of this study was to determine the frequency of long-term survivals in patients with glioblastoma multiforme (GBM) treated in FDA and Institutional Review Board monitored phase 2 studies with ANP (antineoplastons A10 and AS2-1). One hundred seventy-three patients with GBM who could be evaluated were accrued to FDA-monitored phase 2 trials. Seventy-nine patients were admitted to the study protocols (SP), and an additional group of 94 patients were treated under special exception (SE) because of low Karnofsky performance status (KPS), below 60. Ninety-eight percent of patients failed prior surgery, radiation therapy, and/or chemotherapy. ANP was given intravenously daily in escalating doses. The median duration of ANP administration was 4 months for SP and 3 months for SE, and the average dosage of A10 was 6.18 (SP) and 6.92 (SE) and of AS2-1 was 0.26 (SP) and 0.25 (SE) g/kg/d. Responses were assessed by gadolinium-enhanced MRIs and PET scans (as necessary). Long-term survival was defined as patients surviving 3 years after initial diagnosis. There was 15.5% long-term survival in the SP group and 7.1% in the SE group. The maximum survival in the SP group was more than 12 years and in the SE group was more than 10 years. Survival was significantly reduced in the SE group, which consisted of patients with lower KPS. The data indicate that more than 15% of evaluable patients with GBM treated with ANP in phase 2 studies were long-term survivors. The results are significantly worse in a group of patients with lower KPS, but compare favorably with radiation therapy and chemotherapy.

69. RESPONSES OF THE ADULT MAMMALIAN CENTRAL NERVOUS SYSTEM TO EXPERIMENTAL INTRACRANIAL GLIOMA

M. Barish,[1] J. Najbaur,[2] E. Garcia,[2] M. Metz,[2] and K. Aboody[2];
[1]Division of Neurosciences, Beckman Research Institute and [2]Division of Hematology and HCT, City of Hope National Medical Center, Duarte, California, USA

In the brain, tumor cells and normal cells are the interacting elements of a two-part system connected by competition for physical space, extracellular matrix components, and secreted soluble factors. Our goal is to understand how normal brain cells respond to, and feed back onto, tumor cells. In the present experiments, U251 human glioma cells were injected into cortices of adult nude mice to induce experimental glioma. Brains were examined over time when tumors were 0.5 to 4 mm in diameter and occupying an increasing portion of the frontal area. Markers of cell type and status are examined by immunofluorescence in horizontal sections. Nestin immunoreactivity (ir) was detected in tumor and host cells and separated using species-specific antibodies (Chemicon; MAB353 for mouse, MAB5326 for human). On the ipsilateral side, nestin ir was observed in host cells along both lateral and medial ventricular walls, in cells positioned between the tumor mass and the pia, in cells dispersed around the tumor and just inside its edge, and in the corpus callosum and adjacent tumor. Some nestin-immunoreactive (nestin-ir) cells, especially those in the parenchyma lateral to the ventricle and inside the tumor mass, had the morphology of neural progenitor cells (NPCs). Other nestin-ir cells, particularly those interposed between the

tumor and the pia, had the fibrous morphology of reactive astrocytes, and some of these cells were also GFAP-ir. The presence of proliferating cells was probed using antibody to PCNA (Chemicon MAB424). In addition to the tumor cells, PCNA-ir was observed in subpopulations of nestin-ir presumptive NPCs and in nestin-ir presumptive reactive astrocytes, as well as in unidentified non-nestin-ir cells dispersed lateral to the ventricle, in the corpus callosum, in the fimbria, and in the dentate gyrus. Our results suggest that the experimental glioma induced appearance of proliferating nestin-ir NPCs, nestin-ir reactive astrocytes, and other cells whose identity has not yet been determined. The position of the reactive astrocytes suggests that mechanical deformation may play an inductive role. NPCs near the ventricle and medial to the tumor mass may be responding to presently unidentified factors emerging from the tumor and/or from adjacent host cells. In neither case did it appear that the tumor environment was highly mitogenic for host cells, which suggests that host cells were not major contributors to the tumor burden.

70. TARGETED THERAPY WITH ANP IN CHILDREN LESS THAN 4 YEARS OLD WITH INOPERABLE BRAIN STEM GLIOMAS

S.R. Burzynski, R.A. Weaver, T.J. Janicki, B. Burzynski, and G. Jurida; Burzynski Clinic, Houston, Texas, USA

The purpose of the study is to evaluate the outcome of patients less than 4 years old with intrinsic diffuse brain stem gliomas (BSG) treated with ANP (antineoplastons A10 and AS2-1) in two FDA and Institutional Review Board monitored phase 2 trials. A total of 10 assessable patients who were less than 4 years old were among 65 participants of phase 2 trials (study and special exception patients): 2 with anaplastic astrocytomas, 1 with pilocytic astrocytoma, and 7 who had no biopsy because of a dangerous tumor location. Age ranged from 3 months to 3 years. Three patients failed prior radiation and chemotherapy, 1 had stable disease after radiation, 2 had tumor resection, and 4 were not treated prior to ANP. ANP was given intravenously daily through a subclavian venous catheter and a double channel infusion pump. The median duration of ANP administration was 9½ months, and the average dosage of A10 was 12.16 g/kg/day and of AS2-1 was 0.41 g/kg/day. Responses were assessed by gadolinium-enhanced MRIs and confirmed by PET scans in some cases. The overall survival at 2 years was 50% and at 5 years 20%, and the maximum survival is 6+ years. Median progression-free survival was 2 years and 2 months. Complete response was achieved in 30%, stable disease in 40%, and progressive disease in 30%. Serious toxicities included reversible anemia and hypokalemia. There were no chronic toxicities. ANP targets the *AKT2, RAS, p53, and p21* pathways, and its administration results in substantial survival and response rates in a small group of young children who do not have a favorable prognosis for standard therapy.

71. INHIBITION OF INSULIN-LIKE GROWTH FACTOR I RECEPTOR SIGNALING INCREASES CHEMOSENSITIVITY OF PEDIATRIC CNS ATYPICAL TERATOID/RHABDOID TUMOR CELLS

T. Shalaby, J. D'cunia, and *M. Grotzer*; University Children's Hospital, Oncology, Zurich, Switzerland

Central nervous system (CNS) atypical teratoid/rhabdoid tumors (ATT/RhT) are among the pediatric malignant tumors with the worst prognosis and fatal outcome. To date there are no explanations for their remarkable resistance to cytostatic drugs and radiotherapy. Insulin-like growth factor I receptor (IGF-IR) protects cancer cells from apoptosis induced by a variety of anticancer drugs and radiation, but when impaired by inhibitors, tumor cells undergo massive apoptosis, resulting in an inhibition of tumorigenesis and metastases in experimental animal models. IGF-IR was found to be clearly overexpressed in ATT/RhT compared to near normal brain samples and to other pediatric CNS neoplasms as confirmed by Western blotting and immunohistochemistry. Moreover, we found IGF-I and IGF-II mRNA in an ATT/RhT cell line indicating the presence of an autocrine/paracrine IGF-I/II/IGF-IR loop in ATT/RhT. Human BT-12 and BT-16 ATT/RhT cells were treated with IGF-IR antisense or scrambled control oligonucleotides for different time periods. Antisense treatment of ATT/RhT cells for 48 h resulted in significant downregulation of IGF-IR mRNA and IGF-IR protein expression, both in BT-12 and in BT-16 cells. IGF-IR antisense treatment resulted in inhibition of cellular proliferation and induction of apoptosis. Moreover, chemosensitivity of ATT/RhT cells to doxorubicin and cisplatin was found to be significantly increased upon treatment with IGF-IR antisense oligonucleotides. This suggests the presence of an autocrine/paracrine IGF-I/II/IGF-IR loop in ATT/RhT that may be responsible for the low susceptibility of ATT/RhT cells to undergo apoptosis. Inhibition of IGF-IR represents a novel therapeutic strategy in childhood CNS ATT/RhT that warrants further investigation.

72. HYPOXIA SENSITIZES HUMAN MALIGNANT GLIOMA CELLS TOWARD CD95L-INDUCED CELL DEATH

J. Steinbach,[1] H. Wolburg,[2] A. Klumpp,[1] and M. Weller[1]; [1]Abt. Allgemeine Neurologie and [2]Institute of Pathology, University of Tübingen, Tübingen, Germany

Death ligands such as CD95 ligand (CD95L) have limited activity against glioma cells under normoxic conditions (Weller et al., J. Clin. Invest. 94, 954, 1994). However, many glioma cell lines can be sensitized toward death ligand–induced apoptosis by inhibition of epidermal growth factor receptor (EGFR) (Steinbach et al., Brain Pathol. 12, 12, 2002). Hypoxia is a critical aspect of the microenvironment of gliomas. We have established a paradigm for the investigation of hypoxia-induced cell death in glioma cells in vitro, which faithfully reproduces many aspects of human glioma pathology (Steinbach et al., Cell Death Differ. 10, 823, 2003). Here, we investigated the effect of co-exposure to acute hypoxia and CD95L in three human malignant glioma cell lines with different susceptibility to CD95L under normoxic conditions. Hypoxia sensitized all three cell lines toward CD95L-induced cell death. Co-exposure resulted in apoptotic changes in the early phase, with gradual conversion to secondary necrosis with increasing length of hypoxia. The mitochondrial injury induced by hypoxia was enhanced by co-treatment, and caspase cleavage became prominent. Inhibition of the EGFR, while sensitizing glioma cells to CD95L under normoxia, protects glioma cells from hypoxia by reducing energy consumption (Steinbach et al., Cancer Res. 64,1575, 2004). However, the opposing effects of EGFR signaling on death induced by CD95L or hypoxia were neutralized by co-exposure to hypoxia and CD95L. Further, inhibition of protein synthesis by cycloheximide also reduced glucose consumption and conferred protection from hypoxia, but did not modulate CD95L-induced cell death under hypoxic conditions. These results suggest that death ligands may be useful to target hypoxic tumor cells resistant to conventional therapies or to complement strategies aiming at the induction of tumor hypoxia.

73. CONSTITUTIVE INTEGRIN ACTIVATION BY RAP-GTPASE ON LEUKEMIC CELLS PROMOTES PROGRESSION OF LEPTOMENINGEAL LEUKEMIA

D. Brandsma,[1,2] L. Ulfman,[3] J. Reijneveld,[1] M. Bracke,[4] M. Taphoorn,[1] J. Zwaginga[6,7] M. Gebbink,[5] H. de Boer,[8] and E. Voest[2]; Departments of [1]Neurology, [2]Medical Oncology, [3]Pulmonary Diseases, [4]Pharmaco-epidemiology and Pharmacotherapy, and [5]Haematology, University Medical Center Utrecht, Utrecht; [6]Department of Haematology, Academical Center Amsterdam, Utrecht; [7]Sanquin Research, Amsterdam; [8]Department of Nephrology, Leiden University Medical Center, Leiden; The Netherlands

Leptomeningeal metastases are a serious neurological complication in cancer patients and are associated with a dismal prognosis. Tumor cells that enter the subarachnoid space adhere to the leptomeninges and form tumor deposits. The role of integrins in tumor cell adhesion to leptomeninges is largely unknown. We studied the role of integrin expression and activation in the progression of leptomeningeal metastases. Therefore, we used a suspension (L1210-S) and an adherent (L1210-A) variant of a mouse acute lymphocytic leukemic cell line. Static adhesion levels of L1210-A cells on a leptomeningeal cell layer were significantly higher than for L1210-S cells. All mice that were intrathecally injected with L1210-A cells died rapidly because of leptomeningeal leukemia. In contrast, 45% of long-term survivors were seen after intrathecal injection with L1210-S cells. β_1-, β_2- and β_3-integrins were in a constitutive active state on L1210-A cells and in a low but inducible state on L1210-S cells, as determined by adhesion assays on surfaces coated with β_1 integrin ligand (collagen), β_2 integrin ligand (ICAM-1), and β_3 integrin ligand (vitronectin). Expression levels of these integrins were comparable in the two cell lines. The discrepancy in integrin activation state on the two cell lines is due to a difference in the activation of the small GTPase Rap, which is involved in integrin inside-out signaling. Rap in L1210-A cells is in a constitutive active state, whereas in the L1210-S cells Rap is off, but inducible. Our data indicate that constitutive integrin activation on leukemic cells promotes leptomeningeal leukemia progression by increased adhesion to the leptomeninges via an aberrantly regulated Rap GTPase signaling pathway.

74. INHIBITION OF THE MAMMALIAN TARGET OF RAPAMYCIN SENSITIZES GLIOMA CELLS TO ANTICANCER DRUGS

K. Sakurada,[1] Y. Sonoda,[1] C. Kitanaka,[2] W. Mori,[1] and T. Kayama[1]; [1]Neurosurgery; [2]Molecular Cancer Science, Yamagata University, Yamagata, Japan

The phosphatase and tensin homolog deleted from chromosome 10 (PTEN) functions as a tumor suppressor by negatively regulating the growth/survival signals of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. The PI3K/Akt pathway in PTEN-deficient tumors may be one of the key targets for anticancer therapy. Nevertheless, how PI3K/Akt pathway contributes to clinical drug resistance is unclear. The mammalian target of rapamycin (mTOR) is a serine-threonine kinase that functions downstream from Akt in PI3K/Akt/mTOR signaling pathway commonly activated in GBM. In this study, we analyzed the effects of mTOR inhibitor rapamycin on apoptosis and cytotoxicity induced by several kinds of chemotherapeutic agents. Human malignant glioma cell lines T98G, U251MG (PTEN-deficient cells), and A172 (PTEN-wild-type cells) were used for this study. We examined effects of the mTOR inhibitor rapamycin on apoptosis and cytotoxicity induced by chemotherapeutic agents including antimicrotuble agent vincristine, a topoisomerase II inhibitor etoposide, and a DNA cross-linking agent cisplatin (cis-diamminedichloroplatinum), and we compared the rapamycin-induced enhancement of effects of those agents. Among chemotherapeutic agents, vincristine has the strongest cytotoxicity to the glioma cells. The rapamycin itself inhibited growth of glioma cells, but did not cause apoptosis. In addition, the rapamycin could enhance the cytotoxicity by vincristine. These findings show that mTOR is a possible target for therapy in patients with gliomas, and mTOR inhibition in combination with chemotherapeutic agents could be potent modalities for patient with malignant gliomas.

75. CASPASE-8 STATUS OF EX VIVO GLIOMAS

D. Ashley, C. Riffkin, A. Muscat, and *C. Hawkins;* Royal Children's Hospital, Children's Cancer Centre, Melbourne, Australia

We have identified that a significant proportion of glioma patient samples carry mutations in the caspase-8 gene, some of which act as dominantly interfering inhibitors. Malignant glioma has a dismal prognosis, partly because of the inability of chemotherapy and radiotherapy to induce apoptosis in the tumor cells. Death ligands, including TRAIL/Apo2L, are attractive candidate therapeutic agents for unresponsive cancers like malignant glioma, as they induce apoptosis via a pathway that is distinct from that triggered by chemotherapy and irradiation. Caspase-8 is an apoptotic protease that plays a crucial role in apoptosis mediated by death ligands. Mutations or downregulation of caspase-8 have been reported in neuroblastoma and other tumor types, prompting its designation as a tumor suppressor gene. In this study, we analyzed the status of caspase-8, and other apoptosis pathway components, in ex vivo high-grade glioma specimens. Caspase-8 protein levels were frequently low or undetectable. We also amplified and sequenced the caspase-8 mRNA expressed by the tumors. A significant proportion of the gliomas bore mutations, some of which acted as dominant negative inhibitors in death ligand apoptosis assays. These data have significant implications for our understanding of the process of gliomagenesis and the potential use of death ligands like TRAIL for treatment of patients with malignant glioma.

76. EXPRESSION LEVELS OF THE TRANSFORMING GROWTH FACTOR-BETA MEDIATOR SMAD3 DETERMINES GLIOMA PHENOTYPE IN A GENETICALLY DEFINED HUMAN GLIOMA MODEL AND A TRANSGENIC MURINE GLIOMA MODEL

E. Reese,[1] A. Hjelmeland,[1] R. McLendon,[2] E. Holland,[5] D. Bigner,[2] X. Wang,[3] and *J. Rich*[4]; Departments of [1]Surgery, [2]Pathology, [3]Pharmacology & Cancer Biology, and [4]Medicine, Duke University Medical Center, Durham, North Carolina; [5]Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, New York; USA

Transforming growth factor-β (TGFβ) plays an important role in gliomas through secretion of angiogenic factors, increased invasion, and immune escape. We previously demonstrated that TGFβ potently inhibits cellular proliferation of astrocytes associated with a G_1 cell cycle arrest and induction of $p15^{INK4B}$ cyclin-dependent kinase inhibitor expression. This process is dependent on SMAD3, as TGFβ largely failed to inhibit the growth of SMAD3–/– astrocytes or to induce $p15^{INK4B}$. As most glioma cell lines resist TGFβ-mediated growth inhibition, we mimicked the activation of TGFβ signaling in brain tumors by expressing constitutively active TGFβ ligand in a genetically defined glioma model that we have previously reported. Early passages of this line were growth restricted, but later passages escaped the antiproliferative effects of TGFβ and developed a much greater proliferative potential. Both early and late passage glioma cultures expressing constitutively active TGFβ ligand expressed lower levels of the receptor SMADs (SMAD2 and 3), but the escape from TGFβ growth suppression was accompanied only by further SMAD3 expression loss in later passages. This finding is consistent with reports of decreased SMAD3 expression in human brain tumors. TGFβ exerts the majority of its effects through interactions with the microenvironment. Therefore, we modified a previously developed transgenic glioma model in which an avian retroviral receptor is expressed in a tissue-specific manner (Nestin tv-a). We interbred the Nestin tv-a mice with Smad3 null mice to determine the impact of SMAD3 expression on glioma formation. Nestin tv-a SMAD3–/– mice were generated at less than Mendelian ratios but were viable. We generated tumors through intracranial implantation of retroviruses expressing PDGF at P_0. Nearly all mice so treated developed neurological symptoms and tumors with histopathology similar to human gliomas. The loss of SMAD3 did not impact the latency of tumor development. Despite the limited number of Smad3–/– mice examined, the loss of SMAD3 was associated with increased tumor grade relative to SMAD3+/+ and +/– mice. No SMAD3–/– mice lack tumors, whereas other genotypes lacked tumors in a fraction of mice. Tumor generation continues, and we are characterizing the impact of targeted disruption of SMAD3 on tumor angiogenesis and invasion. In conclusion, loss of SMAD3 may contribute to the shift of TGFβ from a tumor suppressor to tumor enhancer in gliomas in both human cellular and transgenic murine glioma models. This work was also supported by NIH grant NS047409. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

77. TRAIL IS NONTOXIC TO NORMAL HUMAN ASTROCYTES

D. Song,[1] J. Song,[1] T. Wilson,[2] V. Yong,[2] and C. Hao[1]; Departments of [1]Pathology & Laboratory Medicine and Hematology & Oncology, Emory University, Atlanta, Georgia, USA; [2]University of Calgary, Clinical Neuroscience, Calgary, Canada

Tumor necrosis factor (TNF) family members such as TNF and FasL can induce apoptosis in cancer cells; however, their toxicity to normal tissues has limited their usefulness in clinical application. TNF-related apoptosis-inducing ligand (TRAIL) is a new member of the TNF family, and its clinical application currently is under a similar debate. We have reported that the recombinant soluble TRAIL (amino acids 114-281) can induce apoptosis in human malignant glioma cells. Here, we report that TRAIL is nontoxic to normal human astrocytes. Normal human fetal astrocytes were prepared and cultured in RPMI-1640 medium supplemented with 10% FBS. Cell death was determined by crystal violet assay, and caspase cleavage was examined on Western blots. Small interfering RNA (siRNA) was generated to target specific genes. The normal human astrocytes were treated with FasL and TRAIL, and cell death analysis showed that the astrocytes are resistant to TRAIL and FasL-induced cell death. Treatment of the astrocytes with a pharmacologic inhibitor KN93 to calcium/calmodulin-dependent protein kinase II (CaMKII) sensitized the astrocytes to FasL-induced apoptosis through caspase-8-initiated caspase cascade, as evident by the cleavage of caspase-8, caspase-3 and DNF fragmentation factor 45 (DFF45). Treatment of the astrocytes with KN93 inhibited expression of cellular Fas-associate death domain-like, IL-1b-converting enzyme-inhibitory protein (c-FLIP) and phosphoprotein enriched in diabetes (PED) in human astrocytes. Transfection of siRNAs specifically targeting *c-FLIP* and *PED* gene sensitized the astrocytes to FasL-induced apoptosis. The results indicate that CaMKII-mediated c-FLIP and PED pathway modulates FasL signaling in human astrocytes. In contrast, neither KN93 nor siRNA targeting c-FLIP and PED sensitized the human astrocytes to TRAIL-induced apoptosis. Flow cytometry analysis revealed cell surface expression of Fas, but not DR4 and DR5 in the human astrocytes. The study identifies the different modulation mechanisms between TRAIL and FasL signaling pathways in normal human astrocytes. Lack of the expression of TRAIL receptors in human astrocytes contributes to TRAIL resistance.

78. STAGE-SPECIFIC EMBRYONAL ANTIGEN EXPRESSION IN GLIOBLASTOMA CELLS: POSSIBLE ROLE IN TUMOR MAINTENANCE

V. Tabar, G. Panagiotakos, and P. Gutin; Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Stage-specific embryonal antigens (SSEAs) are a group of carbohydrate molecules that are characteristically expressed on the surface of embryonic stem cells during development, or at the undifferentiated stage in vitro. They are rapidly downregulated at the blastocyst stage and upon ES cell differentiation. While their role is unclear, this temporal regulation is very

consistent and highly predictive of the differentiation status of ES-derived progeny. Surprisingly, when we performed histochemical screens on a group of glioblastoma specimens (n = 12), we found consistent expression of SSEA1, SSEA3, and SSEA4. Gene expression profiles in the same group demonstrate the presence of other genes highly specific to ES cells, such as Oct4, Nanog and bmi1. To further investigate the parallel expression of these "stem cell-specific factors" in ES cells and gliomas, we performed FACS on several cell groups: undifferentiated human ES cells (NIH WA-01 and WA-09), three different series of human ES-derived neural precursors (NPC), and cells dissociated from four glioblastomas. Our preliminary data confirms a very high level of expression of SSEA-4 in the undifferentiated hES cells (88.17%) while few cells expressed SSEA-1 (0.98%). CD133 was also significantly expressed in these cells (59.08%). Upon neural induction the marker profile is dramatically changed, with downregulation of SSEA-4 to 3.7%, upregulation of SSEA-1 to 23.18%, with CD133 at 24.06%. Interestingly, SSEA-4 expression is significantly upregulated in gliomas (average of 19.17%), a phenomenon not previously described in somatic tissues or in other tumors. The levels of SSEA-1 in tumors are somewhat decreased when compared to NPCs (9.81% vs. 22.67%), and CD133 is dramatically reduced to 0.2%. Limiting dilution assays were performed on the fractionated glioma populations. In the one-cell wells a ratio of 1 sphere per cell was established for the SSEA4+ cells vs. 0.5 to 1 for SSEA1+ and 0.2 to 1 in the SSEA4- fraction. Subcutaneous injections in NOD/SCID mice revealed a parallel efficiency at tumor formation at dilutions from 500 down to 20 cells/animal/injection. Interestingly, differences among the positive and negative SSEA4 fractions were not noted upon initial injection in mice, but rather after the second passage, whereby SSEA4- cells failed to produce tumors when injected at dilutions of 100 up to 500 cells/injection. These results were independent of CD133 expression, recently reported as a "cancer stem cell" marker, and are suggestive of a complex hierarchy within glioma tumors, whereby some cell populations (e.g., SSEA4+) are capable of efficient tumor amplification and maintenance parallel to the role of transit-amplifying progenitors described within the subventricular zone of the developing or adult brain. Such cells are indistinguishable from "stem cells" as currently defined by neurosphere and tumor formation assays. Additional studies are conducted to further elucidate the expression of "stem cell" genes among the populations capable of self-renewal, and the relationships of SSEA4 expression to that of CD 133.

79. NEURAL STEM/PROGENITOR CELL MIGRATION AND GLIOMA TARGETING IS STIMULATED THROUGH THE EGF-PI3K SIGNALING CASCADE

S.E. Kendall,[1] K.S. Aboody,[2] M. Metz,[2] J. Najbauer,[2] E. Garcia,[2] and *C.A. Glackin*[1]; Departments of [1]Molecular Medicine and [2]Hematology/HCT and Neuroscience, Beckman Research Institute, City of Hope National Medical Center, Duarte, California, USA

Recent advances in neural stem/progenitor cell (NSPC) biology offer novel strategies to specifically target invasive gliomas through chemotactic migration. We have previously demonstrated the inherent tumor-tropic properties of transduced and untransduced NSPCs to several tumor types. We are currently utilizing an immortalized human NSPC line, F3.C1 (obtained from Seung U. Kim MD, PhD [UBC]), which targets the invasive U251 invasive experimental glioma in frontal lobe of nude mice. We hypothesize that EGF produced by the tumor facilitates the NSPC chemotactic response, showing that a blocking antibody efficiently inhibits chemotaxis toward U251 cells in modified Boyden chamber assays. Furthermore, overexpression of a dominant negative EGFR significantly reduces the chemotactic response. In contrast, overexpression of a mutant constitutively active EGFR most commonly associated with high-grade glioma, EGFRvIII, enhanced NSPC migration irrespective of ligand binding. The enhanced migration conferred by EGFRvIII on high-grade gliomas is believed to converge on PI3K signaling. Overexpression of a dominant negative p85*f*430-615 lacking the p110-binding domain was effective in blocking migration of F3-EGFRvIII and chemotaxis of F3.C1 cells toward U251 cells. The activation of PI3K recruits the formation of an active signaling complex at the EGFR cell surface, which includes Shp2. Overexpression of Shp2 C459S phosphatase dead mutant in F3.C1 cells was effective in blocking the chemotactic response to U251 and enhanced migration by F3-EGFRvIII, which suggests that growth factor signaling converges on Shp2 activation to promote cell migration. EGF signals converge on Shp2, and this leads to subsequent divergence by activating MAPK signaling, cdc42, and Rac1, while inhibiting RhoA. The inhibitory effects observed through Shp2 C459S reduce ERK activation and partially reduce the chemotactic response, which suggests additional routes to enhance cell migration. Constitutively active Shp2 E76A enhances cell migration of F3.C1 cells and reduces migration with constitutively active RhoA Q63L and thus enhances actin stress fibers. The migratory response is further reduced when cells are cotransfected with both RhoA Q63L and dominant negative Rac1 N17C. Collectively, these results demonstrate that the signaling cascade converges onto PI3K leading to activation of Shp2, thereby providing the necessary molecular changes to permit chemotaxis toward an EGF gradient.

80. SURVIVAL RATES FOR LOW-GRADE GLIOMA PATIENTS IN AN INTRA-OPERATIVE MRI

E. Claus, A. Horlacher, L. Hsu, R. Schwartz, D. Dello-Iacono, F. Talos, F. Jolesz, and P. Black; LEPH, Yale University, New Haven, Connecticut, USA

No age-and histologic-adjusted assessments of the association between extent of resection and risk of either recurrence or death exist for neurosurgical patients undergoing resection of a low-grade glioma using intraoperative magnetic resonance guidance. The data include 156 patients undergoing surgical resection of a unifocal, supratentorial low-grade glioma in the magnetic resonance operating suite at Brigham and Women's Hospital between January 1, 1997, and January 31, 2003. Estimates of disease-free and overall survival probabilities are calculated by using Kaplan-Meier methodology. The association between extent of resection and these probabilities is measured by using a Cox proportional hazards model. Observed death rates are compared to those expected using age- and histologic-specific survival rates obtained from the Surveillance, Epidemiology, and End Results Registry (SEER). Patients who underwent subtotal resection were at 1.4 (95% confidence interval [CI], 0.7–3.1) times the risk of recurrence and 4.9 (95% CI, 0.61–40.0) times the risk of death relative to patients undergoing gross total resection. The one-, two-, and five-year age- and histologic-adjusted death rates for patients undergoing surgical resection using intraoperative MRI guidance, 1.9% (95% CI, 0.3%–4.2%), 3.6% (95% CI, 0.4%–6.7%), and 17.6% (95% CI, 5.9%–29.3%), respectively, were significantly lower than those reported using national databases. The data presented here suggest a possible association between surgical resection and survival for neurosurgical patients undergoing surgery for a low-grade glioma under intraoperative MRI guidance. Further study within the context of a large, prospective, population-based project is needed to confirm these findings.

81. TEMPORAL TRENDS IN INCIDENCE OF PRIMARY GERM CELL TUMORS OF THE BRAIN AND CENTRAL NERVOUS SYSTEM IN THE UNITED STATES

J. Propp[1] and B. McCarthy[2]; [1]School of Public Health—Division of Epidemiology; University of Illinois at Chicago, Chicago, Illinois; [2]Central Brain Tumor Registry of the United States, Chicago, Illinois; USA

The objective of this study was to describe temporal trends in incidence of all primary (nonmalignant and malignant) germ cell tumors (GCTs) of the brain and central nervous system (CNS) in the United States (US) by using a large population-based series of tumors from the Central Brain Tumor Registry of the United States (CBTRUS). Brain and other CNS tumors have increased in the United States over the last two decades, both overall and for certain histologies. The observed increase is attributable in part to changes in diagnostic tools and tumor classification and coding. In the United States, the overall incidence rate for GCT of the brain/CNS is 0.08/100,000 person-years (py) (CBTRUS, 1997–2001). The rate is highest in children 0 to 19 years and young adults 20 to 34 years (0.18 and 0.10/100,000 py, respectively). Rates are higher in males than in females and in whites than in blacks. CBTRUS compiled data from six state cancer registries for tumors diagnosed from 1985 to 1999. Multiplicative Poisson regression was used to calculate the average annual percent change (AAPC [95% CI]) in incidence rates over the time period while controlling for age, sex, race, and microscopic confirmation, and to statistically compare trends over time. Joinpoint regression analysis was utilized to identify sharp changes in incidence over time. A total of 140 GCTs of the brain/CNS were diagnosed during the 15-year time period; 76% were in males, 85% in whites, and 90% of the tumors were microscopically confirmed. Sixty-nine percent were diagnosed in children (0–19 years), 23% in young adults (20–34 years), and 8% in persons 35 years and older. Incidence of all primary GCT of the brain/CNS did not significantly increase over the time period (AAPC = 3.0% [–0.9%–6.9%]). Incidence rate trends varied by sex, with females experiencing a significant increase over the time period (AAPC = 10.7% [2.5%–19.6%]) and males experiencing no significant change (AAPC = 0.8% [–3.6%–5.2%]). Because GCTs are more common in the younger compared to the older age groups, we focused our analyses upon children and young adults. Among children (0–19 years) there was a significant positive time trend (AAPC = 5.1% [0.5%–9.9%]), and among young adults (20–34 years) there was no significant change in incidence over time (AAPC = –0.4% (–8.6%–7.7%)] and no variations in trend by sex. Data will be presented to best reflect significant temporal trends and/or sharp changes in incidence that emerged for these tumors. This analysis adds to the scant literature on trends in incidence of benign and malignant GCT of the brain/CNS.

82. PRIOR HOSPITALIZATION FOR EPILEPSY AND SUBSEQUENT RISK OF GLIOMA AND MENINGIOMA

J. Schwartzbaum,[1] F. Jonsson,[2] A. Ahlbom,[2] S. Preston-Martin,[3] B. Malmer,[4] S. Lonn,[2] K. Soderberg,[2] and M. Feychting[2]; [1]Epidemiology and Biometrics, Ohio State University, Columbus, Ohio, USA; [2]Karolinska Institute, IEM, Epidemiology, Stockholm, Sweden; [3]University of Southern California School of Medicine, Los Angeles, California, USA; [4]Radiation Sciences, Umea University Hospital, Umea, Sweden

We conducted a case-control study to evaluate the preclinical association between epilepsy and primary adult brain tumors. We first identified all 1,501 low-grade glioma (LGG) and 4,587 high-grade gliomas (HGG) and 4,193 meningioma cases reported to the Swedish Cancer Registry from 1987 to 1999. Next, controls (137,485) were randomly selected from the continuously updated Swedish Population Registry and matched to cases diagnosed that year on age and sex. Finally, cases and controls were linked to the Swedish Hospital Discharge Registry (1969–1999). We found that eight or more years before HGG diagnosis (or control reference year) there was an elevated risk of HGG among people discharged with epilepsy (odds ratio [OR] = 3.01, 95% confidence interval [CI], 1.73–5.22). Two to three years before HGG diagnosis this risk increased (OR = 5.33, 95% CI, 3.58–7.93) and was especially strong among people under age 55 years (OR = 13.49, 95% CI, 6.99–25.94). During this two- to three-year prediagnostic period we also found an increased risk of HGG among people discharged with meningitis (OR = 3.02, 95% CI, 1.06–8.59) or viral encephalitis (OR = 12.64, 95% CI, 2.24–71.24). Results are similar for glioblastoma multiforme, LGG, and meningioma. The occurrence of excess epilepsy eight or more years before HGG diagnosis suggests a relatively long preclinical phase for these tumors.

83. RECORD OF CENTRAL NERVOUS SYSTEM PRIMARY TUMORS IN FRANCE: PRELIMINARY RESULTS

L. Bauchet,[1] V. Rigau,[2] H. Mathieu-Daude,[3] D. Figarella-Branger,[4] D. Hugues,[5] M. Fabbro,[6] C. Campello,[7] and F. Segnarbieux[1]; [1]Neurosurgery and [2]Neuropathology, Centre hospitalo-universitaire, Montpellier; [3]Registre des tumeurs de l'Herault, Epidemiology, Montpellier; [4]Centre hospitalo-universitaire, Neuropathology, Marseille; [5]Groupe de Neurooncologie du Languedoc Roussillon, Epidemiology, Montpellier; [6]Centre Regional de Lutte Contre le Cancer, Oncology, Montpellier; [7]Centre Hospitalo-universitaire, Neurology, Nimes; with the participation of the Association des Neuro-Oncologues d'Expression Française, Société Française de Neurochirurgie, and Société Française de Neuropathology; France

This work aims at prospectively recording all primary tumors (PT) of the central nervous system (CNS) in France for which histological diagnosis is available. The main objectives are to create a national registry and a network in order to (1) perform epidemiological studies, (2) implement a new database and use it for setting up both clinical and basic research protocols, and (3) harmonize the health care of patients affected by CNS-PT. All French neuropathology and neurosurgery departments have designated a correspondent to participate to the program. A data file is completed by the clinician and the neuropathologist for every patient. The file contains socio-demographic, clinical, radiologic, and anatomopathologic data. The Tumor Registry from Herault based in Montpellier (south of France), which is recording data files, has extensive expertise in working with tumor data and holds the required authorizations for recording nominal data. Over the first year, 4094 cases have been recorded from 51 national and private institutions in France, which includes 53% women and 47% men. Tumor localizations were supratentorial (80%), infratentorial (14%), and spinal (7%). Surgical operations were tumor resections (76%) and biopsies (24%). Histology revealed glioma (48.4%), other neuroepithelial tumors (3.7%), meningioma (32%), neurinoma (7.8%), lymphoma (3.1%), and others (5%). Detailed results will be presented during the congress. Our objective of recording all primary CNS tumor cases nationwide is long and difficult. During the first year program, the number of recorded cases has increased. Preliminary results are encouraging and stimulating for the long-term goal of creating a National Registry (based on histological data at a first step) and a National Network for patients affected by CNS-PT.

84. DESCRIPTIVE EPIDEMIOLOGY OF PRIMARY OLIGODENDROGLIOMAS IN THE UNITED STATES

J. Propp[1] and B. McCarthy[2]; [1]University of Illinois at Chicago, School of Public Health—Division of Epidemiology; [2]Central Brain Tumor Registry of the United States, Chicago, Illinois; USA

The objective of this study was to estimate the incidence, to describe temporal trends in incidence, and to estimate survival rates for primary oligodendrogliomas (OLGs) in the United States. CBTRUS compiled population-based data on all primary brain and central nervous system (CNS) tumors diagnosed between 1997 and 2001 from 15 state cancer registries. Overall and sex-, race-, and age-specific incidence rates of OLG were estimated. Age-adjusted rates were standardized to the Year 2000 U.S. standard population. For the analysis of time trends in incidence, CBTRUS compiled population-based data from 6 state cancer registries for tumors diagnosed from 1985 to 1999. Multiplicative Poisson regression was used to calculate the average annual percent change (AAPC [95% CI]) in incidence rates over the time period while controlling for age, sex, race, and microscopic confirmation, and to statistically compare trends over time. Relative survival rates for primary OLG for cases diagnosed between 1973 and 2001 in nine Surveillance, Epidemiology, and End Results (SEER) Program areas were also estimated. Tumors of interest were OLG (ICDO-3 morphology code 9450/3) and anaplastic OLG (9451/3, 9460/3). Overall incidence rate of OLG was 0.37/100 000 person-years (py) (CBTRUS, 1997–2001; N = 1585). Median age at diagnosis was 41 years. Rates were higher in males than in females (0.40 vs. 0.34/100 000 py) and in whites than in blacks (0.40 vs. 0.14/100 000 py). Rate was lowest in the 0 to19-year age group (0.08/100 000 py) and highest in the 35- to 44-year age group (0.66/100 000 py). Overall incidence rate of anaplastic OLG was 0.17/100 000 py (CBTRUS, 1997–2001; N = 738). Median age at diagnosis was 48 years. Rates were higher in males than in females (0.19 vs. 0.16/100 000 py) and in whites than in blacks (0.18 vs. 0.09/100 000 py). Rate was lowest in the 0 to 19-year and 85-year and older age groups (0.02/100 000 py) and highest in the 55- to 64-year age group (0.32/100 000 py). Incidence of OLG (N = 617) and anaplastic OLG (N = 153) increased over the time period 1985–1999 (AAPC = 6.9% [5.1%–8.8%]) and 22.3% [17.7%–27.2%]), respectively). One-, five-, and 10-year survival rates following diagnosis of OLG were 89%, 70%, and 53%, respectively (SEER; N = 1748), and for anaplastic OLG were 77%, 42%, and 30%, respectively (SEER; N = 340). Additional rates by age, sex, and race will be presented. These and further descriptive epidemiologic studies may facilitate the identification and elucidation of risk factors for these tumors.

85. RACIAL DISPARITIES IN PATIENT OUTCOME AFTER CRANIOTOMY FOR TUMORS IN THE UNITED STATES, 1988–2000

F. Barker, W. Curry, and B. Carter; Department of Neurosurgery, Massachusetts General Hospital, Boston, Massachusetts, USA

Racially based disparities in American health care are well documented. We studied disparities in outcomes after 40,101 craniotomies for brain tumors (primary tumors, metastases, meningiomas, and acoustic neuromas) in the United States, 1988–2000. The following analytical methods were used: multivariate proportional-odds ordinal logistic regression corrected for clustering by hospital; random-effects meta-analysis and bootstrapped heterogeneity estimation. Data source was the Nationwide Inpatient Sample (HCUP, AHRQ, Rockville, Maryland). Analyses adjusted for age, sex, insurance, income in zip code of residence, geographic region, admission type and source, medical comorbidity, and hospital volume of care. In-hospital mortality and adverse discharge disposition were both more likely in black patients than in others for all tumor types. Odds ratios for mortality ranged from 1.5 (primary tumors) to 6.3 (acoustic neuromas); combined mortality OR for all tumor types was 1.70 (95% confidence interval [CI], 1.4–2.1, $P < 0.001$, no heterogeneity). Odds ratios for adverse discharge disposition ranged from 1.4 (primary tumors, acoustics) to 1.5 (meningiomas); combined adverse discharge disposition OR for all tumor types was 1.41 (95% CI, 1.3–1.6, $P < 0.001$, no heterogeneity). Adjusted for primary insurance and income in zip code of residence, black patients were more likely to present as emergency cases: OR, 1.7 (primary tumors) to 4.6 (acoustic neuromas); combined OR, 2.1, significant heterogeneity detected. Medical comorbidity was more severe in black patients: OR 1.3 (metastases) to 2.1 (acoustic neuromas); combined OR, 1.6, significant heterogeneity detected. Black patients were significantly more likely to suffer adverse outcomes after tumor craniotomy in adjusted analyses. Black patients were also more likely to present as emergency admissions and to have significant medical comorbidities than patients of other races, with the largest disparities observed for benign tumors.

86. PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF POLYMORPHISMS AT THE GLUTATHIONE S-TRANSFERASE M1, M3, P1, AND T1 GENE LOCI IN HUMAN ASTROCYTOMAS

F. Ali-Osman,[1] J.E. Herndon II,[2] L. Stephenson,[1] F. Davis,[4] B. McCarthy,[4] D. Reardon,[1] A. Friedman,[1] R. McClendon,[5] H. Friedman,[1] and D.D. Bigner[1, 3]; Departments of [1]Surgery, [2]Biostatistics, and [3]Pathology, Duke University Medical Center, Durham, North Carolina; [4]Department of Epidemiology, University of Illinois, Chicago, Illinois; USA

The glutathione S-transferase (GST) family of genes encodes proteins that metabolize and inactivate a wide variety of carcinogenic and anticancer agents and thus are potential determinants of individual risk to cancer caused by these agents and of the outcome of chemotherapy. This study examines the prevalence of polymorphisms at the loci of four GST genes, namely, GSTM1, GSTM3, GSTP1, and GSTT1, as a function of age, gender, and histological grade in astrocytomas patients and the relationship of these genetic polymorphisms with patient survival. Genomic DNA from peripheral blood of 334 patients with grades 1–IV astrocytomas was used to analyze the four GST genes for deletions and/or polymorphisms. Demographic and GST genotype data were stratified by histological grade and correlated with patient survival/death. Of the 334 patients, 62% were male and 38% female. Two thirds had glioblastoma muliforme, 16% anaplastic astrocytoma, less than 10% low-grade astrocytoma. Patients of age >50 years had a 2-fold higher rate of glioblastoma compared to those under 50 years, while lower grade tumors were more prevalent in the under 50-year group. GSTM1 null and positive genotypes were present in equal proportions. Among the GSTM1+ve patients, the GSTM1A allele was 2-fold more frequent than GSTM1B, with GSTM1A/B heterozygosity in only 5%. The GSTM3 gene was deleted in 2% of the patients, and the GSTM3A allele was 4-fold more frequent than the M3B allele. The GSTP1A allele (Ile104/Val113) was present at 66%, GSTP1B (Ile104/Ala114) at 29%, and GSTP1C (Val104/Val113) at 9%. Despite its low frequency, the GSTP1C allele was 2-fold more prevalent in glioblastoma patients than in others. No relationship was observed between GSTM1 and GSTM3 polymorphisms and survival. These analyses of the GSTM1A/B alleles and the GSTM3 genes are the first to be reported for malignant gliomas. In contrast, the GSTP1C allele was associated with early death. We conclude that significant differences exist in the prevalence of GST alleles and genotypes in different histological grades of astrocytomas and that the GSTP1C and GSTT1 null genotypes are associated with decreased survival and early death in this tumor type. The findings provide novel insights into the potential significance of these polymorphisms and into the possible mechanistic role of these genes in astrocytomas and are an important contribution to efforts at defining the role of genetic polymorphisms in brain tumor etiology and prognosis. This research was supported by grants RO1 CA91438, RO1 CA79644, and P50-CA108786 from the NIH (USA).

87. IS ANDROPAUSE PROTECTIVE IN THE DEVELOPMENT OF MENINGIOMAS IN MEN?

J. Grutsch[1], C. Lis[1], J. Propp[2], B. McCarthy[2], and *F. Davis*[2]; [1]Cancer Treatment Centers of America, Zion, Illinois; [2]University of Illinois at Chicago, Epidemiology and Biostatistics, Chicago, Illinois, USA

Clinical, epidemiological, and hormonal receptor data imply a link between meningiomas and ovarian hormones. The fact that a third of meningiomas occur in men and active androgen receptors are found in nearly 70% of tumors arising in males suggests that there may be additional hormonal influences. We indirectly investigated the protective effect of andropause on the age-specific incidence of meningiomas arising within the cerebral hemispheres and spine by evaluating the relationship between age at diagnosis and meningioma. Population-based incidence data from the Central Brain Tumor Registry of the United States (CBTRUS) was obtained, and linear regression methods were applied. The rate of increase in incidence rates accelerated in males after age fifty-five. Age-specific linear regressions found that the slope of the line under age fifty-five was 2.5 and for those over age fifty-five was 3.8. Considering tumor location, there was no exponential increase in spinal meningioma rates until age 50 where, up to age 75, the slope was 5.2. The risk of male meningioma also accelerates after age fifty in the cerebral hemispheres. These male increases, rather than a female decrease, account for the declining female/male ratios in patients over 55 years of age. These results suggest the existence of multiple genetic/etiologic pathways leading to the development of meningiomas. In addition, the etiology of meningiomas may be contingent upon gender and anatomical location.

88. THE ROLE OF ENVIRONMENTAL AND HORMONAL FACTORS IN THE ETIOLOGY OF RADIATION-ASSOCIATED AND SPORADIC MENINGIOMAS

S. Sadetzki, P. Flint-Richter, and B. Oberman; Cancer and Radiation Epidemiology, Gertner Institute, Sheba Medical Center, Ramat-Gan, Israel

Current knowledge about the etiology of meningiomas is sparse, with ionizing radiation being the only well-established risk factor. The aim of this study was to comprehensively assess the contribution of environmental and hormonal risk factors in radiation-associated meningiomas (RAM) and sporadic meningiomas. Between 1948 and 1960 about 20,000 children in Israel were treated with ionizing radiation to the scalp for tinea capitis (TC). An additional unknown number of children were treated abroad. This research was designed as a nested 4-group case-control study balanced for irradiation that enabled the estimation of the main effect of each suspected risk factor as well as its interaction with radiation. The total study population included 526 subjects: 161 RAM cases that were irradiated for TC in childhood and subsequently developed meningioma, 85 sporadic cases, 145 irradiated controls, and 135 nonirradiated controls. The latter 3 groups were individually matched to the RAM cases by gender, year of birth, and continent of origin. Data on smoking, alcohol consumption, history of head trauma and asthma, parity, age at menarche and menopause, and use of exogenous hormones was collected by using a personal interview. In a multivariate analysis of risk factors in men, smoking was associated with a significant increased risk for meningioma (OR = 2.50; 95% CI, 1.19–5.27), while alcohol consumption decreased the risk (OR = 0.12; 95% CI, 0.02–0.87). For both of these factors a dose-response association was found. In women, a significant interaction between active smoking and radiation was observed. In the subgroup of nonirradiated women, smoking was associated with a decreased risk for meningioma (OR = 0.34; 95% CI, 0.13–0.88. The risk decreased with increasing smoking pack-years (*P* for trend = 0.003). This protective effect was not seen among irradiated women. In the analysis of hormonal factors, we observed a protective effect for variables that reflect a reduced exposure to endogenous and exogenous feminine hormones. This study design enabled the assessment of risk factors (other than radiation) for meningiomas and the evaluation of interactions between radiation and other determinants in the development of this tumor. The dose-response effect observed for both smoking and alcohol in men supports the causal interpretation of these results. The protective effect of smoking in nonirradiated women may be explained by the antiestrogenic effect of smoking. The negative association found between exposure to feminine hormones and meningiomas is biologically plausible, considering the higher incidence of this tumor in women and the existence of steroidal hormone receptors in the tumor tissue. As more mechanistic information on susceptibility and etiology of the disease become available, risk assessment and possible primary and secondary prevention could be considerably improved.

89. POLYMORPHISMS ASSOCIATED WITH ASTHMA ARE INVERSELY RELATED TO GLIOBLASTOMA MULTIFORME RISK

J. Schwartzbaum,[1] A. Ahlbom,[2] B. Malmer,[3] S. Lonn,[2] A. Brookes,[4] W. Debinski,[6] R. Henriksson,[3] and M. Feychting[2]; [1]Epidemiology and Biometrics, Ohio State University, Columbus, Ohio, USA; [2]Epidemiology, Karolinska Institute, IEM, Stockholm, Sweden; [3]Radiation Sciences, Umea University Hospital, Umea, Sweden; [4]Genetics, University of Leicester, Leicester, England; [5]Statistics, Ohio State University, Columbus, Ohio, USA; [6]Brain Tumor Center of Excellence, Wake Forest University, Winston-Salem, North Carolina, USA

A reduced risk of primary adult brain tumors is observed among people reporting asthma, hayfever, and other allergic conditions; however, findings may be attributable to prediagnostic effects of tumors or recall bias. To determine whether asthma and allergic condition polymorphisms are inversely related to glioblastoma multiforme (GBM) risk, we conducted a population-based case-control study of 111 GBM patients and 422 controls. We identified five single nucleotide polymorphisms (SNPS) on three genes previously associated with asthma (interleukin [IL] 4RA, IL13, ADAM33) and one gene associated with inflammation (COX2). Confirming previous literature, we found that self-reported asthma, hayfever, and eczema reduce GBM risk (e.g., asthma and hayfever at time of interview, odds ratio [OR] = 0.38, 95% confidence interval (CI), 0.18–0.83). In addition, IL4RA ser478pro TC, CC and IL4RA gln551arg AG, AA are associated with increased GBM risk (OR = 1.64; 95% CI, 1.05–2.55; and 1.61; 95% CI, 1.05–2.47) while IL13 -1112 CT, TT is associated with decreased GBM risk (0.56, 95% CI, 0.33–0.96). Each of these polymorphism-GBM associations is in the opposite direction of a corresponding polymorphism-asthma association, thereby confirming previous findings that asthmatics and people with allergic conditions have lower GBM risk than do people without these conditions. Because we used germline polymorphisms as biomarkers

for asthma and allergic conditions, our results cannot be attributed to recall bias or effects of GBM on the immune system. Our findings have implications for asthma or allergy treatments that inhibit IL13 production.

90. FEASIBILITY OF WEB-BASED VERSUS TELEPHONE INTERVIEWS AS A DATA COLLECTION METHOD IN EPIDEMIOLOGY STUDIES OF BRAIN TUMOR PATIENTS

G. Rauscher,[1] K. Rankin,[1] S. Erdal,[2] B. McCarthy,[1] and *F. Davis*[1]; [1]Epidemiology and Biostatistics, and [2]Environmental and Occupational Health Sciences, University of Illinois at Chicago, Chicago, USA

As part of a pilot study conducted for a large case-control study of neurocarcinogens, gene polymorphisms, and adult brain cancer, we have developed a Web-based data collection instrument intended to allow patients to participate at their own pace in a comfortable environment, while minimizing study data collection effort. Among 223 respondents who completed the Web-based survey and 67 respondents who completed a telephone survey, we examined characteristics associated with the choice of survey mode. We also examined the reliability of responses for 140 respondents who completed both the Web-based survey and a short Web-based resurvey, as well as the reliability of responses for 47 respondents who completed both a phone survey and phone resurvey. Compared to telephone survey participants, Web-based survey participants were less likely to be divorced or widowed (7% vs. 20%, respectively), more likely to have family incomes over 100,000 (40% vs. 23%), and less likely to have never searched the Internet (4% vs. 24%). Individuals completing the Web-based survey, however, were also slightly more likely to perceive the interview as difficult to complete (9% vs. 5%). The reliability (Kappa or Spearman rank correlations) for responses to 5 questions on history of smoking and oral contraceptive use ranged from 0.84 to 0.97 for Web responses and 0.56 to 0.92 for phone responses. Responses to questions intended for use in constructing variables for exposures to neurocarcinogens were less reliable. These included questions on living arrangements (heating sources, drinking water sources, dwelling type, living on a farm, daycare as a child), methods of food preparation, intake of fresh fruits and vegetables, and use of indoor pesticides. Reliability for the 23 exposure variables examined ranged from 0.17 to 0.78 (median, 0.54) for Web responses and from -0.02 to 1.0 (median, 0.51) for phone responses. Phone respondents answered a median of 8 of 35 questions with concordance at both surveys, whereas Web respondents answered a median of 23 of 35 questions with concordance at both surveys. Results will be presented by case and control status and controlled by age. Preliminary results suggest that a Web-based survey format is feasible in some research settings and that it has some advantages over traditional formats. However, to obtain information on all demographic subgroups and to minimize biases in studies, more than one mode of data collection may be optimal.

91. AGE-RELATED PATTERNS IN CNS TUMOR INCIDENCE WITH A FOCUS ON THE ADOLESCENT AND YOUNG ADULT POPULATION

D. Walker,[1] C. Stiller,[2] L. Ries,[3] A. Bleyer,[4] and A. Bendel[5]; [1]Children's Brain Tumour Research Centre, University of Nottingham, Nottingham, UK; [2]Childhood Cancer Research Group, Oxford, UK; [3]National Cancer Institute, Bethesda, Maryland, USA; [4]The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA; [5]Children's Hospital & Clinics, Minneapolis, Minnesota, USA

Brain tumor incidence data were analyzed from two large population-based databases, the Surveillance, Epidemiology, and End Results Program (SEER) and the Central Brain Tumor Registry of the United States (CBTRUS), to determine the incidence of brain tumors according to histological subtype and age at diagnosis. The focus of the study was to analyze incidence of CNS tumors in the adolescent and young adult (AYA) population and to look for any patterns of incidence that might explain mechanisms of tumorigenesis. Incidence of CNS tumors during 1975 to 1998 in SEER registries was determined for each 5-year age group from 0 to 44 years. SEER groups CNS tumors into broad histological categories: astrocytomas (grade I–IV), other gliomas (oligoastrocytoma and oligodendroglioma), PNET, ependymoma and germ cell tumors. Additional incidence data were obtained from the published 1995–1999 statistical report of the CBTRUS, to supplement the SEER data, and to provide age-related incidence data for specific histological subtypes, including meningioma, craniopharyngioma, mixed glioma, and each grade of astrocytoma and oligodendroglioma. Four patterns of tumor incidence were recognized from the SEER and CBTRUS databases: (1) peak incidence in the 15-to 19-year age group (germ cell tumors), (2) falling incidence with aging (grade I astrocytoma and PNET), (3) rising incidence with aging (grade II–IV astrocytoma, oligodendroglioma, oligoastrocytoma and meningioma), and (4) stable incidence with aging (craniopharyngioma and ependymoma). The pattern of age-related incidence of CNS tumors suggests that their development is driven, in part, by factors linked to the completion of the brain's growth and development, by hormones key to sexual maturation, and by factors that influence adult aging. The peak incidence of germ cell tumors in the AYA years justifies them being taken as the model tumor for AYA neuro-oncology. Their rarity justifies a worldwide clinical trial strategy building upon evidence of rising survival rates (germinoma >90%, nongerminomatous germ cell tumor >60% 10 year). Quality of survival is widely recognized as a priority, although it has not yet been selected as a primary outcome measure. We propose a worldwide, combined age approach to the investigation of CNS germ cell tumors aimed at optimizing quality of survival.

92. EPIDEMIOLOGICAL AND CYTOGENETICAL INVESTIGATIONS IN THE GROUP OF THE CHERNOBYL CLEAN-UP WORKERS WITH THE DISEASES OF NERVOUS SYSTEM

E. Diomina, *N. Ryabchenko*, E. Smekhova, and M. Diomina; Department of Radiobiology, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine

The increase of the case rate of malignant pathologies in Ukraine is significantly connected with the rise of the cancerogenic loading on the population. Special attention to the cancerogenic effects of radiation was given after the accident in Chernobyl. The purpose of the present investigation was to study the frequency of the diseases of nervous system (cancer and noncancer pathologies), peculiarities of cytogenetical effects in blood lymphocytes of clean-up workers of the Chernobyl catastrophe, and their dependence on the dose of radiation exposure. Epidemiological studies (more than 20,000 clean-up workers with documented doses of exposure) and metaphase analyses of radiation-induced chromosome aberrations in peripheral blood lymphocytes (cytogenetical investigation of 2000 clean-up workers with documented doses of irradiation) were conducted. It was established that diseases of nervous system took the first place in the structure of the development of diseases of the clean-up workers of the Chernobyl catastrophe during all years of our investigation (1990–2002). Statistically significant tendency of the increase of the diseases of nervous system (unstochastic effects) with the dose of irradiation (1 85 cGy) was observed irrespectively of the age of clean-up workers. As for neuro-oncological pathologies (stochastic effects), their highest frequency was registered in the group of the clean-up workers irradiated in a range of low doses (1–5 cGy). Dose dependence of the frequency of radiation markers (dicentric chromosomes) in the lymphocytes of peripheral blood was observed only in a group of clean-up workers with neuro-oncological pathologies, when the lack of anticancer protection of the organism was caused by the radiation injury in low doses. Cytogenetical criteria for the formation of the groups with the increased neuro-oncological risk and the improvement of the monitoring of the health status of clean-up workers were determined. Low doses of ionizing radiation are statistically significant factors of cancer risk, including development of neuro-oncological pathologies.

93. THE RISK FOR MALIGNANT PRIMARY ADULT-ONSET GLIOMA IN A LARGE, MULTI-ETHNIC, MANAGED CARE COHORT: CIGARETTE SMOKING AND OTHER LIFESTYLE BEHAVIORS

J.T. Efird,[1] G.D. Friedman,[2] S. Sidney,[3] A. Klatsky,[3] L.A. Haurel,[3] N.V. Udaltsova,[3] S. Van Den Eeden,[3] and L.M. Nelson[2]; [1]John A. Burns School of Medicine, Office of the Dean, Honolulu, Hawaii; [2]Stanford School of Medicine, Health Research and Policy, Division of Epidemiology, Palo Alto, California; [3]Kaiser Permanente Medical Care Program, Division of Research, Oakland, California; USA

The purpose of this study was to determine the risk for malignant primary adult-onset glioma (MPAG) associated with cigarette smoking and other lifestyle behaviors in a large, multi-ethnic, managed-care cohort. The study population included a cohort of 133,811 subscribers to the Kaiser Permanente Medical Care Program of Northern California (KPMCP-NC) who had received a multiphasic health checkup and questionnaire (MHC) between 1977 and 1985, were at least 25 years old at their start of follow-up, and had no prior history of benign or malignant brain tumors. In this cohort, patients were followed for up to 21 years for the development of MPAG. Risk for MPAG among women increased with increasing packs of cigarettes smoked per day (P for trend = 0.04), adjusting for cigar and pipe smoking, patient age, sex, race, education, alcohol use, and coffee consumption. A similar pattern was not observed for men. Individuals who smoked marijuana at least once a month, adjusting for cigarette smoking (packs smoked per day) and for the factors noted above, had a 2.8 (CI, 1.3–6.2)-fold increased risk for MPAG. Relative risk for MPAG increased with increasing consumption of coffee (P for trend = 0.05). Cigarette smoking was associated with an increased risk for MPAG among women but

not among men. Individuals who smoked marijuana at least once a month had an increased risk for MPAG, although no dose-response relation was observed. Drinkers of >7 cups of coffee per day had a 70% increased risk for MPAG and smaller risk elevation for lower consumption. Alcohol use was not associated with an increased risk for MPAG.

94. MATERNAL DIET DURING PREGNANCY AND ITS ASSOCIATION WITH MEDULLOBLASTOMA

G.R. Bunin,[1] L.H. Kushi,[2] P.R. Gallagher,[3] L.B. Rorke-Adams,[4] M.L. McBride,[5] and A. Cnaan[3]; [1]Oncology, Children's Hospital of Philadelphia, Philadelphia, USA; [2]Research, Kaiser Permanent, Oakland, California, USA; [3]Biostatistics and Epidemiology and [4]Pathology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; [5]Cancer Control Research Unit, British Columbia Cancer Agency, Vancouver, Canada

Fruit, vegetables, vitamin C, and folate during pregnancy have been suggested as protective factors for medulloblastoma, a common brain tumor in children. The authors sought to replicate these findings and investigate other aspects of diet. Mothers of 315 cases under age six at diagnosis and of 315 controls were interviewed about their pregnancy diet. The authors observed modest, inverse associations for fruits/juices (odds ratio [OR] for highest compared to lowest category = 0.6, 95% confidence interval [CI], 0.3–1.1) and vitamin C (OR = 0.6; 95% CI, 0.4–1.1). In contrast to the previous study, folate and vegetables showed no association. As hypothesized, cured meats were not associated with medulloblastoma, unlike previous findings for other childhood brain tumors. An inverse association with non-fresh peaches and similar fruits (OR = 0.5; 95% CI, 0.3–0.8) and a positive association with nonchocolate candy (OR = 1.7, 95% CI, 1.0–3.0) replicated previous findings. Vegetable fat (OR = 1.7; 95% CI, 1.0–2.9) and French fries (OR = 2.4, 95% CI, 1.2–4.9) were associated with medulloblastoma. The lack of association with cured meats suggests that medulloblastoma differs in etiology from astrocytoma (for which associations with cured meats have been observed in several studies). The results for vitamin C, fruit/juices, non-fresh peaches, and candy replicate previous findings for medulloblastoma and suggest that these aspects of diet are worthy of more detailed investigation.

96. ORGANOTYPIC SPHEROIDS OF GLIOBLASTOMA: A NOVEL HIGH-THROUGHPUT DRUG SCREENING SYSTEM

P. De Witt Hamer,[1] A. Van Tilborg,[1] C.J.F. Van Noorden, [2]A.H. Zwinderman,[3] D. Troost,[4] and S. Leenstra[1]; [1]Neurosurgery, [2]Cell Biology & Histology, [3]Clinical Epidemiology & Biostatistics, and [4]Neuropathology, Academic Medical Centre, Amsterdam, The Netherlands

The plethora of newly developed potential therapeutics for glioblastoma requires a test model that adequately quantifies response and is biologically valid. Historically, monolayer cultures and commercially available cell lines are used for this purpose. However, there is marked discrepancy in responses of these widely used test systems and human tumor responses, which illustrates the biological invalidity of these cell-suspension test models. This is partially explained by clonal selection and loss of intercellular cross-talk. To circumvent these problems, surgical specimens are cultured in medium as explants on the short term and grown as organotypic spheroids as previously described by Bjerkvig et al (J. Neurosurg. 72, 463, 1990). Limitations for use in drug experiments were based on (1) lack of quantitation of response using only qualitative morphological assessment, (2) heterogeneity of spheroids with undetermined implications for design of experiments, and (3) only hypothesized superior biological validity as compared with widely used and easily quantitated cell-suspension models. First, therefore, a semiautomated method has been developed that facilitates quantification of viability, proliferation, and apoptosis, and this appears to be a valid quantitative approach to determine response. Images of enzyme-histochemically and immunohistochemically stained cryostat sections of spheroids are digitally captured and analyzed using automated image cytometry. Second, the natural variance of measurements dictates the number of spheroids to be used in experiments. This is shown to be within ranges that allow for high-throughput screening. Third, to demonstrate the biological validity of the spheroid test model, DNA microarrays experiments are performed to compare the genetic profile of the original tumor with both monolayer culture and organotypic spheroids from the same material. It is hypothesized that the organotypic spheroids show good correlation with the original tumor, in contrast to the monolayer culture. In conclusion, an organotypic spheroid test system for in vitro drug screening in malignant glioma has been developed that rapidly quantifies response, allows for high-throughput screening, and appears to be superior to cell-suspension models in genetic profile as compared with the original tumor.

97. MOLECULAR PROFILING AND CHARACTERIZATION OF SYNTHETIC ASTROCYTES, DIFFERENTIATED FROM EMBRYONIC STEM CELLS IN VITRO

D. Kamnasaran[1] and *A. Guha*[1,2]; [1]Neurosurgery Division, Toronto Western Hospital; [2] Neurosurgery Division, Labatts Brain Tumor Centre, HSC, Toronto; Canada

The pluripotency of embryonic stem (ES) cells is determined by the ability to differentiate into derivatives of the three germ layers. We have determined an optimal protocol for the in vitro synthesis of astrocytes from murine ES cells harboring wild-type (wt) and p53 (±) genetic backgrounds and undertaken expression and tumorigenicity studies. Astrocytic differentiation of the ES cells, with GFAP positive and negative OLIG2, NESTIN plus OCT2 expression, was shown in more than 90% of cells. RT-PCR for these and other neural-glial lineage markers further supported that these were synthetic derived astrocytes from the ES cells. The majority of the synthetic astrocytes from wt-ES cells displayed morphology similar to Type-1 astrocytes. cDNA microarray analyses on Affymetrix arrays were performed. After normalizing and scaling of the data, the transcriptomes of synthetic astrocytes with wt or p53 (±) genetic backgrounds were found to be very similar ($r = 0.89$). However, several genes were differentially expressed ($P < 0.001$), most likely representing p53 response genes. For some of these differentially expressed genes, verification was obtained by real-time PCR. The transcriptomes of the synthetic astrocytes were compared to the published transcriptomes of in vivo astrocyte cultures established from various murine embryonic, postnatal, and adult brain sections (PNAS 101, 8384). We calculated the extent of similarity (Pearson's correlation coefficient (r), $P < 0.001$) with possible astrocyte specific genes (~325 transcripts) identified by having an expression pattern similar to GFAP, using a recursive-supervised machine (R-SVM) class prediction analysis. Wt- synthetic astrocytes were found most similar to astrocytes from the adult corpus callosum ($r = 0.281$), while synthetic p53 (±) astrocytes were most similar to astrocytes from the cortex (P2) ($r = 0.212$). By comparison, using 156 astrocyte-specific markers identified by R-SVM analysis, both wt- and p53 (±) synthetic astrocytes were found to be most similar to astrocytes from the cortex (P2) ($r = 0.287$, $r = 0.315$). Finally, both wt- and p53 (±) synthetic astrocytes were tested for in vivo intracranial transformation in Nod-Scid mice. Intracranial injections into these mice with wt- or p53 (±) ES cells resulted in teratomas within 3 to 5 weeks. No tumors developed with wt- or p53 (±) synthetic astrocytes. Study is ongoing with synthetic derived astrocytes from ES cells, of varying glioma relevant genetic backgrounds, which we believe provide a relatively quick bioassay to understand the roles of these genetic alterations in glial differentiation and transformation.

98. REGIONAL BRAIN SPHEROID CULTURE: AN IN VITRO 3D MODEL TO STUDY SPECIFICITY OF TUMOUR INVASION

T.L. Parker,[1] K. Searle,[1] L. Storer,[2] and D. Walker[2]; [1]Institute of Neuroscience, School of Biomedical Sciences, University of Nottingham Medical School, Queens Medical Centre, Nottingham; [2]Division of Child Health, University of Nottingham, Nottingham; UK

Dimensionality plays a major role in cellular behavior, and in vitro tumor invasion studies have been hampered by the lack of suitable 3D models of tumor/host brain interaction. Allowing enzyme dissociated cells to reform into 3D spheroids has provided the means of developing such a model. Spheroid cultures of enzymatically dissociated fetal rat cerebellum, brainstem, and cerebral cortex (E18) were morphologically and immunohistochemically characterized at 3, 5, and 10 days in vitro. Comparisons between 3D spheroids, 2D monolayer, and undissociated brain tissue demonstrate that spheroid cultures begin to differentiate and express neurofilament proteins and glial fibrillary associated proteins (GFAPs) in addition to forming synaptic contacts and extracellular collagen. Neural cells appear to differentiate at a higher rate than glial. The developmental profile of neurons and glia in the 3D regional brain spheroids mimics the in vivo cytoarchitecture to a greater extent than 2D brain cell cultures. The level of GFAP immunoreactivity (IR) is significantly greater than neurofilament (NF) immunoreactivity (IR) in all monolayer cultures (50%), compared to 8.3% NF in vitro. Levels do not significantly change over time in vitro. NF IR reaches 97% and GFAP 65%. Adult rat brain tissue has a significantly greater IR to NF (88%) than GFAP (51%). Therefore, the ratio of NF:GFAP IR in regional brain spheroids approaches that found in adult rat tissue. The 3D spheroid microenvironment results in cellular selection that more closely reflects adult tissue cellular composition. By using a 3D spheroid co-culture model of host brain/Daoy cells (medulloblastoma cell line), it was found that the Daoy cells preferentially invaded cerebellum and brainstem, and not the cerebral cortex, possibly reflecting differing regional cellular microenvironments. Therefore, this 3D model appears to mimic in situ medulloblastoma behavior. The viability and reproducibility of these regional brain spheroid cultures make them a useful tool for further inves-

tigations into developmental biology, neurotoxicity, and antitumor therapy in different brain regions.

99. HEMATOPOIETIC STEM CELLS HOME TO MALIGNANT GLIOMAS

G. Tabatabai,[1] O. Baehr,[1] R. Moehle,[2] I. Eyuepoglu,[3] A. Boehmler,[2] J. Wischhusen,[1] J. Rieger,[1] I. Bluemcke,[4] M. Weller,[1] and W. Wick[1]; [1]Laboratory of Molecular Neuro-Oncology, Department of General Neurology and Hertie Institute for Clinical Brain Research, Tuebingen; [2]Department of Internal Medicine II, University of Tuebingen, Tuebingen; [3]Departments of Neurosurgery and [4]Neuropathology, University of Erlangen, Erlangen; Germany

Stem and progenitor cells (PCs) of various lineages have become attractive vehicles to improve therapeutic gene delivery to cancers, notably glioblastoma. Here we report that adult human and murine hematopoietic PCs display a tropism for intracerebral gliomas but not for normal brain tissue in mice. Organotypic hippocampal slice culture and spheroid confrontation assays confirm a directed PC migration toward glioma cells ex vivo and in vitro. RNA interference-mediated disruption of transforming growth factor beta (TGF-b) synthesis by the glioma cells strongly inhibits PC cell migration. We delineate a CXC chemokine ligand (CXCL) 12–dependent pathway of TGF-b-induced PC migration that is facilitated by MMP-9-mediated cleavage of stem cell factor (SCF). Moreover, neutralizing antibodies to CXCL12 strongly reduce PC homing to experimental gliomas in vivo. Thus, we define here the molecular mechanism underlying the glioma tropism of the probably most easily accessible PC population suitable for cancer therapy, that is, adult hematopoietic PC. This work was supported by the Landesstiftung Baden-Württemberg, State of Baden-Württemberg, Germany (P-LS-AS/HSPA7-12).

100. ACTIVATION OF PROTEOLYTIC PATHWAYS BY POLYPHENOLIC PHYTOCHEMICALS FOR APOPTOSIS IN HUMAN GLIOBLASTOMA T98G CELLS

S. Ray,[1] A. Das,[1] S. Karmakar,[1] S. Patel,[2] and N. Banik[1]; Departments of [1]Neurology and [2]Neurosurgery, Medical University of South Carolina, Charleston, South Carolina, USA

Glioblastoma, the deadliest and most prevalent human brain tumor, still remains refractory to almost all conventional chemotherapeutic agents. Therefore, alternative and innovative therapeutic strategies need to be developed for treating this deadly disease in humans. Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers. Flavonoids such as apigenin (APG), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG), and genistein (GST) are polyphenolic phytochemicals, which ubiquitously occur in fruits and vegetables. Flavonoids have been shown to suppress proliferation of various cancer cells, inhibit growth factor signaling pathways, suppress expression of antiapoptotic proteins, and also induce apoptosis, indicating that they may have untapped therapeutic value. Very recently, these phytochemicals have also been found to reverse both radioresistance and chemoresistance in patients undergoing glioblastoma treatment. In the current investigation, we have examined the molecular events relating to mitochondria-mediated apoptosis in human glioblastoma T98G cells following exposure to APG, EGC, EGCG, and GST. We used trypan blue dye exclusion test for assessing cell viability, Wright staining for determining distinct morphological characteristics of apoptosis, modified version of the telomerase repeat amplification protocol (TRAP) assay for detecting telomerase activity, fura-2 assay for quantifying intracellular free [Ca^{2+}], Western blottings for examination of expression of specific proteins, and also a colorimetric assay for estimating caspase-3 activity in T98G cells following exposure to the flavonoids. Trypan blue dye exclusion test showed that the number of viable T98G cells was decreased in a dose-dependent manner when T98G cells were exposed to these phytochemicals. Wright staining indicated predominantly apoptotic morphology in T98G cells exposed to 100 mM APG, 50 mM EGC, 50 mM EGCG, or 100 mM GST, for 24 h. We applied a modified version of the TRAP assay to phytochemical treated T98G cells to determine any downregulation in the activity of telomerase, an enzyme responsible for lending an unlimited capability of proliferation to the cancers including glioblastoma. Apoptosis in T98G cells following exposure to these phytochemicals was associated with an increase in intracellular free [Ca^{2+}], as determined by fura-2 assay. Western blot analyses showed an increase in Bax:Bcl-2 ratio, as well as overexpression of calpain and caspase-3 that were also activated to cleave 270 kD a-spectrin at specific sites for generation of 145 kD spectrin breakdown product (SBDP) and 120 kD SBDP, respectively. Further, colorimetric assay using a synthetic substrate specific for caspase-3 confirmed activation of caspase-3 in apoptotic T98G cells following exposure to 100 mM APG, 50 mM EGC, 50 mM EGCG, or 100 mM GST for 24 h. Taken together, these results strongly suggest that selective polyphenolic phytochemicals can be used for activation of proteolytic activities of calpain and caspase-3 for apoptosis in human glioblastoma T98G cells. This investigation was supported in part by the R01 grants from the NCI and NINDS of the NIH and also a grant from the State of South Carolina.

101. COMBINATION OF HISTONE DEACETYLASE INHIBITORS AND RADIATION OFFERS A PROMISING THERAPY FOR NEUROBLASTOMA

K. Hovinga,[1] M. Entin-Meer,[1] X. Yang,[1] N. Jelluma,[1] J. Chen,[1] W. Weiss,[2] L. Chesler,[2] A. Nudelman,[3] A. Rephaeli,[3] and D. Haas-Kogan[1]; [1]Department of Radiation Oncology and Comprehensive Cancer Center and [2]Department of Neurology, Pediatrics, and Neurological Surgery, University of California San Francisco, San Francisco, California, USA; [3]Pharmacology and Experimental Oncology, Felstein Medical Research Center, Tel Aviv, Israel

Neuroblastoma, a malignancy arising from neural crest cells, exhibits resistance to current therapies. HDAC inhibitors have emerged as exciting new cancer therapies and have demonstrated antineoplastic effects against neuroblastoma. Pivaloyloxymethyl butyrate (AN-9, Pivanex) was derived from the HDACI butyric acid (BA) and shows more efficient delivery and higher intracellular concentrations in comparison with BA. We sought to test the combination of the AN-9 and radiation in the treatment of neuroblastoma in vitro. The neuroblastoma cell line SHEP21, constructed to contain inducible MYCN, was treated with different doses of radiation and AN-9. Effects on cell viability and apoptosis of AN-9, radiation. and combinations thereof were tested in high and low Mycn-expressing cells by using MTS assays and FACS analyses. The effect of AN-9 on the Mycn expression was examined by Western blot. We also addressed the influence of the order of administration of AN-9 and radiation on antineoplastic activity. Consistent with published literature, AN-9 inhibited Mycn expression. Combinations of AN-9 and radiation were more effective than either treatment alone. The combined effect was additive in MTS cell viability assays. FACS analyses demonstrated that high-Mycn-expressing cells were markedly more susceptible to apoptosis than their low-Mycn-expressing counterparts. The order of administration significantly influenced antineoplastic efficacy, as exposure to AN-9 after radiation was more effective than the reverse order. AN-9 and radiation exhibit additive antineoplastic effects in neuroblastoma, a promising clinical approach, as these two therapies have nonoverlapping toxicities. Although high-Mycn-expressing neuroblastoma cells were much more sensitive to radiation-induced apoptosis, AN-9 inhibited Mycn expression. It follows that delivering radiation prior to AN-9 would maximize the efficacy of combined HDACI and radiation treatments. Combination therapy using HDACIs and radiation represents an exciting new therapeutic approach to neuroblastoma.

102. A NOVEL THREE-DIMENSIONAL "ALL HUMAN" IN VITRO BRAIN TUMOR INVASION MODEL

S.A. Murray,[1] H.K. Rooprai,[1] R. Selway,[2] and G.J. Pilkington[1]; [1]Cellular & Molecular Neuro-oncology Group, School of Pharmacy & Biomedical Sciences, University of Portsmouth, Portsmouth; [2]Department of Neurosurgery, Kings College Hospital, Denmark Hill, London; UK

In order to study brain tumor invasion in vitro, previous three-dimensional model systems have utilized pre-cultured chick heart fragment and rat brain as targets in co-culture with human brain tumor. We have now succeeded in establishing a novel three-dimensional model whereby human neoplastic glial cell spheroids are juxtaposed with spheroids derived from non-neoplastic human brain tissue. Spheroids are developed from either established glioma-derived cell lines or from primary cultures of glioma biopsy as well as brain resected from patients who have undergone surgery for epilepsy, short-term astrocyte-rich cell cultures derived therefrom. Using the "hanging drop" method, 45,000 cells were inversely suspended from a petri dish in 20 ml of DMEM. After either 24 h (fast-growing cells) or 48 h (slower growing cells) in their gravity pools, spheroids are transferred to agar-coated petri dishes for a further 24 h prior to confrontation. Cells were tracked with the use of fluorescent cell trackers; progress was recorded every 3 days for up to 15 days. Fluorescent cell trackers were also employed for time-lapse video microscopy in the most invasive combination over 5 days. We are further developing the model in utilizing human serum instead of fetal calf serum, which has been shown to alter antigenic expression and growth rate of human glioma cells in two-dimensional cultures within our laboratories. The effects of various agents that may impede local brain tumor invasion are currently under investigation. To these ends we are utilizing scanning and transmission electron microscopy, confocal microscopy, total internal reflected fluorescence (TIRF) microscopy, and

live cell imaging utilizing Improvision Velocity and Openlab software on a Zeiss inverted axiovert 200M microscope. This work was supported by the Dr Hadwen Trust

103. AN IN VITRO DEMONSTRATION OF SYNERGISTIC GLIOMA CELL KILL BY THE SELECTIVELY REPLICATION COMPETENT ICP34.5-NULL HSV MUTANT, HSV1716, AND IONIZING RADIATION

S. Harrow,[1] R. Rampling,[1] A. Mace,[2] J. Plumb,[3] S. Brown,[2] and J. Harland[2]; [1]Clinical Oncology, [2]Neurology, [3]Medical Oncology, The University of Glasgow, Glasgow, Scotland, UK

Here we demonstrate, in vitro, in the human glioma cell line U373, a synergistic relationship between cell kill from the oncolytic ICP34.5 null herpes simplex virus mutant, HSV1716, and ionizing radiation. Additive cell kill is shown in another human glioma cell line, MOG. Cell kill from HSV1716 and ionizing radiation was investigated in vitro using the MTS assay, a colorimetric method of determining the number of viable cells. HSV1716 and ionizing radiation were combined to determine cell kill in the human glioma MOG and U373 cell lines. Experiments combining HSV1716 and ionizing radiation indicated additional cell kill in U373 cells by day 6 compared to either modality in isolation. Isobologram analysis confirmed a synergistic relationship between ionizing radiation and HSV1716 when the U373 cells were irradiated one hour prior to virus inoculation. In the MOG cell line the relationship in terms of cell kill appears to be additive. Phase 1 studies in glioma patients have failed to demonstrate toxicity when HSV1716 is injected into tumor or normal brain, nor when they proceeded to receive further immunosuppressive treatment in the form of ionizing radiation or chemotherapy. This supports the use of HSV1716 in conjunction with standard therapies but leaves open the question as to whether combined therapy is likely to be additive or synergistic. Contradictory data have been published on the possible synergy between ICP34.5 null HSV and ionizing radiation in cell kill. In general, although the relationship between the two modalities is dependent on factors such as the HSV mutant used, the target cell type, and the scheduling of the virus and radiation treatments, the in vivo data indicate that ICP34.5 null HSV and ionizing radiation act synergistically in tumor cell kill. However, to our knowledge, there has been no published data to date demonstrating synergy between the two modalities in vitro. It has been proposed that the additional cell kill demonstrated by various oncolytic HSV null mutants when combined with radiation is due to the upregulation by radiation of certain cellular proteins such as mammalian ribonucleotide reductase or GADD34, which complement the missing gene in the attenuated virus. Multicycle growth experiments showed that HSV1716 fails to replicate as efficiently as wild-type HSV in U373 cells. However, pre-irradiating U373 cells failed to result in enhanced viral replication, suggesting that increased viral replication might not be the reason for the increased cell kill. The demonstration of a synergistic relationship between ionizing radiation and HSV1716 in vitro is of interest if these modalities are to be combined in clinical practice and justifies further investigation of the mechanisms involved.

104. PHYSICAL AND NUMERICAL MODELING CEREBROSPINAL FLUID BEHAVIOR FOR DRUG DELIVERY TO THE LEPTOMENINGES

D. Walker, S. Ammourah, A. Aroussi, M. Garnett, D. Giddings, L. Howden, H. Power, and M. Vloeberghs; Children's Brain Tumor Research Centre, University of Nottingham, Nottingham, UK

Leptomeningeal dissemination (LMD) of tumor is a high risk in hematological malignancies, primary brain tumors and parameningeal tumors of childhood and adolescence, justifying either prophylactic or radical CNS directed chemoradiotherapy. In leukemias, combined systemic and intrathecal therapy has replaced cranial radiotherapy as prophylaxis; in primary brain tumors, reducing doses of craniospinal or focal radiotherapy in combination with systemic chemotherapy is being explored. In this latter group, intrathecal therapy has not been extensively studied because of uncertainties of drug selection and delivery. The purpose of this cross-disciplinary study at the University of Nottingham is to reconsider the design of intrathecal drug delivery methods for prevention of LMD. An MRI scan of the entire CNS was obtained and data used to create 3D models of the ventricles and subarachnoid space (SAS). Work by Aroussi and Vloeberghs has provided information about the flow in the cerebral aqueduct and likely behavior of drugs injected into cerebral ventricles. Current research is preparing increasingly realistic physical models of the CNS fluid flows, and CFD models using commercial engineering packages (Fluent). The existing physical model is constructed from thin-walled acrylic sheet; it contains the ventricles, a simplified brain surface, and spinal cord and the boundary of the spine and skull. It is suspended in a transparent tank on a mobile frame allowing rotation of the model and visual inspection of flow tracers. The CFD model solves fluid flow equations and can be programmed to induce motion of the model boundaries, thus allowing investigation of arterial pulsation of the brain and respiratory effects in the spine. For effective intrathecal delivery, the flow induced by CSF production alone is not sufficient, and the flow emitted from the foramina of Lushka and Magendie is transported mainly around the cranial SAS; diffusion is much less than the likely convection of fluid from the foramina to the arachnoid villi. Computational work shows flow patterns that may be produced by pulsations driven by physiology exterior to the SAS. Experimenting with models is a relatively cheap technique for illuminating the likely behavior of highly complex fluid flow in this nearly inaccessible region of the human body. The models will provide insight into current drug delivery behavior and potential new delivery methods.

105. LACTACYSTIN EXHIBITS POTENT ANTITUMOR ACTIVITY IN AN ANIMAL MODEL OF MALIGNANT GLIOMA WHEN ADMINISTERED VIA CONTROLLED-RELEASE POLYMERS

F. Legnani,[1] G. Pradilla,[2] Q. Thai,[2] B. Tyler,[2] G. Zamora,[1] S. Gaini,[3] F. DiMeco,[1] H. Brem,[2] and A. Olivi[2]; [1]Department of Neurosurgery, Istituto Neurologico C. Besta, Milan, Italy; [2]Department of Neurosurgery, Johns Hopkins University, Baltimore, Maryland, USA; [3]Università degli Studi di Milano, Neurosurgery, Milan, Italy

Lactacystin, a proteasome-inhibitor, has been shown to induce apoptosis of experimental gliomas in vitro. However, its systemic toxicity prevents further clinical use. To circumvent this problem, lactacystin can be delivered locally to the tumor site. We tested the efficacy of lactacystin incorporated into controlled-release polymers for treating experimental gliomas in vitro and in vivo. 9L-Gliosarcoma and F98-glioma cell lines were treated with lactacystin (10–100 μg/ml) for 72 h in vitro. Cell viability was measured with MTT assays. The toxicity of lactacystin/polycarboxyphenoxypropane-sebacic-acid (pCPP:SA) polymers was established in vivo by using Fischer-344 rats that were intracranially implanted with lactacystin polymers loaded from 0.1% up to 2% lactacystin by weight (w:w). The efficacy of 1, 1.3, 1.5 and 1.7% lactacystin/pCPP:SA-polymers was determined in Fischer-344 rats intracranially challenged with 9L and treated either simultaneously or 5 days after tumor implantation. Lactacystin was cytotoxic in 9L cells, causing a 16 ± 8% ml that increased to 78 ± 4% at 100 μg/ml. Similarly, lactacystin inhibited growth of F98 by 18 ± 8% at 10 μg/ml and 74 ± 2% at 100 μg/ml in vitro. Polymers released lactacystin for 21 days and intracranial implantation in rats did not generate local nor systemic toxicity at doses lower than 2% (w:w). Treatment with lactacystin/pCPP: SA polymers with loading concentrations of 1.0, 1.3, and 1.5% prolonged survival of animals intracranially challenged with 9L when polymers were inserted in the day of tumor implantation. Lactacystin exhibits potent cytotoxic-activity against 9L and F98 in vitro. Furthermore, lactacystin can be efficiently incorporated and delivered using controlled-release polymers, and at the proposed concentrations, lactacystin polymers are safe for CNS delivery and prolong survival in the 9L model. These findings support the role of proteasome inhibitors in the treatment of malignant gliomas when administered using local drug delivery.

106. GENETIC IDENTIFICATION OF GLIOBLASTOMA MAINTAINING CELLS IN A XENOGRAFT MODEL

C. Glanz,[1] J. Rebetz,[1,2,4] Y. Stewénius,[3] D. Gisselsson,[3] A. Darabi,[1] E. Englund,[5] L.G. Salford,[1] B. Widegren,[1,2,4] and *X. Fan*[1,2,6]; [1]The Rausing Laboratory, Division of Neurosurgery, Lund University Hospital, Lund; [2]Section of Immunology, [3]Department of Clinical Genetics, [4]Division of Genetics, Department of Cell and Molecular Biology, [5]Department of Pathology, [6]Section of Molecular Medicine and Gene Therapy, Lund Strategic Research Center for Stem Cell Biology and Cell Therapy, Lund University, Lund; Sweden

Glioblastoma (GBM) can consist of heterogeneous cell populations. A small CD133+ stem cell–like population has been demonstrated to be responsible for GBM maintenance. We have developed a serial transplantable xenograft GBM model to characterize the genetic background and constitutive signaling pathways in GBM maintaining cells. Freshly isolated GBM cells from a 47-year-old female patient at recurrence were subcutaneously injected into SCID-Beige mice. Xenograft GBM formed in initial SCID-Beige mice were serially transplanted in SCID-Beige or NOD/SCID mice without cell sorting for up to 4 passages. Freshly isolated GBM cells and its xenograft cells at different passages were analyzed for cell surface marker expression. Similar patterns of heterogeneous cell populations were observed both in the GBM and its xenografts at all passages. CD44 and epidermal growth factor receptor were expressed in 90% of the fresh GBM and the xenograft GBM cells. The stem cell marker CD133 was expressed in 70% of the primary GBM and about 30% of the xenograft GBM cells,

the platelet-derived growth factor receptor-a (PDGFRa) in 34% of the primary GBM and about 20% of the xenograft GBM cells. However, the immature neural ganglioside recognized by A2B5 was detected in 63% of the primary GBM but not in the xenograft GBM cells. The same pattern of heterogeneous cell populations was maintained in all passages of xenograft GBM, suggesting that the GBM cell population hierarchy is to a great extent maintained in this xenograft model and that a fraction of GBM cells were capable of maintaining the xenograft GBM by self-renewal. G-band karyotyping showed that the primary GBM cells exhibited great intercellular heterogeneity with a wide variety of related subclones, all of which showed complex karyotypes, including several whole-chromosome losses and unbalanced translocations. In contrast, late passage xenograft cells showed almost no intercellular variation and exhibited a complex karyotype almost identical to one of the original GBM subclones, identified in 3 out of 25 cells in the primary GBM cells. This indicates that most subclones in the primary GBM did not contribute to the maintenance of xenograft GBM. Interestingly, the PDGFRa expression in the in vitro cultured xenograft GBM cells was specifically downregulated by a 6-day cyclopamine treatment in a dose-dependent manner. Concomitantly, a 2.5-fold reduction of cell proliferation ($P < 0.01$, n = 3) was observed at 10 mM cyclopamine treatment. Thus, constitutively active sonic hedgehog signaling critically contributes to GBM cell proliferation via PDGFRa expression in this tumor system. Taken together, our data suggest that even though GBMs consist of heterogeneous cell populations, only a small fraction of these cells is responsible for tumor cell regeneration

107. COMPUTERIZED TIME-LAPSE MICROSCOPY OF HUMAN GLIOMA INVASION IN ORGANOTYPIC BRAIN SLICE CULTURES

B.W. Kristensen,[1] C. Bonde,[3] K. Eriksen,[1] C. Andersen,[2] H.D. Schrøder,[1] and J. Zimmer[3]; [1]Institute of Pathology and [2]Department of Neurosurgery, Odense University Hospital, Odense; [3]Department of Anatomy and Neurobiology, University of Southern Denmark, Odense; Denmark

The purpose of this study was to establish a computerized time-lapse microscopy assay in order to monitor and characterize the growth of human gliomas in organotypic brain slice cultures. Recent results have shown that human glioma invasion in such cultures correlates with the histological typing of the gliomas, suggesting that this model might be of considerable value in studying the mechanisms of brain tumor invasion. Human glioma biopsy specimens were produced from freshly resected grade II–IV gliomas and incubated with the fluorescent dye DiI for live cell labeling. Then the specimens were implanted in organotypic rat brain slice cultures grown on semiporous membranes for 1 to 2 weeks according to standard protocols. The slice cultures with implanted glioma biopsy specimens (n = 5–10 per case) were thereafter transferred for time-lapse fluorescence microscopy by using an automatic inverted fluorescence microscope placed in a specially constructed CO_2 incubator. Controlled by a computer program developed locally by Bonde et al., the cultures were photographed by fluorescence and phase contrast microscopy every 30 min for up to 2 weeks, only interrupted by change of culture medium. Larger series of slice cultures with implanted glioma tissue (n = 12–36 per case) were photographed by conventional inverted fluorescence and phase contrast microscopy with 4- to 6-day intervals during 2 weeks. The results show that the invasion of human glioma cells into organotypic brain slice cultures can be monitored for up to 2 weeks by conventional and time-lapse microscopy in the present setup, demonstrating how both glioma cell density and distance of invasion increased with time. In contrast, when glioma biopsy specimens were placed directly on semiporous membranes, no outgrowth of glioma cells on the membrane was seen. The present study shows that computerized time-lapse microscopy of fluorescently labeled glioma cells implanted in rat brain slice cultures provides a model for monitoring quantitative and qualitative growth characteristics. This work was supported by the Danish MRC, the Danish Cancer Research Foundation, Foundation in the memory of Alice Brenå, Grant in the memory of Einar Willumsen, Anniversary Grant of King Christian IX and Queen Louise, King Christian X Foundation, Simon Foughner Hartmanns Foundation and EU 5th FP (QLK3-CT-2001-00407).

108. HUMAN GLIOBLASTOMA T98G CELLS XENOGRAFTED IN ATHYMIC NUDE MICE OVERWHELMINGLY COMMITTED APOPTOSIS AFTER TREATMENT WITH ALL-*TRANS*-RETINOIC ACID AND TAXOL

S. Ray,[1] S. Karmakar,[1] A. Das,[1] N. Banik,[1] and S. Patel[2]; [1]Departments of Neurology and [2]Neurosurgery, Medical University of South Carolina, Charleston, South Carolina, USA

Glioblastoma is the most prevalent and highly malignant brain tumor in humans. It is not yet amenable to any currently available therapeutic strategy and thus remains as a challenge both to basic scientists and to physicians. Therefore, new therapeutic approaches need to be explored for effective treatment of glioblastoma. We used all-*trans*-retinoic acid (ATRA) and taxol (TXL) alone and also in combination for controlling the growth of human malignant glioblastoma T98G cells xenografted in athymic nude mice. For xenotransplantation of glioblastoma, 6 week-old athymic nude mice (Charles Rivers) were subcutaneously (sc) injected with a (1:1) mixture of exponentially growing T98G (6 million cells/mouse) and Matrigel. Animals with 3-week-old glioblastoma xenografts were randomly divided into 4 groups: control, ATRA, TXL, and ATRA plus TXL. Animals in the control group did not receive any therapy. Each animal in the other groups received intraperitoneally (ip) a daily dose of ATRA (1.5 μg/kg), or TXL (45 μg/kg), or ATRA (1.5 μg/kg) plus 4 h later TXL (45 μg/kg) for 7 days. Histopathological and biochemical experiments were conducted to evaluate the efficacy of different treatments. Histopathological examination of H&E stained tumor sections revealed that control tumors maintained characteristic growth of glioblastoma, ATRA alone inhibited tumor cell proliferation and caused astrocytic differentiation, TXL alone induced apoptosis to some extent, and ATRA plus TXL caused significant amounts of apoptosis in differentiated cells. Differentiation of cells was associated with the downregulation of telomerase activity. In situ immunofluorescent labeling showed calpain overexpression in apoptotic cells, suggesting a role for calpain in mediation of apoptosis. Further, in situ TUNEL and double immunofluorescent labeling confirmed cell death with an increase in calpain expression in tumor sections treated with TXL, or ATRA plus TXL. Western blot analyses showed changes in expression of Bax and Bcl-2 proteins leading to increased Bax:Bcl-2 ratio, cytochrome c release from mitochondria, activation of calpain and caspase-3 for degradation of 270 kD α-spectrin at the specific sites to generate 145 kD spectrin breakdown product (SBDP) and 120 kD SBDP, respectively, in the course of apoptosis. Our investigation in an animal model revealed that treatment of xenografted glioblastoma with ATRA plus TXL induced differentiation and apoptosis for controlling malignant growth.

109. INTERACTIONS OF NEURAL STEM CELLS AND GLIOMA CELLS IN A THREE-DIMENSIONAL ORGANOTYPIC BRAIN SLICE CO-CULTURE SYSTEM

O. Heese, D. Zirkel, M. Westphal, and K. Lamszus; University Hospital Hamburg-Eppendorf, Neurosurgery, Hamburg, Germany

Various in vivo studies have demonstrated a migration tendency of neural stem cells (NSCs) toward intracranial gliomas, making these cells a potential carrier for delivery of therapeutic genes to disseminated gliomas. We analyzed NSC migration in response to glioma stimuli in an organotypic brain slice model in order to mimic the in vivo microenvironment including the three-dimensional architecture of murine brain tissue. The chemotactic effects of 5 glioma cell lines and their responding conditioned media on the murine NSC line C17.2 in a co-culture organotypic brain slice system were assessed. NSCs and glioma cells were identified inside the standardized murine brain slice by pre-implantation staining with DiI and DiO. Migration of NSCs and glioma cells inside the brain slices was characterized and quantified by using a confocal laser microscope on days 2, 6, and 12. C17.2 NSCs migrate inside the brain slices and seem to follow preserved anatomic structures. Migration of the NSCs was modified by conditioned media of glioma cells. Conditioned media of two glioma cell lines augmented migration of NSCs up to 50% compared to controls. In two gliomas, conditioned media stimulation was only moderate (20%). Conditioned media of one cell line produced inhibition of NSC migration. Co-culturing of NSCs and glioma cells inside the brain slice resulted in a directed migration of both cell types toward each other in 3 of 5 glioma cell lines. The organotypic brain slice model displays several advantages over less complex in vitro migration models since a physiologic microenvironment of brain tissue and a three-dimensional architecture are preserved. Migration of NSCs toward gliomas in our assay system seems to depend on individual phenotypic characteristics and growth factor release patterns of the target tissue.

110. INTERSTITIAL PHOTODYNAMIC THERAPY OF RECURRENT MALIGNANT GLIOMAS USING 5-AMINOLEVULINIC ACID (5-ALA)

J. Mehrkens,[1] W. Stummer,[3] T. Beck,[2] J. Tonn,[1] and F. Kreth[1]; [1]Department of Neurosurgery and [2]Laser-Research-Laboratory, Ludwig-Maximilians-Universität, München; [3]Department of Neurosurgery, Heinrich-Heine-University, Dusseldorf; Germany

Photodynamic therapy (PDT) might have the potential to improve local tumor control in selected patients: In PDT a photosensitizer (PS) is transferred selectively in tumor cells and activated by light of an appropriate wavelength, which leads to cytotoxic reactions. However, uncertainties concerning dosimetry and PS-distribution as well as therapy-related side effects have limited the clinical impact of PDT. These disadvantages might be overcome by the concept of stereotactic interstitial PDT (iPDT) using 5-ALA as PS. iPDT was considered to be indicated for patients with a minimum Karnofsky performance status (KPS) of 70 with a "circumscribed" recurrence of a malignant glioma after prior multimodal therapy with a maximum diameter of 3 cm. All operations were performed under general anesthesia. Patients received 20 mg/kg 5-ALA 1 h preoperatively. After tumor histology had been verified by stereotactic biopsy, 3D-multimodal-treatmentplanning (CT, MRT, FET-PET) followed by stereotactic implantation of up to 6 laser-probes was performed. Irradiation time was 60 min (Ceralas PDT Diode Laser: wavelength 633 nm, power 200 mW/cm [BioLitec AG, Jena, Germany]). Therapy effects were documented by MRI (T1, T2 ± contrast) 24 h, 4 weeks, and then in 3 month-intervals postoperatively. Between October 2002 and December 2003, 10 adult patients (mean age 54, range 31–72 years; mean tumor volume 7.9 ml, range 2.1–26 ml) were included. The applied volume-dose was in the range of 1000 to 1500 J/cm^3. Early MRI follow-up within 24 h post-treatment showed a complete resolution of the contrast-enhanced lesion in 7 patients and a partial response in the other 3 patients. There was no enhanced treatment–induced brain edema. Thus, steroids were only applied for 3 days, as routinely done in other stereotactic procedures in our institution. The estimated 1-year survival rate was 70% (3 patients had died on last follow-up). There was no surgery- or treatment-related morbidity or mortality. Stereotactic iPDT using 5-ALA is a minimally invasive and low-risk therapy. The multimodal 3-D treatment planning for the first time allows for an exact three-dimensional dosimetry and irradiation of a defined tumor volume.

111. HSV1716 INJECTION INTO BRAIN ADJACENT TO TUMOR FOLLOWING SURGICAL RESECTION OF HIGH-GRADE GLIOMA: SAFETY DATA AND LONG-TERM SURVIVAL

S. Harrow,[1] V. Papanastassiou,[2] J. Harland,[3] R. Mabbs,[5] R. Petty,[3] M. Fraser,[2] D. Hadley,[4] J. Patterson,[6] S. Brown,[5] and R. Rampling[1]; [1]Beatson Oncology Centre, Clinical Oncology, and Departments of [2]Neurosurgery, [3]Neurology, and [4]Neuroradiology, The University of Glasgow, Glasgow, Scotland; [5]Cruscade Laboratories Ltd., Glasgow, Scotland, [6]Clinical Physics, South Glasgow University Hospitals, Glasgow, Scotland; UK

In this study the authors have demonstrated that herpes simples virus 1716 (HSV1716) is safe following injection into brain adjacent to excised high-grade glioma, in patients who proceeded to receive further immunosuppressive radiotherapy or chemotherapy. Twelve patients, six with recurrent disease and six with newly diagnosed high-grade glioma, underwent maximal resection of the tumor. Following resection, 1 ml of 10^5 pfu of HSV1716 was injected into the brain adjacent to tumor. Aliquots of 0.1 to 0.2 ml of HSV1716, were injected into eight to ten sites of the brain adjacent to tumor with the intent of infecting residual tumor cells. Patients were reviewed daily in the first week, weekly for six weeks, then twice monthly for the first year, and then on a six-month basis. Clinical and neurological parameters were recorded and blood samples were obtained for hematological, biochemical, and serological assessment. In addition, MRI, or CT if MRI was not tolerated, with and without contrast, was performed pre- and postoperatively, and then in line with the clinical assessment review schedule. Patients also underwent SPECT (single photon emission computed tomography) with thallium-201 used to identify areas of high cellular metabolic activity reflecting high-grade tumor growth. Patients underwent imaging outwith protocol when clinically indicated. There was no clinical evidence of toxicity during the period of formal follow-up or during the period associated with the administration of HSV1716. Longitudinal follow-up, until February 2003, has allowed assessment of overall survival compared to that of similar patients not treated with HSV1716. Three patients remain alive and clinically stable at 15, 18, and 22 months postsurgery and HSV1716 injection. Remarkably, the first patient in the trial, who had extensive recurrent disease pre-procedure, was alive at 22 months following injection of HSV1716 and 29 months following first diagnosis. Imaging demonstrated a reduction of residual tumor over the 22-month period despite no further medical intervention since surgery and HSV1716 injection. In this study we demonstrate that on the basis of clinical observations, there has been no toxicity following the administration of HSV1716 into the resection cavity rim in patients with high-grade glioma. The survival and imaging data, in addition to the lack of toxicity, give us confidence to proceed to a clinical trial to demonstrate efficacy of HSV1716 in glioma patients.

112. TEMOZOLOMIDE/PEGYLATED LIPOSOMAL DOXORUBICIN (PLD) IN PROGRESSIVE GLIOBLASTOMA MULTIFORME (GBM)

G. Dresemann; Onkologische Abteilung, Franz-Hospital Duelmen, Duelmen, Germany

GBM still is one of the most aggressive malignancies, with a median survival of 1 year. Two-year progression-free survival is about 11% in newly diagnosed cases, and 5-year overall survival less than 5%, so most of the patients (pts) will experience a progression within the first 2 years. Although magnetic resonance imaging (MRI) seems to indicate deficiency in the blood-brain barrier (BBB), only drugs penetrating the intact BBB have in vivo efficacy in GBM, with a median survival benefit of about 3 months only. Temozolomide (T) is one the most effective drugs in progressive GBM. Long-term survival, however, remains limited. Doxorubicin, a very effective drug in GBM in vitro, does not penetrate the BBB and therefore did not show in vivo effectivity. Pegylated liposomal doxorubicin (PLD) is able to penetrate the BBB and has shown modest activity in temozolomide refractory GBM. The drug profiles of T and PLD highly suggest additive activity without overlapping toxicity. From August 2001 to May 2004, 40 pretreated (surgery, irradiation therapy, 1 chemotherapeutic regimen) GBM pts were treated with T/PLD for progression. T was given on days 1–5, 8–12, 15–19 dosed 200 mg per day for pts up to 70 kg of bodyweight and 250 mg per day for pts over 70 kg, combined with PLD 30 mg/m^2 body surface on day 1 intravenously to be repeated every 4 weeks. Prolonged dosage of T was chosen because of enzyme induction with the potency of increased efficacy. Blood cell counts were taken weekly, and MRI scans were performed every 8 weeks for disease monitoring. T/PLD caused °III hematotoxicity in 6/40 pts °II hematotoxicity in 16/40 pts, there were no febrile neutropenias, 4/40 pts had °III nausea/vomiting requiring 5HT3 antagonists. One of 40 pts had a complete response lasting for 33 months, 4/40 had a partial response with a median duration of 23 months (8–25), 24 pts had a stable disease with a median duration of 6 months (3–15), and 11 pts were primary progressive. Median progression-free survival (PFS) was 5 months, median overall survival (OS) 7 months, PFS after 12 months was 6/40 pts, and OS at 12 months was 9/40 pts. T/PGD was well tolerated and effective in this group of 40 progressive pretreated GBM pts. These data should be evaluated by further clinical and pharmacological examination.

113. CONVECTION-ENHANCED DELIVERY OF PACLITAXEL IN PATIENTS WITH RECURRENT GLIOBLASTOMA MULTIFORME: A PHASE 1/2 STUDY

R. Goldbrunner,[1] P. Tanner,[1] M. Holtmannspoetter,[2] G. Poepperl,[3] W. Rachinger,[1] F. Kreth,[1] and J. Tonn[1]; Departments of [1]Neurosurgery, [2]Neuroradiology, and [3]Nuclear Medicine, Universiteitsklinikum Grosshadern, Munchen, Germany

Critical issues in chemotherapy of malignant gliomas are systemic toxicity and the ability of bypassing the blood-brain barrier. To overcome these issues, direct drug delivery into the brain parenchyma is increasingly used. Utilizing slight pressure gradients, convection can be used to achieve a distribution volume sufficient to cover the brain tumor and the surrounding tissue. Effective dose, efficacy, and safety of convection enhanced delivery (CED) of paclitaxel (Taxol) in patients with recurrent glioblastoma multiforme (GBM) are evaluated in a phase 1/2 trial. Eight patients with recurrent GBM were enrolled in this study each completing up to 2 cycles of intratumoral paclitaxel infusion. One to two catheters were placed stereotactically into the solid tumor on the basis of MRI and FET-PET data. Paclitaxel dissolved in Cremophore at a concentration of 0.25 to 0.5 mg/ml was infused in a rate of 0.3 ml/h for 5 days, resulting in a total dose of 9 to 18 mg paclitaxel. Convection was monitored by MRI including DWI and DTI. Follow-up including MRI and FET-PET was performed at day 28, then bimonthly for 1 year. Tumors were located within or next to speech or central areas in 7/8 patients. Initial MRI studies demonstrated induction of intratumoral necrosis in 7/8 patients. Partial response (as shown by MRI and PET) was seen in 7/8 patients, stable disease in 1/8. Median progression-free survival was 7.0 months, and median overall survival was 10.0 months (range, 4–15 months). Permanent neurological deficit was seen in 2 patients, temporary deficits during paclitaxel infusion in 4/8. There were no signs of any systemic toxicity. According to these preliminary results, CED

of paclitaxel is a very effective therapy for patients with recurrent glioblastoma multiforme with an acceptable safety profile. Long-term observations with a larger number of patients will follow.

114. PERITUMORAL CONVECTION-ENHANCED DELIVERY (CED) OF IL13-PE38QQR (IL13PE): RESULTS OF MULTICENTER PHASE 1 STUDIES IN RECURRENT HIGH GRADE GLIOMA (HGG)

S. Kunwar,[1] Z. Ram,[2] J.H. Sampson,[3] M. Prados,[1] S. Chang,[1] F.F. Lang,[4] J.M. Piepmeier,[5] P.H. Gutin,[6] D. Croteau,[7] and R.K. Puri[8]; [1]University of California, San Francisco, California, USA; [2]Tel Aviv University, Tel Aviv, Israel; [3]Duke University Medical Center, Durham, North Carolina, USA; [4]M.D. Anderson Cancer Center, Houston, Texas, USA; [5]Yale University School of Medicine, New Haven, Connecticut, USA; [6]Memorial Sloan-Kettering Cancer Center, New York City, New York, USA; [7]NeoPharm, Inc., Lake Forest, USA; [8]CBER, U.S. FDA, Bethesda, Maryland, USA

IL13PE is a recombinant protein consisting of IL13 and truncated *Pseudomonas* exotoxin that has selective antitumor activity against malignant glioma cells overexpressing the IL13 receptor. We assessed the safety and efficacy of peritumoral CED of IL13PE in patients with recurrent HGG. Three Phase 1 studies evaluated peritumoral CED of IL13PE following tumor resection. Eligibility criteria included: age >18, KPS >70, and a tumor amenable to resection. The first study (002) determined the maximum tolerated infusate concentration (MTD) of IL13PE, using a dose escalation design. Infusion duration and feasibility and accuracy of postresection catheter positioning were also investigated. A second study (103R02) expanded the patient population treated at the MTD, and a third study (105) further assessed drug distribution at the MTD. Patients underwent tumor resection followed by a single 4–6 day infusion of 0.25–1.0 μg/ml of IL13PE (total dose = 18–72 μg) and were followed with serial MRI scans. Enrollment is complete; 51 patients received study drug via peritumoral infusion. Forty-five patients had histologically confirmed recurrent glioblastoma multiforme (GBM: study 002, n = 38; 103R02, n = 3; 105, n = 4). Maximum tolerated infusate concentration was 0.5 μg/ml. Most frequent adverse events (AEs) were headaches and hemiparesis. There were 2 grade 4 toxicities at the highest concentration, and 9 grade 3 AEs reported in the study group. Concentration-dependent imaging changes related to study drug were also observed. Median overall survival (OS) for GBM patients was 45.9 weeks. Median OS for GBM patients with peritumoral infusate concentration of 0.5 μg/ml (n = 24) is currently 70.3 weeks. Eight patients remain progression free (24+ to 174+ weeks, median = 69 weeks). Initially, 50% of intraoperatively placed catheters were positioned accurately; accuracy increased to 90% when postresection stereotaxis was utilized. IL13PE appears to have a favorable risk-benefit profile, and prolonged overall survival has been observed. Postresection catheter placement appears to enhance positioning accuracy and, potentially, drug distribution and OS. Design of the ongoing international phase 3 trial to determine efficacy and safety of IL13PE in first relapse GBM is based on these findings.

115. INDIVIDUALIZED CHEMOTHERAPY FOR MALIGNANT ASTROCYTOMAS BASED ON O6-METHYLGUANINE-DNA METHYLTRANSFERASE METHYLATION ANALYSIS

T. Watanabe, A. Ogino, T. Ohta, A. Yoshino, and Y. Katayama; Nihon University School of Medicine, Neurological Surgery, Tokyo, Japan

The correlation between O6-methylguanine-DNA methyltransferase (MGMT) methylation and responsiveness to nitrosourea chemotherapy observed in recent clinical studies suggests that use of this alkylating agent should be reserved for MGMT-methylated tumors. MGMT appears not to be linked to platinum resistance, which makes platinum chemotherapy a good candidate for the treatment of MGMT-unmethylated tumors. We instituted a preliminary trial of individualized chemotherapy based on MGMT methylation status combining interferon and radiation therapy for patients with malignant astrocytomas. A total of 20 patients with newly diagnosed malignant astrocytomas were enrolled onto the study. Seven patients with MGMT-methylated tumors were treated with the procarbazine, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-2(2-chloroethyl)-3-nitrosourea (ACNU), and vincristine (PAV) regimen. An objective response to the PAV therapy was noted in all 3 patients with measurable residual tumor (2 complete responses and one partial response). Five patients continued to be disease free as of 10, 17, 19, 22, and 26 months after initiation of the PAV therapy. The 13 patients with MGMT-unmethylated tumors were treated with the carboplatin and etoposide (CE) regimen. Only one (14%) of 7 patients with assessable disease partially responded to the CE therapy. Three patients were free from progression at 11, 14, and 20 months, whereas the remaining 10 patients progressed early following commencement of the CE administration. Our results provide support for the responsiveness of MGMT-methylated malignant astrocytomas to the PAV regimen, but do not justify the usefulness for the CE regimen in unmethylated tumors.

116. A PHASE 1/2 TRIAL OF TEMOZOLOMIDE AND VINORELBINE IN PATIENTS WITH RECURRENT BRAIN METASTASES

A.M.P. Omuro, J.J. Raizer, and L.E. Abrey; Memorial Sloan-Kettering Cancer Center, Department of Neurology, New York, New York, USA

Treatment options for recurrent brain metastasis (BM) are limited. Temozolomide (TMZ) is an alkylating agent with efficacy in BM. Vinorelbine is a lipophilic agent with efficacy in a variety of solid tumors and can potentially cross the blood-brain barrier. We designed a phase 1/2 trial for recurrent BM utilizing vinorelbine in combination with TMZ in a protracted schedule. Patients with solid tumors and recurrent or progressive BM were eligible. One cycle was defined as 28 days. TMZ was given on a dose of 150 mg/m^2 on days 1 to 7 and 15 to 21. Vinorelbine was given on days 1 and 8 at escalating doses for the phase 1, with a starting dose of 15 mg/m^2 and increments of 5 mg/m^2 for each cohort of 3 patients, until maximum tolerated dose (MTD) or a dose of 30 mg/m^2 was reached. For the phase 2, 20 pts would be treated at the MTD; if two or more major responses were seen, sample size would be increased to 35. Twenty-seven pts have been enrolled (phase 1, 18 pts; phase 2, 9 pts). Median age was 60 (40–76); 11 pts were men, median KPS was 80 (70–100). Primary cancer was lung in 16 pts (non-small cell, 13 pts), breast in 8, renal in 1, endometrial in 1. During the phase 1, no dose-limiting toxicity was observed and the phase 2 dose of vinorelbine has been 30 mg/m^2. Myelotoxicity has been the main adverse event, with grades 3 or 4 neutropenia in 7 pts, lymphopenia in 14, anemia in 2, and thrombocytopenia in 3. Response has been evaluated in 18 pts (complete response, 1; partial response, 1; minor response,1; stable disease, 5; progressive disease, 10). Median time to progression was 2 (1–6) months. This regimen is well tolerated, and preliminary results suggest promising efficacy. The phase 2 sample size will be increased to 35 pts. Updated results will be presented.

117. AN UPDATE OF PHASE 2 TRIAL RESULTS: PATIENTS WITH RECURRENT MALIGNANT GLIOMA TREATED WITH IODINE 131-LABELED MURINE ANTITENASCIN MONOCLONAL ANTIBODY 81C6 INTO THE RESECTION CAVITY

D. Reardon, G. Akabani, A. Friedman, H. Friedman, R. McLendon, J. Quinn, J. Rich, J. Vredenburgh, M. Zalutsky, and D. Bigner; Duke University Medical Center, Durham, North Carolina, USA

We previously established the maximum tolerated dose of iodine 131-labeled murine antitenascin antibody 81C6 (^{131}I-81C6) administered into a surgically created resection cavity (SCRC) for the treatment of recurrent malignant glioma in adult patients to be 100 mCi. The current phase 2 study is designed to evaluate the clinical activity of a 100-mCi dose of ^{131}I-81C6 administered to patients with recurrent malignant glioma and to further evaluate the safety and toxicity of this approach. Eligibility criteria included the following: adults with recurrent malignant CNS tumor; gross total resection; lack of communication between the resection cavity and the CSF space; KPS greater than 60%; and adequate bone marrow, hepatic, and renal function. To date 43 patients have been enrolled, including 34 with either GBM or gliosarcoma, 6 with AA, 2 with AO, and 1 with metastatic adenocarcinoma. Ninety-three percent received prior external beam radiation, and 51% were treated with prior chemotherapy. The median age was 54.5 years (range, 26–77) and 63% were males. All patients received 100 mCi except for two patients who received 67 mCi and 75 mCi respectively because of the limited size of the SCRC. Reversible hematologic toxicity occurred in 27% of patients, and 5 patients (15%) developed delayed neurotoxicity. However, only one patient required reoperation for symptomatic radionecrosis. The median survival of all patients and of those with GBM was 67.5 and 63.2 weeks, respectively. For patients with GBM the probability of 1-year survival is 0.56 (95% CI, 0.41–0.78). These encouraging results suggest that further study of ^{131}I-81C6 for patients with recurrent CNS tumors is warranted.

118. PROGNOSTIC RELEVANCE OF CLINICAL AND THERAPEUTICAL ASPECTS IN PATIENTS WITH GLIOBLASTOMA MULTIFORME. ANALYSIS OF 99 PATIENTS

A. Mangiola,[1] C. Anile,[1] P. De Bonis,[1] B. Pettorini,[1] G. Sabatino,[1] S. Mannino,[1] C. Pedone,[2] and G. Maira[1]; [1]Neurosurgery, Neurological Sciences; [2]Geriatrics Gerontological, Geriatrics and Physiatric Sciences, Catholic University, Rome, Italy

Glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults. Despite advances in research and treatment, the prognosis for these patients remains poor, with a median survival of 1 year. Current management strategies used for GBM include surgery followed by adjunctive radiation therapy and chemotherapy. There is controversy among the neurosurgeons regarding surgical strategies. The purpose of this study was to identify clinical and therapeutic predictors of survival. We retrospectively analyzed 99 consecutive patients with primary supratentorial GBM who underwent tumor removal at our institution between 1999 and 2003. After surgery patients were treated with adjuvant therapy: radiotherapy, chemotherapy, radioimmunotherapy. Of 99, 23 underwent a reintervention after recurrence. Based on different therapeutic strategies and clinical aspects different subgroups have been defined. We determined the median survival of each one using the Kaplan-Maier method. In concordance with literature we have obtained that age and total removal are predictors of survival. Other data differ from literature: survival according to Karnofsky Performance Scale score, epilepsy as first symptom, and functional location and presence of central necrosis were not statistically significant. The most interesting results come from therapeutics. Patients who underwent total en bloc resection had a survival of 19 months, while total inside-out resection group had a survival of 12 months ($P = 0.0005$). Patients who underwent one surgical treatment had a survival of 10.5 months, and patients who underwent more surgical treatments had a survival of 22 months $P = 0.002$). Survival according to adjuvant therapy has been 23 months (radiotherapy, chemotherapy, radioimmunotherapy), 18 months (radiotherapy, chemotherapy), and 14 months (radiotherapy) ($P = 0.002$). In our series the "en bloc" resection represents an important prognostic variable. When tumor recurs, a re-intervention has to be considered. The treatment of GBM remains multidisciplinary. In our experience the best adjuvant treatment is radio + chemo + radioimmunotherapy.

119. PHASE 1 STUDY RESULTS OF GEFITINIB (IRESSA; ZD1839) PLUS RAPAMYCIN IN THE TREATMENT OF PATIENTS WITH RECURRENT MALIGNANT GLIOMA

D.A. Reardon, J. Rich, J. Quinn, J. Vredenburgh, A. Desjardins, S. Sathornsumetee, S. Gururangan, P. Lyons, D. Bigner, and H. Friedman; Duke University, Durham, North Carolina, USA

Aberrant signaling of the PI3K/Akt pathway is common in malignant glioma. The primary objective of this phase 1 study is to determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of Gefitinib, an inhibitor of the EGFR receptor tyrosine kinase, when combined with rapamycin, a macrolide antibiotic capable of inhibiting mTOR, a critical downstream regulator of PI3K/Akt signaling, among patients with recurrent malignant glioma. Eligibility criteria include the following: recurrent malignant glioma; fewer than 4 prior episodes of recurrence; greater than 18 years of age; KPS greater than 60%; adequate hepatic, renal, pulmonary and bone marrow function. Patients previously treated with EGFR or mTOR-directed therapies are excluded. Patients are stratified based on concurrent use of enzyme-inducing anticonvulsants (EIAC; phenytoin, carbamazepine, and phenobarbital). A standard "3+3" dose escalation design was employed with both strata independently escalated. Gefitinib and rapamycin are administered on a continuous daily dosing regimen. Each treatment cycle is 28 days, and patients are evaluated for response every other cycle. Twenty-three patients have been enrolled to date, including 20 with recurrent GBM and 3 with recurrent AA. The median age is 51 (range, 33–66); 65% are male, and 52% are on EIAC. Accrual and dose escalation are ongoing, and the MTD has yet to be defined for either stratum. The only DLT to date was an episode of grade 3 mucositis. Pharmacokinetic sampling has been collected in approximately half of patients. Six patients have discontinued therapy due to progressive disease, while 17 continue on study having received 1 to 3 cycles of therapy. One marked radiographic response has been observed to date. An update of the outcome and toxicity of Gefitinib plus rapamycin for patients with recurrent malignant glioma will be presented based on additional follow-up and enrollment.

120. ENHANCED DELIVERY OF CHEMOTHERAPY BY OSMOTIC BLOOD-BRAIN BARRIER DISRUPTION (BBBD) WITH DEFERRED RADIOTHERAPY FOR TREATMENT OF PRIMARY CNS LYMPHOMA (PCNSL): ANALYSIS OF 33 PATIENTS

T. Siegal,[1] E. Shalom,[1] E. Linetsky,[1] A. Taliansky,[1] A. Lossos,[1] F. Bokstein,[1] N. Levin,[1] A. Schwartz,[2] and J. Gomori[2]; [1]Leslie and Michael Gaffin Center for Neuro-Oncology; [2]Neuro-Radiology Unit, Hadassah Hebrew University Hospital, Jerusalem, Israel

The optimal therapy for PCNSL has not yet been established. The addition of high-dose chemotherapy (chTx) to radiotherapy substantially improves median survival, but the combined therapy carries a high risk of severe treatment-induced delayed neurotoxicity. Several studies tried to use intensive chTx as the sole treatment for PCNSL but their limitations proved to be either a low response rate, short duration of response, or a high rate of treatment-related death (10%). A single institution's experience with delivery of chTx in conjunction with osmotic BBBD, without subsequent radiotherapy, was reported to yield a survival rate similar to that observed in standard regimens that use both chTx and radiation therapy. This BBBD-enhanced therapy was not associated with delayed neurotoxicity. We report the experience at Hadassah University Hospital with BBBD-enhanced chTx without radiotherapy in PCNSL. Pts with non-AIDS related PCNSL were prospectively treated with methotrexate-based chTx as first-line treatment or with carboplatin-based therapy if they failed any previous methotrexate treatment. Both regimens were given in conjunction with BBBD and with no subsequent radiotherapy. We treated 33 pts whose median age was 48 (range, 23–68). CSF spread and intraocular lymphoma were each present in 12% of the pts. The mean number of treatment cycles/pt was 7, with 15% of cycles based on carboplatin with a total of 474 BBBD procedures. A complete response was achieved in 23 pts (70%). Median follow-up is 27 months (range, 5–94), and the median survival is 39 months with a 5-year survival rate of 41%. Three pts died of causes other than disease progression; one was treatment related. Other procedure-related complications included 2 minor strokes, 2 arterial injuries successfully repaired by stenting, and one reversible brainstem lesion induced by carboplatin. The median time to tumor progression has not yet been reached (13/33 progressed) with 24- and 36-month PFS rates of 69% and 57%, respectively. Of pts followed for more than 24 months, none demonstrated delayed leukoencephalopathy, and there was no progressive cognitive loss on clinical and neuropsychological testing. BBBD-enhanced chTx without subsequent radiotherapy requires the expertise of a multidisciplinary team to treat pts with PCNSL. It results in PFS and survival outcomes similar to those with standard therapy that use radiotherapy, but offers a favorable long-term cognitive outcome.

121. TEMOZOLOMIDE (TMZ) FOR 21/28 DAYS IN PATIENTS WITH PROGRESSIVE GLIOBLASTOMA (GBM): PRELIMINARY RESULTS OF PHASE 2 STUDY OF GICNO (ITALIAN NEURO-ONCOLOGY GROUP)

A. Brandes,[1] G. Cavallo,[2] A. Tosoni,[1] M. Ermani,[3] L. Scopece,[2] E. Franceschi,[2] L. Nicolardi,[1] A. Talacchi,[4] V. Blatt,[1] and L. Crinò[2]; [1]Medical Oncology, Ospedale Busonera, Padova; [2]Department of Medical Oncology, Ospedale Bellaria, Bologna; [3]Department of Neurological Sciences, Azienda Ospedale Università, Padova; [4]Departmentt of Neurosurgery, University Hospital, Verona; Italy

The activity of TMZ, at a standard schedule of 200 mg/m^2 for five days every 28 days, appears encouraging in patients (pts) with relapsed and newly diagnosed malignant gliomas. A continuous dose schedule of TMZ leads to DNA repair enzyme AGAT level depletion in tumor cells. AGAT, by removing TMZ-produced methyl adducts, contributes to resistance to alkylating agents. The aim of the present study was therefore to determine the efficacy and safety of continuous low-dose TMZ administration in patients with GBM. Twenty pts (11 males, 9 females; median age, 56 years [range, 31–71]) with GBM progressive/recurrent after surgery and radiotherapy received oral TMZ 75 mg/m^2 for 21 consecutive days, every 28 days. All pts were chemo-naïve. Preliminary data on efficacy and safety were available for 20 pts. PFS-6 was 34% (95% CI, 18%–66%), and median TTP was 15 weeks (95% CI, 12.4–NA). A total of 80 cycles were delivered (mean 4 per pt; range, 1–8). Grade 2 and 3 lymphopenia was observed in 15% and 30% of pts, respectively. Neutropenia was G3 in 1 patient (5%) and G4 in 1 (5%). Hepatic G1 and G3 toxicity was observed in 15% and 5% of patients respectively. The TMZ dose was reduced to 75% in one cycle (1.2%) because G3 neutropenia and G3 lymphopenia occurred. This regimen appears to be well tolerated and active in patients with progressive GBM.

122. MODERATE HEMATOLOGICAL TOXICITY OF DOSE-INTENSIFIED TEMOZOLOMIDE (TMZ) IN PATIENTS WITH RECURRENT GLIOBLASTOMA

W. Wick and W. Weller; Department of General Neurology and Hertie Institute for Clinical Brain Research, University of Tuebingen, Tuebingen, Germany

A one-week-on/one-week-off TMZ schedule had shown clinical efficacy and moderate hematological toxicity in 21 patients with recurrent glioblastoma. In a reassessment of our database, 39 patients with recurrent or progressive glioblastoma treated with the one-week-on/one-week-off regimen of TMZ, starting at 150 mg/m^2 with dose adaptation in 25 mg/m^2 steps, have been evaluated according to leukocyte and platelet counts which were determined in weekly intervals. The analysis similarly to the published data demonstrated a promising progression-free survival rate of 43% at 6 months. The survival rate at 12 months from recurrence was 21%. Hematological toxicity in a total of 382 treatment weeks was low. Thirty-two of 39 patients never experienced leukocyte counts below 3,000/μl or platelet counts below 100,000/μl. Thrombopenia necessitated platelet transfusions in 5 patients. Two patients experienced grade 3/4 hematological toxicity according to the common terminology criteria for adverse events later than 3 months after they started on TMZ. All other patients suffered such toxicity within the first weeks of TMZ. None of the 13 patients receiving TMZ for more than 6 months experienced grade 3/4 hematological toxicity. Our present extended analysis of the continuous one week on/one week off schedule of TMZ confirms the safety and efficacy of this regimen in recurrent or progressive glioblastoma.

123. LOOK FOR ANXIETY IN THE YOUNG AND DEPRESSION IN THE PREVIOUSLY DEPRESSED IN POSTOPERATIVE PATIENTS WITH BRAIN TUMORS

L. Kilbride,[1] and R. Grant[2]; [1]School of Acute & Continuing Care Nursing, Napier University, Edinburgh, Scotland; [2]Edinburgh Centre for Neuro Oncology, Lothian University NHS Trust, Edinburgh, Scotland; UK

Anxiety and depression can be symptoms that limit the quality of life of patients if not adequately diagnosed or treated. The period between surgery and radiotherapy for patients with brain tumors is a period where anxiety and depression commonly occur. We prospectively studied patients at 3 time points after surgery to study the frequency of anxiety and depression, the most likely time point for developing symptoms and any features that might act as a predictor of anxiety or depression. Fifty-one consecutive patients with intrinsic brain tumors gave consent to be involved in the study. Only 34/51 completed the Hospital Anxiety and Depression (HAD) scale at three points. A full history was taken, and a HAD scale score was obtained postsurgery, three weeks postsurgery, and pre-radiotherapy. A HAD score of 11 was considered abnormal. Thirty-eight patients (74%) completed the first HAD questionnaire, 34 (67%) completed the second, and 34 (67%) completed the third. Of 37 patients, 23 (62%) improved in functional improvement over the study period, and 5 patients (13%) deteriorated. Six of 11 of the patients (55%) who functionally deteriorated through the study did not complete the HAD scale. Five of 38 patients (13%) were anxious postsurgery, and four of these patients continued to be anxious throughout the study period. Eight of 34 (23%) were anxious pre-radiotherapy. All patients reporting heightened levels of anxiety were aged 65, and the anxiety levels were not related to initial functional impairment or change in function. Five patients had a significant depression at one or more time between surgery and radiotherapy. Four of the 5 patients with depression had a past history of depression. Anxiety was more common in younger patients. Anxiety was slightly more frequent pre-radiotherapy. A past medical history of depression is a predictor of significant depression in the postoperative period.

124. ADVERSE EFFECTS OF ANTIEPILEPTIC DRUGS IN BRAIN TUMORS: PRELIMINARY DATA OF A MULTI-INSTITUTIONAL SURVEY. ARE NEW ANTIEPILEPTIC DRUGS LESS TOXIC?

M. Maschio, M. Riva, R. Soffietti, R. Rudà, M. Scerrati, A. Mauro, C. Bompressi, L. Provinciali, F. Imbesi, *C. Carapella*, A. Pace, and B. Jandolo, on behalf of the Italian Association of Neuro-Oncology (AINO) and the Italian Society of Neurology (SIN)

Adverse effects of antiepileptic drugs (AEDs) in patients affected by brain tumors are more frequent than in nontumoral epileptic patients (24% vs. 12%, respectively, Glantz et al). Several classical AEDs (phenobarbital, phenytoine, and carbamazepine) are potent enzyme-inducing drugs, have serious CNS side effects that may alter quality of life and interfere with anticancer treatments, and may presents hematological and systemic toxicities. There are few studies focused on incidence of side effects from new AEDs in neuro-oncology. We report preliminary data of an Italian multicentric study based on antiepileptic treatment of a large population of brain tumor patients treated with new AEDs. The aim of the study was to evaluate the incidence of adverse effects in brain tumor patients treated with oxcarbazepine (OXC), lamotrigine (LTG), vigabatrin (GVG), topiramate (TPM), gabapentin (GBP), and levetiracetam (LEV). Two hundred thirty patients were analyzed (148 M, 93 F), mean age 50 years (range, 16–80). Histology was glioblastoma in 112 patients, anaplastic astrocytoma in 59, and low-grade glioma in 68. Patients were treated in monotherapy in 170 cases: OXC, 69; LTG, 40; GVG, 9; TPM, 51; and GBP, 1. In 60 cases, patients were treated in polytherapy with new AEDs in add-on to classical AEDs (PB in 26 patients; CBZ, 26; OXC, 75; TPM, 76; LTG, 52; GVG, 11; DHT, 11; LEV, 10; VPA, 3; GBP, 1). Overall incidence of adverse effects was 13.5% (31/230 patients). The incidence of side effects in patients treated in monotherapy with new AEDs was 7.0% (12/170). More frequent side effects included skin reaction in 17 (7.4%), mostly in patients treated in association with carbamazepine or phenobarbital; other side effects were sedation, weight loss, hyponatriemia, and liver toxicity. In 14 patients, the severity of side effects led to discontinuation of treatment. Despite the limits of a retrospective analysis from a multi-institutional series of patients, our data seems indicate that new AEDs are better tolerated than standard antiepileptic drugs. Given the relevant influences of anticonvulsant treatment on quality of life of brain tumor patients, and the important interactions with anticancer treatments, larger studies are needed to compare tolerability and efficacy of new AEDs with respect to standard AEDs.

125. PHASE 2 STUDY OF TOPOTECAN IN COMBINATION WITH CONCURRENT RADIOTHERAPY IN ADULTS WITH GLIOBLASTOMA WITH INCOMPLETE RESECTION

T. Lesimple,[1] M. Ben Hassel,[1] D. Gédouin,[1] J. Bay,[2] D. Frappaz,[3] C. Linassier,[4] G. Piot,[5] M. Fabbro,[6] S. Saikali,[7] and Y. Guégan[7]; [1]Neurooncology, Rennes; [2]Medical Oncology, Clermont-Ferrand; [3]Pediatric Oncology, Lyon; [4]Medical Oncology, Tours; [5]Medical Oncology, Le Havre; [6]Medical Oncology, Montpellier; [7]Neurosurgery, Rennes; France

In a previous phase 1 study of topotecan (TPT) administered as a continuous infusion (CIV) for 5 days every 2 weeks in combination with concurrent radiotherapy (RT), the recommended dose of TPT was 0.9 mg/m^2/day (Lesimple et al., J. Neurooncol. 65, 2003). The antitumor activity (measured by 12-month overall survival [OS]) and the safety of TPT were assessed in patients (pts) with histologically proven and previously untreated glioblastoma multiforme (GBM): After partial resection or stereotactic biopsy, pts received cranial RT (60 Gy/30 fractions/40 days) and 3 cycles of 0.9 mg/m^2/day of TPT (Hycamtin, Glaxo-SmithKline, Marly le Roi, France) as CIV from day 1 to 5 on weeks 1, 3, and 5 during RT. A total of 50 pts were entered, and 37 pts (M/F: 24/13; median age: 59, range: 42-69; PS 0/1-2: 17/20) were analyzed here. Twenty-one pts had stereotactic biopsy and 16 had partial resection. Grade 3–4 hematological toxicity was observed in 15 patients: neutropenia (16%, associated with fever in 4%), lymphopenia (15%), thrombopenia (5%) and anemia (3%). Grade 3–4 nonhematological toxicity consisted of hyperglycemia (4 pts); vomiting, diarrhea, hypokalemia (2 pts); and hyponatremia, GGT elevation, catheter-related infection, nausea, somnolence and thrombotic microangiopathy (1 patient each). One patient experienced a partial response (3.7% of 27 evaluable pts for response) and 11 pts (40.7%) had stabilization, with a time to progression of 12 weeks. One-year OS rate was 41.7% for the 37 pts (median OS, 41 weeks). TPT in combination with RT was well tolerated but had modest activity in partially resected GBM in terms of response rate and 12-month OS.

126. TREATMENT WITH 3-WEEK COURSES OF DAILY TEMOZOLOMIDE AS SECOND-LINE CHEMOTHERAPY OF RECURRENT HIGH-GRADE GLIOMAS

R. Gaspar, A. Guerrero, and J. Ponce; Department of Medical Oncology, Hospital Universitario La Fe, Valencia, Spain

Several studies suggest that continuous treatment with temozolomide (TMZ) may be more effective than the standard 5-day schedule. We present our results with 3-week courses, daily TMZ, in recurrent high-grade gliomas (HGG). Between March 2003 and September 2004, 17 patients (pts) were included. Patient characteristics were as follows: 9 males, 8 females; median age, 39 years (range, 18–59); histology: 7 glioblastomas, 4 anaplastic astrocytomas, 1 anaplastic ependymoma, and 5 suspected HGG by neuro-imaging and clinical criteria. Previous treatments of recurrence were as follows: TMZ (200 mg/m^2 for 5 d every 28 d) in 13 cases; CPT-11 (125 mg/m^2 d, 1, 8, 22, and 29 every 36 d) in 3 cases; PCV in 1 case. At progression, pts were treated with TMZ, 75 to 80 mg/m^2/d for 21 d every 4

weeks. Ninety cycles of TMZ were administered (median, 4 cycles; range, 2–11 cycles). No grade 3 or 4 toxicity has been observed. According to Macdonald's criteria, partial response was achieved in 2 pts, stable disease was achieved in 9 pts, and 5 pts progressed. Both partial responses, lasting 4+ and 6+ months, have been observed in patients who progressed during treatment with standard 5-day courses of TMZ. Nine of the 17 pts are still on treatment; 4 additional pts have been recently included. We conclude that TMZ given continuously for 21 d every 4 weeks is well tolerated. Responses can be achieved in pts who have progressed with the standard 5-day schedule.

127. PREDICTORS OF SURVIVAL AND TUMOR RECURRENCE IN A COHORT OF 530 PATIENTS WITH GLIOBLASTOMA MULTIFORME: A REPORT FROM THE BRAIN CANCER REGISTRY OF THE NATIONAL NEUROLOGICAL INSTITUTE CARLO BESTA, ITALY, 1997 TO 2002

G. Filippini, M. Farinotti, C. Falcone, C. Politi, and R. Farina; Istituto Nazionale Neurologico, Neuroepidemiology, Milan, Italy

Glioblastoma multiforme (GBM) is the most frequent brain tumor and accounts for approximately 12%–15% of all intracranial neoplasms and 50%–60% of all glial tumors. Aims of our study were to examine if resection is better than biopsy, to verify the role of extent of resection in relation to survival and tumor recurrence/progression in patients with GBM, and to evaluate the added beneficial value of radiotherapy and chemotherapy. All patients were consecutively registered from January 1997 to December 2002 in the Brain Cancer Registry of the National Neurological Institute Carlo Besta, Milan. During this period, all adult patients newly diagnosed with histologically verified cerebral GBM were included and followed up until December 2003. Data about Karnofsky performance status (KPS), type of initial surgical procedure, therapies, survival, and recurrence of disease were studied. Analysis was done using both Cox and logistic regression models. Five hundred thirty patients (60% males, 40% females) were included; mean age at intervention was 56 years (16–81 years). Median clinical follow-up time was 48 weeks. Median survival time was 58 wks (95% CI, 54–62). Survival probability was 55% at 1 year and 7% at 3 years. In multivariate analysis, age resulted as one of the most significant prognostic factors for survival of pts with GBM, with a hazard ratio (HR) of 1.32 (95% CI, 1.04–1.68) for age 53 to 61 years and 1.83 (95% CI, 1.40–2.39) for age >61 years compared with 15 to 52 years (reference category). The intervention most significantly associated with survival was radiotherapy with HR = 4.17 (95% CI, 2.98–5.85) for no radiotherapy. With respect to extent of resection, HR was 2.05 (95% CI, 1.36–3.09) for biopsy and 1.25 (95% CI, 0.90–1.74) for partial resection (reference category: total resection). Median time to recurrence/progression (TTP) was 24 weeks (95% CI, 22–26). In multivariate analysis, the most significant prognostic factor for TTP was radiotherapy with HR = 3.86 (95% CI, 2.85–5.22) for no radiotherapy. Biopsy, compared to surgical intervention, was significantly associated with the following: sex (females vs. males), with a relative risk (RR) of 2.15 (95% CI, 1.16–3.98); number of lesions (multiple vs. single), with RR = 4.04 (95% CI, 2.03–8.05); tumor size (overlapping lesion vs. single lesion), with RR = 3.66 (95% CI, 1.88–7.12); and side (bilateral/median vs. unilateral), with RR = 7.92 (95% CI, 3.36–18.65). Partial resection, compared to a total one, was significantly associated with KPS (≤70 vs. >70), with RR = 2.34 (95% CI, 1.15–4.79), and tumor size (overlapping lesion vs. single lesion), with RR = 1.97 (95% CI, 0.98–3.96). We analyzed data from the largest data set on GBL in Italy. Radiotherapy and total surgical resection were significant independent predictors of survival of patients with GBM, but measurements of morbidity and quality of life associated with treatments are now critical issues for these patients.

128. EPILEPTIC SEIZURES DURING FOLLOW-UP OF PATIENTS TREATED FOR PRIMARY BRAIN GLIOMA

J. Hildebrand, C. Lecaille, J. Perennes, and J. Delattre; Hôpital de la Salpêtrière, Paris, France

Epileptic seizures are common in patients with brain glioma and may significantly alter the quality of life of these patients. The aim of our study is to determine the presentation, the incidence, and the severity of epileptic seizures in follow-up of patients treated for primary glioma. Two hundred thirty-four consecutive patients attending an outpatient clinic for chemotherapy of supratentorial brain glioma were examined. One hundred eighty-three (78.2%) experienced tumor-related seizures, and 51 (22.8%) did not. All epileptic patients were on antiepileptic drugs (AEDs). Compared with patients without epilepsy, the group of epileptic patients was characterized by a higher proportion of low-grade gliomas (epilepsy in the course of the malignant disease. Generalization occurred in 50% of early seizures, but in only 19.1% of patients with seizures persisting after the initiation of AEDs and specific antitumor therapies. The reduction of seizure generalization was statistically significant ($P = 0.001$). Despite AED administration and various antitumoral treatments, half of the patients had seizure within one month and two thirds within three months preceding the last evaluation. Most tumor-related seizures first appear in the early course of the disease, usually as a presenting manifestation. AEDs combined with specific antitumoral treatments significantly reduce the rate of seizure generalization. However, the majority of patients continue to present mostly focal epilepsy during therapeutic follow-up.

129. IMAGING RESPONSE IN PHASE 2 TEMOZOLOMIDE TRIALS—REPORT INITIAL TUMOR SIZE

C.F. Schwindack,[1] C. Graham,[1] and R. Grant[2]; [1]Wellcome Trust Clinical Research Facility; [2]Division Clinical Neurosciences, Western General Hospital, Edinburgh, Scotland, UK

Imaging response is an important measure in most trials of malignant glioma. In a previous paper of RMP-7 and carboplatinum, we found age was related to tumor grade and initial size of tumor in glioblastoma (GBM), but not anaplastic astrocytoma (AA). Initial tumor size was important for response in GBM, and age was linked to speed of response in AAs, but numbers were too small in the GBM group to allow analysis. This study analyzes imaging response data from three trials of temozolomide. Imaging response data from 3 clinical trials of temozolomide were supplied by Schering Plough Research International (SPRI). Inclusion criteria included recurrent supratentorial AA or GBM, age > 18 years, and Karnofsky performance status ⩾70. Temozolomide was administered during the first 5 days of a 28-day cycle (200 mg/m^2/day or 150 mg/m^2/day if prior chemotherapy). Macdonald imaging criteria for response were used. Speed of response (fast/slow) was determined by tumor size after 2 cycles. Change in size and speed of response to treatment were related to size, age, initial tumor size, and tumor type. Data on 301 patients were analyzed. There were 108 patients with AA (median age, 41.5 years) and 193 GBM (median age, 55 years) with a significant age difference ($P > 0.001$; 95% CI, -15.0 to -9.00). Median Karnofsky was 85 for AA and 80 for GBM ($P = 0.03$; 95% CI, 0.00–10.00). Median initial tumor size was 9.94 cm^2 in AA and 15.51 cm^2 in GBM ($P = 0.001$; 95% CI, -6.40 to -1.65). Of the patients with AA, 34% showed a response compared to 9.7% with GBM (odds ratio, 0.20; 95% CI, 0.10–0.40; $P < 0.0001$). Size: Initial median tumor size was 8.48 cm^2 in responders with AA versus 10.81 cm^2 in nonresponders ($P = 0.59$; 95% CI, -5.10–2.50) and 6.25 cm^2 in responders with GBM versus 15.63 cm^2 in nonresponders ($P = 0.04$; 95% CI, -10.75 to -0.32). There was no statistically significant difference in median initial tumor size of those with a fast response compared with a slow response (AA, $P = 0.22$; GBM, $P = 0.83$). Age: There was no association between age and initial tumor size in AAs ($P = 0.40$) or GBMs ($P = 0.96$). GBM patients who were responders were younger ($P = 0.01$; 95% CI (2–14), but median ages for responders with AA were no different than nonresponders ($P = 0.57$; 95% CI, -7.4). Age did not influence speed of response: AA ($P = 0.20$; 95% CI, -12–4) or GBM ($P = 0.94$; 95% CI, -18–18). Patients with a GBM are likely to be older and have larger tumors. Patients with a small GBM are more likely to respond than those with a large GBM. Within the tumor group, however, there was no relationship between age and initial tumor size. Studies evaluating response should record age and initial tumor size, and trials should stratify for it.

130. PRIMARY TEMOZOLOMIDE CHEMOTHERAPY IN ELDERLY PATIENTS WITH PRIMARY CNS LYMPHOMA (PCNSL)

U. Herrlinger, M. Uhl, and M. Weller; University of Tübingen, General Neurology, Tübingen, Germany

In some PCNSL patients over 60 years, standard high-dose methotrexate (HD-MTX) therapy may not be applicable due to comorbidities such as impaired renal function. This prompts the search for other chemotherapeutic agents that can be applied to these patients instead of HD-MTX. Progression-free survival, overall survival, and toxicity were retrospectively analyzed in patients with primary CNS lymphoma treated with temozolomide alone as primary therapy. We report 7 patients who had histologically confirmed PCNSL (n = 6) or a steroid-responsive lesion highly suggestive of PCNSL by neuroimaging (n = 1). The patients (62–84 years old) received 1 to 8 four-week courses of temozolomide (200 mg/m^2 for 5 days in 6 patients, reduced dose of 150 mg/m^2 in 1 patient). The median number of courses applied was 3. Complete responses (CRs) were achieved in 4 patients, 1 patient had stable disease after 3 courses and therapy was then switched to WBRT due to myelosuppression, 2 patients had primary progressive disease and did not receive any further therapy. In the patients achieving CR, CR persisted for 5, 18+, 21 and 48+ months. After a median follow-up time of 17 months (range, 1–48 months), median survival has

not been reached yet. One patient died after 1 month due to tumor progression, and 6 patients are alive. Acute toxicity consisted of high-grade thrombopenia and leukopenia with subsequent infection in one patient who had received 200 mg/m^2 temozolomide and high-grade leukopenia without further complications in another patient. Temozolomide appears to be an effective and tolerable therapy for elderly patients with PCNSL and comorbidity who cannot receive HD-MTX.

131. ADJUVANT CHEMOTHERAPY WITH TEMOZOLOMIDE AND LIPOSOMAL DOXORUBICINE IN THE FIRST-LINE THERAPY OF PATIENTS WITH GLIOBLASTOMA: A PHASE 2 TRIAL

P. Hau, T. Jauch, U. Baumgart, D. Beier, S. Gänßbauer, H. Koch, C. Wismeth, A. Steinbrecher, and U. Bogdahn; Department of Neurology, University of Regensburg, Regensburg, Germany

Temozolomide (TMZ; Temodal/Temodar) recently showed promising results in the first-line therapy of glioblastoma (Stupp, 2004). Pegylated liposomal doxorubicin (PEG-Dox; Caelyx/Doxil) was successfully evaluated in patients with recurrent high-grade glioma (Fabel, 2001; Hau, 2002). Therefore, a combination of both agents seems promising. Here, we update data on a pilot phase 2 trial using this regimen. We initiated a combination regimen consisting of TMZ and PEG-Dox in the first-line therapy of patients with glioblastoma. TMZ is given during standard radiotherapy (initiation) and on days 1 to 5 in 28 days starting 4 weeks after completion of radiotherapy (maintenance). PEG-Dox is given as a short-time infusion in a dose-escalation regimen once prior to radiotherapy (initiation) and on days 1 and 15 starting 4 weeks after completion of radiotherapy (maintenance). PEG-Dox is escalated for 5 mg/m^2 in groups of three patients with a highest dose of 20 mg/m^2 (group 4). Primary end point is dose-limiting toxicity (DLT) in the toxicity phase and time to progression in the efficacy phase. In the first treatment group (5 mg/m^2 of PEG-Dox), one out of 7 evaluable patients had a dose-limiting toxicity (DLT). In the second, third, and fourth treatment groups using 10, 15, and 20 mg/m^2 of PEG-Dox, the regimen was tolerated without DLT. Concerning efficacy in the "treated-patients" analysis of 17 patients treated so far, 1 had a partial response in MRI, and 12 patients had tumor stabilization 4 weeks after conclusion of radiotherapy. As no DLT was observed in dose group 4, the efficacy phase of the study will be performed with 20 mg/m^2 of PEG-Dox. Accrual started in November 2004. Considering the results of the dose escalation phase of this study, the regimen is feasible, tolerable, and able to induce objective responses and stabilizations in patients with glioblastoma using the standard dose of TMZ and 5 to 20 mg/m^2 of PEG-Dox.

132. SEVERE MYELOSUPPRESSION WITH THE FIRST COURSE OF STANDARD DOSE TEMOZOLOMIDE: THE "X" FACTOR

T.S. Armstrong, R. Manning, and M.R. Gilbert; Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Although myelosuppression is a dose-limiting toxicity of most cytotoxic chemotherapies, a reported benefit of temozolomide is that myelosuppression is relatively uncommon. Most studies of temozolomide report an overall incidence of grade 3–4 myelosuppression of 5% to 8%. In the initial trial of 445 cancer patients treated with temozolomide, an 11% incidence of significant myelosuppression was reported in females, with older female patients who received higher doses having a greater chance of developing both neutropenia and thrombocytopenia. In our practice, we recognized a trend toward female brain tumor patients having a higher incidence of myelosuppression during the first course of chemotherapy with conventional dose temozolomide. Therefore, we analyzed the hematologic toxicity in 18 consecutive patients after their first course of treatment with standard dose temozolomide (200 mg/m^2, days 1–5 of a 28-day cycle). A marked difference in the incidence of clinically significant myelosuppression (grade 3 or 4) was noted between male 14% (1/7) and female 46% (5/11) patients. The solitary male patient with myelosuppression experienced both a grade 4 thrombocytopenia and neutropenia. Of the five female patients with myelosuppression, 4/5 experienced a grade 4 neutropenia and 1/5 a grade 3. Two out of five experienced a grade 4 thrombocytopenia and 2/5 a grade 3. Furthermore, four females required hospitalization for febrile neutropenia, whereas none of the male patients developed this complication. Among Caucasian females, 71% (5/7) developed severe myelosuppression, compared with none of the four nonwhite females. This higher incidence of toxicity was not associated with a higher body surface area (BSA), serum creatinine, serum chemistry, smoking status, or concomitant medications. These intriguing preliminary findings prompted a thorough review of the MDACC brain tumor treatment database that is currently under way and will be reported.

133. NATURAL HISTORY AND PROGNOSTIC FACTORS OF GANGLIOGLIOMAS

A. Carpentier and A. Behin; Hôpital de la Salpêtrière, Fédération de Neurologie Mazarin, Paris; Ganglioglioma Study Group of the Association des Neuro-Oncologues d'Expression Française; France

Gangliogliomas are rare neuroepithelial tumors. This study led by the ANOCEF (a French-speaking association of neuro-oncologists) aims at a better assessment of the features and prognostic factors of gangliogliomas. This retrospective study reviewed 176 cases from 9 French teaching hospitals, diagnosis being based on neuropathological records. A central review of cases was proposed. Significant data, including symptoms, pre- and post-operative neuroimaging, pre- and post-operative evolution, surgery and other treatments, were all recorded. Potential prognostic factors for progression were tested in univariate analysis using log-rank tests and in multivariate analysis using a Cox model. Results concern the first 120 cases of the study; final presentation will include updated figures on the whole series. Median age at onset was 17 years (range, 40 days–68 years). Epilepsy was the revealing symptom in 75% of cases. On CT scan and MRI, 65% of tumors were partly cystic, with contrast enhancement in more than 90% of cases. Edema was absent or not significant in 82% of cases; 92% of cases were supratentorial, mostly temporal. Complete resection was performed in 65%, partial resection in 24% of patients. After surgery, epilepsy disappeared or was significantly reduced in 45% and 33% of cases, respectively. Only 4% of tissue samples showed anaplastic features. Recurrence or progression was observed in 19% of patients. Surgery was performed in 74% of those cases. Nonsurgical therapy included radiotherapy (17 patients) and chemotherapy (7 patients). Initial prognostic factors for recurrence/progression in univariate analysis included incomplete resection ($P = 0.001$), anaplastic features ($P < 0.0001$), presence of edema on imaging ($P = 0.03$) and nonepileptic onset ($P = 0.0001$), the latter remaining the only significant prognostic factor in multivariate analysis. Age, sex, tumor size, cystic or calcified components did not influence recurrence or progression. On the whole, 63% of patients were progression-free 5 years after diagnosis; median survival was not reached. Most gangliogliomas share common, homogeneous features. The frequency of contrast enhancement on diagnosis should be underlined. The importance of initial resection is conspicuous. On recurrence, surgery remains the standard option. Data pertaining to radiotherapy and chemotherapy are too sparse to allow precise recommendations and warrant further studies.

134. DELAYS IN RADIATION THERAPY AFTER SURGERY FOR INTRINSIC BRAIN TUMOR ARE ASSOCIATED WITH CLINICAL DETERIORATION IN ELDERLY PATIENTS

L. Kilbride,[1] I. Whittle,[2] and R. Grant[1]; [1]School of Acute & Continuing Care Nursing, Napier University, Edinburgh; [2]Centre for Neuro Oncology, Lothian Universities NHS Trust, Edinburgh; Scotland

Beneficial effects of steroids and surgery are often counterbalanced by brain tumor progression in the interval between surgery and radiotherapy. We prospectively studied a cohort of patients examined post surgery and pre-radiotherapy to study the influence of the following: age ($\leqslant$60 vs. $>$ 60 years), dexamethasone dose (mg), and time interval until start of radiation ($\leqslant$5 weeks vs. $>$30 weeks). Fifty-one consecutive patients with an intrinsic brain tumor consented to be prospectively, objectively assessed postsurgery and immediately preradiotherapy using the Edinburgh Functional Impairment Test (EFIT). The EFIT consists of the nine-hole peg test (NHPT), timed 10-m walk (TMW), Williams Delayed Recall Test (WDRT), and Boston Aphasia Severity Rating Scale (BASRS). Normal values and values for clinically significant change for these tests have previously been published. Patients were grouped as (a) no deficits, (b) limb deficits only, (c) memory deficits only, (d) combination of limb and memory/speech. An initial dose of dexamethasone $\geqslant$12mg/day was arbitrarily considered "high dose". A waiting time of $>$5 weeks was considered to be "delayed". Of 51 patients, 49 (96%) completed EFIT at both time intervals. There were 28 males and 13 females, 34 patients were $\leqslant$60. Median age was 55; 81% of patients had high-grade glioma, 19% "other". Also, 96% were treated with steroids (39% high dose); 45% of patients had an abnormal NHPT, 76% had abnormal TMW, 43% had abnormal WDRT, and 41% had abnormal BASRS after surgery. Younger patients (91%) as opposed to older patients (81%) were more likely to have a degree of functional impairment postsurgery; 51% of patients improved and 22% deteriorated prior to radiotherapy. Patients $\leqslant$60 years were more likely to improve prior to radiotherapy (59% vs. 33%). Older patients were more likely to deteriorate (40% vs. 15%). There was no clear association between initial or mean daily dose of dexamethasone and type or degree on neurological impairment, although fewer patients who deteriorated with memory or speech impairment were given high doses of dexamethasone than those with limb impairment. Only 37% started radiation therapy within 5 weeks. No patient treated within $\leqslant$5 weeks deteriorated compared with 36% $>$5 weeks. We conclude that most young patients with functional impairment post-surgery for intrinsic brain tumors improve in the period up to radiation therapy. Older patients and

patients waiting more than 5 weeks are more likely to have clinical deterioration. Initial high-dose dexamethasone seems to have no clear advantage over lower doses in the postoperative period. Delays in radiotherapy are associated with clinical (and probable radiological) tumor progression

135. FIRST-LINE CHEMOTHERAPY WITH TEMOZOLOMIDE IN RECURRENT/PROGRESSIVE OLIGODENDROGLIAL TUMORS: A PHASE 2 STUDY

R. Rudà, A. Costanza, E. Laguzzi, E. Trevisan, M. Nobile, and R. Soffietti; Neuro-Oncology Service, University of Torino, Neuroscience, Torino, Italy

There are few data regarding the efficacy of first-line temozolomide in recurrent oligodendroglial tumors, and the responses range from 17% to 54%. The purpose of this phase 2 study was to investigate the efficacy and toxicity of temozolomide "standard schedule" in patients with oligodendroglial tumors at first relapse after surgery alone or surgery and radiotherapy. The inclusion criteria were as follows: age ⩾18 yrs, Karnofsky performance status ⩾60; histological diagnosis of oligodendroglioma or oligoastrocytoma (grade II or III according to WHO classification); tumor progression on MRI; chemotherapy naïve. Temozolomide was administered at 200 mg/m^2 for 5 days in cycles of 28 days up to a maximum of 24 cycles in responding or stable patients or to unacceptable toxicity. Primary end point of the study was response basing on Macdonald's criteria. Thirty-four patients are assessable, 24 males and 10 females, with a median age of 47 (range, 18–73). A median of 10 cycles (range, 1–24) were administered. Responses were as follows: CR, 2/34 (6%); PR, 10/34 (29%); SD, 21/34 (62%); PD, 1/34 (3%). Among patients with SD, 3 had a reduction of tumor volume of 20% to 40% (minor response). The overall response rate (CR + PR) was 35%. The maximum tumor response was observed after 3 cycles in 2/12, 6 cycles in 7/12, 9 cycles in 2/12, and 15 cycles in 1/12. Four of 12 responding (CR + PR) patients (33%) and 5 of stable patients (24%) displayed a significant reduction of seizures. Nine of 23 (39%) pure oligodendrogliomas responded, including the 2 patients with CR (grade III tumors), compared to 3/11 (27%) oligoastrocytomas. Ten of 22 enhancing tumors responded (45%) compared to 2/12 nonenhancing tumors (17%), and 47% of grade III responded compared to 26% of grade II. Median TTP was 14 months (range, 2–29), with a PFS at 6 months of 88% and at 12 months of 59%. Grade III–IV myelotoxicity was observed in 26% of patients. Temozolomide shows activity as first-line treatment in oligodendroglial tumors at first relapse (CR + PR + minor response: 44%). Pure oligodendrogliomas and enhancing and high-grade tumors tend to respond better. A long-term treatment with temozolomide is well tolerated.

136. PHASE 1 CLINICAL TRIAL AND PHARMACOKINETIC STUDY OF KARENITECIN IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMAS: A STUDY OF THE NABTT CNS CONSORTIUM

S. Grossman, J. Supko, K. Carson, S. Phuphanich, T. Batchelor, D. Peereboom, B. Nabors, G. Lesser, S. Desideri, and F. Hausheer. NABTT CNS Consortium, Baltimore, Maryland, USA

Camptothecins have clinical activity in colorectal, ovarian, lung and, to a lesser extent, brain cancers, but their use has been limited by gastrointestinal and hematopoetic side effects. Karenitecin is a highly lipophilic camptothecin derivative, with a 2-(trimethylsilyl)ethyl substituent at the 7-position, rendering the terminal lactone ring much more resistant to inactivating hydrolysis under physiological conditions than most other campotothecins. The pharmacokinetics of several camptothecin analogues is dramatically affected by the concomitant use of hepatic enzyme-inducing antiepileptic drugs (EIAEDs). This study was conducted to (1) determine the maximum tolerated dose (MTD) of karenitecin in adults with recurrent malignant glioma, (2) describe the effects of EIAEDs on its pharmacokinetics, and (3) obtain preliminary evidence of activity. Karenitecin was administered intravenously over 60 min once a day for 5 consecutive days, every 3 weeks. The starting dose was 1.00 mg/m^2 per day. The dose was escalated by using the continual reassessment method independently in cohorts stratified by EIAED use. Three patients were treated at each dose level. Intrapatient dose escalation was not permitted. Treatment was continued until disease progression, treatment-related dose-limiting toxicity, or patient withdrawal. Pharmacokinetic samples were obtained to define the total karenitecin plasma profile for the first dose of drug. We accrued 32 pts (20 males) to this study. Their median age was 52 years (range, 34–72), median KPS was 90 (range, 60–100); 78% of the patients had glioblastoma multiforme and the remainder had anaplastic gliomas. One prior chemotherapy regimen was permitted. Dose levels evaluated in the +EIAED arm were 1.0, 1.5, 1.7, 1.9, and 2.1 mg/m^2 and in the –EIAED cohort 1.0, 1.5, and 1.8 mg/m^2. Myelosuppression was the major toxicity observed (WBC > platelets > RBC), with a short-lived grade 3-4 neutropenia or thrombocytopenia occurring in 28% and 16% of patients, respectively. The MTD was determined to be 2.0 mg/m^2 in +EIAED patients and 1.5 mg/m^2 in the –EIAED patients, a difference of 25%. Statistical comparisons to assess the influence of EIAEDs were based upon data for patients treated with 1.5 mg/m^2 where there were maximal patient numbers (7 +EIAED, 6 –EIAED). In comparison to the –EIAED cohort, the mean (±SD) maximum concentration of drug in plasma was 36% lower (22.9 ± 12.1 vs. 35.8 ± ng/ml), and the total mean total body clearance was 29% higher (13.5 ± 13.5 vs. 10.5 ± 3.3 l/h/m^2) in the +EIAED group. The median survival after starting therapy was 6.5 months (95% CI, 4.0–9.7 months), and 25 of the 32 patients are deceased. No complete or partial responses were observed, although 12 patients were treated at or above the MTD. As observed with other camptothecin analogues, karenitecin elimination is enhanced by EIAED administration. Single agent karenitecin does not appear very active in recurrent high-grade gliomas.

137. LONG-TERM TREATMENT WITH TEMOZOLOMIDE IN HIGH-GRADE GLIOMAS IS FEASIBLE AND LEADS TO LONG-TERM SURVIVAL IN A SIGNIFICANT SUBSET OF PATIENTS

D. Beier,[1] P. Hau,[1] T. Jauch,[1] D. Koch,[2] T. Hundsberger,[3] S. Schuth,[4] and U. Bogdahn[1]; [1]Neurology, University of Regensburg, Regensburg; [2]Neurosurgery and [3]Neurology, University of Mainz, Mainz; [4]Essex Pharma GmbH, Muenchen; and the Long-Term Temozolomide Study Group; Germany

Although survival in patients with high-grade gliomas is relatively poor, warranting intensified treatment approaches, postoperative radiochemotherapy is frequently limited by bone marrow toxicity, usually terminating chemotherapy after 6 cycles of chemotherapy. Clinical studies using temozolomide (Temodar; TMZ) have produced promising results concerning survival and long-term feasibility, which is in part due to the lacking cumulative toxicity of TMZ. Nevertheless, no systematic data concerning long-term feasibility of TMZ have been acquired yet. Within a retrospective field analysis, German neuro-oncologists were approached to report on their treatment strategies with TMZ in high-grade gliomas using a standardized questionnaire. Long-term application of TMZ (more than 12 cycles or more than 12 months of any dose) were analyzed and evaluated for feasibility, efficacy, and tolerability. Altogether, 128 patients with WHO Grade III or IV gliomas who fulfilled the study criteria were identified by 49 neuro-oncologists. Most of the patients were treated with the standard scheme using 150 to 200 mg/m^2, and some were treated according to protocol EORTC 26981/22981 or by modified schemes. In first-line treatment (n = 64), the median number of applied cycles was 13 (range, 12–40), with a mean progression-free duration of 56 weeks (range, 52–160 weeks). Recurrent patients (n = 55) received a median number of 14 cycles (range 12 to 44 cycles), with a median duration of 62 weeks (range, 52–176 weeks). Grade 3 or 4 (NCI-CTC) toxicity concerning the gastrointestinal system (n = 7), leukopenia (n = 13), thrombocytopenia (n = 7), or infection (n = 5) was observed only in a small amount of patients. Nevertheless, toxicity data may underestimate the true incidence of toxicity due to the retrospective character of the study. Although this was a retrospective analysis, we could identify a significant number of patients with long-term application of TMZ. Up to 44 cycles were given, and times of up to 160 weeks in the first-line and 176 weeks in the recurrent setting without signs of tumor progression could be reached, indicating that patients with active high-grade gliomas may have well-controlled disease over significant periods of time.

138. SUBCORTICAL MAPPING OF MOTOR TRACT PATHWAYS IMPROVES SURGICAL REMOVAL OF HUMAN GLIOMAS

L. Bello, F. Acerbi, C. Giussani, G. Carrabba, M. Fava, V. Songa, P. Baratta, R.M. Villani, and S.M. Gaini; Neurosurgery, University of Milano, Milano, Italy

Surgery for lesions involving motor areas or pathways requires the identification of functional cortical areas to reduce tumor resection morbidity. Intraoperative identification of functional descending motor pathways has been recently advocated as a promising technique to further reduce postoperative morbidity. We present our experience with subcortical identification of motor functional tracts during awake surgery for removal of gliomas involving motor tract pathways. There were 45 patients (25 males, 20 females, age ranging from 22 to 45 years) harboring a low-grade (39) or high-grade (6) glioma located in the frontal lobe and involving motor cortex and/or pathways. In 23 patients, the tumor was also involving language areas and/or pathways. Cortical and subcortical mapping was performed by the use of an Ojemann stimulator and using the largest current that did not produce afterdischarge. Cortical areas were found in all cases. Subcortical

stimulation by using the same current threshold was also applied during tumor resection. Subcortical stimulation mapping was alternated by tumor resection in a back and forth fashion. Motor response was monitored clinically as the appearance of an involuntary motor response or by the use of a continuous multichannel EMG recording. For lesions involving language tracts, patients were submitting to periodic counting, confrontation naming, and verbal generation tests. Subcortical motor pathways were found in 55% of patients, and this was associated with a transient neurological deficit in 62.5%. If subcortical sites were not found, the chance of temporary deficits was 8%. Subcortical language sites were identified in 23.8% of cases, with 1 permanent deficit. In case of deficit, the resection was stopped, the patient asked to rest. When the tasks were normalized, subcortical stimulation was reapplied. In case of response, resection continued in the neighboring structures. Patient fatigue was observed in 20% of cases. Extent of removal was total in 68% and subtotal in 15% of cases. Electrical seizures occurred in 2 patients and were successfully controlled with cold water irrigation. Repeated subcortical stimulations should be alternated with tumor resection to follow white fibers closely. Identification of subcortical sites is associated with a higher risk of transient postoperative deficits. Postoperative deficits are low. Repeated subcortical stimulation further improves safety of resection of tumor involving motor pathways.

139. OUTCOME OF PATIENTS UNDERGOING SURGERY FOR BRAIN METASTASES

F. Bokstein[1] and Z. Ram[2]; [1]Neuro-Oncology Service and [2]Neurosurgery, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Brain metastases (BM) represent one of the most severe complications of solid cancers. Surgical treatment of this debilitating condition, if feasible, seeks not only to prolong the survival but also to improve immediately the neurological condition and quality of life of the patients. Our objective is to report the results of surgical treatment of 82 consecutive patients with BM treated in Tel Aviv Sourasky Medical Center since March 2003. Eighty-two patients (47 females and 35 males) with the median age of 61 (range, 44–82) underwent surgical removal of BM. The most common primary cancers were NSCLC (33 pts, 40%) and breast cancer (13 pts, 16%). In 20 patients (24%) BMs were first presentation of their malignancy. At diagnosis of BM, the majority of patients (58, 71%) had active systemic disease. Nine patients (11%) already had BM in the past, and 7 of them (9%) received previously neurosurgical treatment. Sixty patients (73%) had single BM; in 7 patients (9%) there were 2 BM; 14 patients (17%) suffered from multiple (>2) BM. In most cases of multiple BM only one (rarely two) symptomatic lesion(s) responsible for neurological deterioration were removed. The operation resulted in immediate neurological improvement in 72 patients (88%). The condition of 7 patients (9%) did not change. Three patients (3%) died within 2 weeks after the operation. Surgical removal of BM succeeds to achieve in most cases an immediate clinical improvement and should be considered in neurologically symptomatic patients, including patients with active systemic disease and multiple brain metastases.

140. PATTERN OF RECURRENCE IN PATIENTS WITH GLIOBLASTOMA MULTIFORME TREATED WITH AN ANTIANGIOGENIC THERAPY

J. Tuettenberg,[1] R. Grobholz,[2] R. Erber,[1] T. Korn,[1] F. Wenz,[3] and P. Vajkoczy[1]; [1]Neurosurgery; [2]Pathology; [3]Radio-Oncology, Klinikum Mannheim der Universitat Heidelberg, Mannheim, Germany

Glioblastoma multiforme is the prototype of an angiogenic tumor and thus predestined for an antiangiogenic therapy. But a possible escape mechanism of the tumor cells is increased invasion. The aim of this study was to evaluate the progression free- and overall survival in patients with glioblastoma multiforme, treated with a continuous, low-dose chemotherapy with temozolomide and rofecoxib, with special respect to the localization of tumor recurrence. Twenty-two patients with glioblastoma multiforme received after operation and radiation therapy a continuous, low-dose chemotherapy with temozolomide (up to 20 mg/m^2) and the COX-II inhibitor rofecoxib. Clinical and MRI follow-up examination was done every 8 weeks. Mean follow up time was 20 months. Tumor tissue was analyzed for microvessel density, COX-II expression, and VEGF expression in 13 patients. Mean progression-free survival of all patients was 9.7 months, and mean overall survival was 16.9 months. Of 22 patients, 14 (67%) suffered a tumor recurrence distant to the original tumor localization. These patients had a slightly shorter overall survival. Patients with a higher microvessel density responded significantly better to the therapy (PFS 11.7 vs. 6.7 months). There was no relationship of the immunohistochemical markers and the incidence of distant tumor recurrence. Despite the dramatic increase in distant tumor recurrences compared to historical controls, the continuous, low-dose chemotherapy seems to be a promising therapy option in highly vascularized glioblastoma, since the progression free-survival and the overall survival compare very well to actual studies.

141. PHASE II STUDY OF CLORETAZINE FOR THE TREATMENT OF ADULTS WITH RECURRENT MALIGNANT GLIOMA

A. Desjardins, J. Vredenburgh, M. Badruddoja, J. Quinn, D. Reardon, J. Rich, S. Sathornsumetee, K. Penne, D. Bigner, and H. Friedman; Duke University Medical Center, Durham, North Carolina, USA

Alkylating agents are an important class of chemotherapeutic drugs used for the treatment of CNS neoplasms. A novel class, the 1,2-bis(sulfonyl)hydrazines (BCHs), has been synthesized. The BCH compounds produce chloroethylating species but do not generate vinylation or hydroxyethylation events unlike the nitrosoureas. The chloroethylating species preferentially alkylates the O^6-position of guanine. Cloretazine is a novel BCH compound. In preclinical studies of xenograft models of malignant gliomas Cloretazine has demonstrated potent antitumor effect. In the HT29 tumor cell line that expresses O^6-alkylguanine alkyltransferase (AGT), high concentrations of Cloretazine had similar cytotoxicity compared to a cell line that does not express AGT. We performed a phase 2 study of Cloretazine in adult patients with recurrent malignant glioma. Eligibility included adult patients with recurrent malignant glioma. Patients were divided into two strata: stratum 1, recurrent/progressive glioblastoma multiforme (GBM), and stratum 2, recurrent/progressive anaplastic astrocytoma (AA) or anaplastic oligodendroglioma (AO). Each patient was treated with a 15-min infusion of Cloretazine at 300 mg/m^2 every six weeks. Responses were assessed by functional and imaging criteria after one cycle (6 weeks). Patients were treated until disease progression or unacceptable toxicity. The primary end points were to determine the activity, the toxicity, the time to progression, and survival of patients with recurrent malignant glioma treated with Cloretazine. To date, 38 patients have been enrolled, 32 in stratum 1 (planned accrual [PA] = 32), and six in stratum 2 (PA = 38), for a median age of 55.5 years (range, 31–68) and a 71% male population. Thirty-six patients are assessable for response. Twenty-two patients have presented disease progression after the first cycle. Thirteen patients remained stable for at least two cycles (12 weeks), two patients with minimal responses. Toxicities included thrombocytopenia (6 grade 3, 10 grade 4), neutropenia (2 grade 3, 3 grade 4), leukopenia (1 grade 3, 1 grade 4), AST elevation (1 grade 3), ALT elevation (1 grade 3, 1 grade 4), and infection (1 grade 3). Cloretazine shows very limited disease stabilization in recurrent GBM. However, it is too early to determine the response in recurrent AA/AO. Toxicities are limited to hematologic events and transient transaminase elevation.

142. CELLULAR PATHWAYS MEDIATING APO2L/TRAIL-INDUCED APOPTOSIS IN GLIOMA CELLS

J. Rieger, M. Weller, B. Frank, and *W. Wick*; Laboratory of Molecular Neuro-Oncology, Department of General Neurology and Hertie-Institute for Clinical Brain Research, Tuebingen, Germany

Apo2L ligand/TNF-related apoptosis-inducing ligand (APO2L/TRAIL) is a member of the TNF family that has been shown to induce apoptosis in malignant cells but not in most normal cells. Moreover, Apo2L/TRAIL exhibits synergistic effects with irradiation or chemotherapeutic drugs including lomustine (CCNU) and temozolomide. Previous work showed that most glioma cell lines are resistant to APO2L/TRAIL-induced apoptosis unless co-sensitized by cotreatment with an inhibitor of protein synthesis such as cycloheximide (CHX). Resistance is also observed in some cell lines expressing high levels of the agonistic receptors TRAIL-R1 and TRAIL-R2. However, even in the presence of CHX, some cell lines are still resistant to APO2L/TRAIL. Further, the mechanism by which CHX renders resistant cell lines sensitive to APO2L/TRAIL has remained largely unclear. Because PI3K and casein kinase II signaling have been shown to mediate survival, we investigated whether inhibitors of these signaling paths would modulate the sensitivity of glioma cell lines to APO2L/TRAIL. We find that the inhibition of PI3K by LY294002 is less potent in sensitizing glioma cell lines to APO2L/TRAIL than the inhibition of casein kinase II by DRB. In addition, there is a strong correlation between the sensitizing effects of CHX, LY294002, and DRB. Cell lines that are resistant to APO2L/TRAIL, even in the presence of CHX, are not rendered sensitive to APO2L/TRAIL by these inhibitors. This suggests that there are common molecular survival pathways that are modified by inhibition of protein synthesis (CHX), PI3K (LY294002) and casein kinase II (DRB), all resulting in sensitivity to APO2L/TRAIL-induced apoptosis in glioma cells.

143. MATRIX METALLOPROTEINASE INHIBITOR–INDUCED JOINT-RELATED TOXICITY PREDICTS PROLONGED PROGRESSION-FREE SURVIVAL IN RECURRENT HIGH-GRADE GLIOMA TRIALS

M. Groves, Vinaykumar Puduvalli, Charles A. Conrad, Mark R. Gilbert, Kenneth R. Hess, W.K. Alfred Yung, and Victor A. Levin; Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Recent reports have highlighted relationships between a drug's toxicity and its anti-cancer effect. Variability in drug-induced toxicity can be due to drug metabolism, drug-drug interactions, or pharmacogenomic factors. We reviewed outcomes for 93 patients treated on 2 prospectively conducted phase 2 studies evaluating temozolomide (TMZ) (150–200 mg/m^2/day, days 1–5) plus the matrix metalloproteinase inhibitor marimastat (MRM) (25 mg BID days 8–28) for recurrent glioblastoma multiforme or anaplastic glioma. Histologic subgroups were analyzed individually and together, looking for the effect of toxicity due to MRM (presence and grade of joint-related toxicity (JRT)) and its relationship to progression-free survival (PFS). We reviewed outcomes for all 93 patients, 44 with GBM, 23 with AA, 14 with AO, 12 with AOA. PFS was significantly longer in pts with JRT (median 36 weeks vs. 9 weeks, $P < 0.0001$, hazard ratio = 0.3). This difference remained highly significant after adjustment for clinical factors (age, KPS, extent resection, histology, prior nitrosourea, enzyme-inducing anti-epileptic drug [EIAED] status). However, the difference was bigger in patients on EIAEDs (EIAED: 69 weeks vs. 9 weeks, No EIAED: 24 weeks vs. 9 weeks). This interaction was significant after adjustment for the clinical factors ($P = 0.032$), but not prior to adjustment ($P = 0.20$). The adjusted hazard ratio for JRT is 0.4 in pts without EIAED, and 0.1 in pts with EIAED. There was a graded difference in PFS with grade of JRT (0: Median PFS = 9 weeks, 1: 24 weeks, 2: 33 weeks, 3: 38 weeks). Again these differences were more dramatic in patients taking EIAED. After adjustment for clinical factors, this interaction between EIAED use and JRT grade was significant ($P = 0.056$). The adjusted hazard ratios by grade for patients not taking EIAEDs are 0: 1.0, 1: 0.7, 2: 0.5, and 3: 0.3, and for patients taking EIAEDs they are 0: 1.0, 1: 0.5, 2: 0.2, and 3: 0.1. The modifying effect of EIAEDs on JRT was also seen for CR/PR response: In patients not taking EIAEDs, CR/PR is 0% for patients with and without JRT, but in patients taking EIAEDs, 3% of the 29 patients without JRT had CR/PR, while 27% of the 33 patients with JRT had CR/PR (20% in grade 1, 14% in grade 2, and 33% in grade 3). For those patients who developed JRT, median time to its onset was 7.7 weeks (1–109). Median time to the development of the most severe JRT was 15 weeks (1–109). This analysis demonstrates the association of MRM-induced JRT with significantly prolonged PFS in patients with recurrent malignant glioma. Patients with JRT on EIAEDs have further enhancement of PFS. Pharmacokinetic and pharmacogenomic interactions may be involved. Further trials assessing the efficacy of this combination and its relationship to EIAEDs, drug pharmacokinetics, and drug-induced JRT are warranted.

144. TIME TO TUMOR PROGRESSION (TTP) AND QUALITY OF LIFE (QOL) FOLLOWING PROPYLTHIOURACIL INDUCTION OF CHEMICAL HYPOTHYROIDISM IN FAILED MALIGNANT GLIOMAS

E. Linetsky,[1] A. Hercbergs,[2] S. Dotan,[1] E. Shalom,[1] and *T. Siegal*[1]; [1]Leslie and Michael Gaffin Center for Neuro-Oncology, Hadassah Hebrew University Hospital, Jerusalem, Israel; [2]Department of Radiation Oncology, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Under hypothyroid conditions, gene expression of several growth factors and their receptors is downregulated. A previous phase 1/2 clinical study suggested that high-dose tamoxifen given in association with chemical induction of HT prolongs survival of pts with recurrent malignant gliomas. However, a major drawback of induced HT may be a potential negative effect of HT on the QOL of these vulnerable pts. Our objective was to evaluate the toxicity and effect of induced HT on TTP and QOL of pts with failed malignant gliomas. Propylthiouracil was used to induce HT in 20 pts (median age 49 years) with failed malignant gliomas (8 GBM, 7 anaplastic astrocytomas, 5 anaplastic oligoastrocytomas). Tamoxifen (240 mg/kg/d) was given only to pts who became hypothyroid. Clinical evaluation and QOL were assessed monthly by using a standard questionnaire. Thyroid function tests were done weekly until HT was induced (TSH > 8) and then repeated monthly and MRI every 8 weeks. Twelve pts (60%) achieved HT (median TSH 9.8) within a median time of 3 months (range, 1.5–6 months). KPS, age, gender, treatment with enzyme inducing antiepileptic drugs, and baseline TSH levels did not differ between the 2 groups of pts retrospectively stratified as achieving or not achieving HT. Induction of HT often caused mild fatigue but no other clinical symptoms of HT. Median TTP was longer in the HT group (5 months vs. 2.7 months, $P = 0.002$), with 6 months PFS of 33% vs. 0%. Clinical improvement was noted in 8/12 pts (66%) with HT and led to dose reduction or withdrawal of steroid therapy. Marked decrease in seizure activity was noted, and 2 pts became seizure free. MRI showed objective response in 25% of the HT group and stable disease in 75%. At baseline evaluation, the QOL did not differ between pts who later achieved HT and those who did not. In the HT group, QOL improved after 3 months and differed significantly from QOL of the group without HT based on categories of weakness ($P = 0.01$), fatigue ($P = 0.01$), depression ($P = 0.002$), interference with family life ($P = 0.001$), global QOL ($P = 0.02$), and global health ($P = 0.004$) evaluations. Induction of HT is associated with significantly longer median TTP in pts with failed malignant gliomas. Clinical and objective responses are associated with significant improvement in QOL despite the induction of HT. Further studies are warranted to evaluate the benefit of early induction of HT shortly after diagnosis and prior to tumor recurrence.

145. SURGICAL TREATMENT OF LOW-GRADE GLIOMA RECURRENCES

L. Bello,[1] F. Acerbi,[1] C. Giussani,[1] J. Casagrande,[2] P. Casagrande,[2] D. Spagnoli,[1] M. Caroli,[1] R. Campanella,[1] G. Tomei,[2] and S.M. Gaini[1]; [1]Neurosurgery, University of Milano, Milano; [2]Neurosurgery, University of Insubria, Varese; Italy

Treatment of low-grade gliomas recurrences is controversial and consists of a wait-and-see policy or treatment based on surgery, chemo, and/or radiotherapy. The role of surgery, particularly of repeated surgeries, should still be defined. We present the results of the surgical treatment of 158 patients with a recurrent low-grade glioma admitted at our Dpts during the decade 1990 to 2000. They were 68 males and 91 females, age ranging from 22 to 69 years. Follow-up ranged from 50 to 264 months (median 156 months). Only patients with a low-grade astrocytoma, mixed glioma, or oligodendroglioma were included. Time to first recurrence correlated with extent of surgery at first operation, tumor removal, and extension of the tumor toward deep structures. Seventy-eight percent of tumors recurred in the previous location. At surgery, 42% of patients were symptomatic for seizures or neurological deficits, and in 58% an enlarging mass or an enhancing lesion on MR scan was documented. Global 50% survival was 126 months. Survival correlated with malignant transformation. Malignant transformation occurred in 57% of cases, at first or further surgeries, and correlated with extent of surgery at first operation and tumor extension and volume. Survival and malignant transformation correlated also with extent of resection at surgery for recurrence removal. Time to further recurrence correlated with extent of resection. A second surgery or further surgeries were performed in 39%, 22, and 4.5% of cases. Further surgeries did not result in an increase of morbidity (0.7% mortality, 11% of transient morbidity). Time to recurrence decreased with the increase in the number of surgeries (from 72 to 23 months). Percentage of total removal decreased as well (from 51 to 26%). In case of early recurrence, with no findings of malignant transformation, early surgery was associated with a longer survival than when surgery was postponed and a wait and see policy was adopted. When a recurrence appeared, the combination of surgery eventually followed by chemotherapy was associated with a longer survival than when surgery followed chemotherapy. Awake surgery and cortical and subcortical stimulations reduced morbidity and improved resection of lesions close to or involving functional motor or language areas of pathways. Surgery and repeated surgeries for low-grade glioma recurrences are safe, control or relieve symptoms, and may postpone the use of adjuvant therapies until the time of appearance of malignant transformation.

146. RESPONSE OF RECURRENT PILOCYTIC ASTROCYTOMA IN ADULTS TO TEMOZOLOMIDE (TMZ)

D. Macdonald; Medical Oncology, London Regional Cancer Centre, London, Canada

The objective of this study was to evaluate the activity of temozolomide (TMZ) chemotherapy for recurrent pilocytic astrocytoma in adults. Pilocytic astrocytomas (PA) are WHO grade I tumors that usually occur in children. Surgical resection is often curative. Incompletely resected PAs are often treated with radiotherapy (RT). Hypothalamic and optic pathway PAs in children often respond to carboplatin- or lomustine-based chemotherapy, but side effects are common and sometimes serious. There is little information on chemotherapy in adult PA. TMZ is a new, well-tolerated, oral chemotherapeutic agent with activity in newly diagnosed and recurrent malignant gliomas and in low-grade gliomas. Thus, it was appropriate to evaluate TMZ in PA. The design of the study was a case series. Two adults with recurrent PA responded to TMZ. A man (case 1), age 54, had a cerebellar PA partly resected in 1995 and then received RT. Multifocal recurrence was biopsy-confirmed in February 2002. He received 12 cycles of standard oral TMZ (200 mg/m^2/day × 5 days, every 28 days). A partial response (PR) was seen after 6 cycles and a complete response (CR) after 12 cycles. He remains in CR over 20 months off treatment. A woman (case 2), age 27, had partial resection of a right temporal PA in 1987. MRI progres-

sion was biopsy-confirmed in January 2000 and treated with RT. Symptomatic MRI progression was treated with standard TMZ starting November 2001. A minor response was seen after 9 cycles and a PR after 12 cycles. She received 24 cycles of TMZ with continuing PR more than 10 months off treatment. TMZ was well tolerated in both patients. Temozolomide is active in recurrent PA, producing sustained responses with little toxicity, but prolonged treatment may be needed. Temozolomide should be evaluated in newly diagnosed pilocytic astrocytoma.

147. SEQUENTIAL ADMINISTRATION OF TEMOZOLOMIDE AND FOTEMUSTINE IN DE NOVO GBM PATIENTS

N. Oktar, N. Ozdamar, and T. Dalbasti; Department of Neurosurgery, Ege University, Faculty of Medicine, Izmir, Turkey

The DNA repair enzyme O6-alkylguanine-DNA alkyl transferase (AT) mediates resistance to chloroethylnitrosoureas. Agents depleting AT such as DTIC and its new analogue temozolomide (TMZ) can reverse resistance to chloroethylnitrosoureas. There are some reports concerning the pharmacokinetics and sequential administration of temozolomide and fotemustine treatment in malignant melanoma and malignant glioma patients. In this clinical study response to sequential administration of p.o. TMZ and infusional fotemustine was evaluated in de novo GBM patients after irradiation. Forty patients with primary glioblastoma multiforme were included in this study. In group I, ten patients were treated with surgery and radiation therapy alone. In group II, ten patients received 200 mg/m^2 TMZ for 5 days in 26-day intervals for 6 months. In group III, ten patients received i.v. 100 mg/m^2 fotemustine every week for 3 weeks and then in three-week intervals. In group IV, ten patients were treated with a 200-mg/m^2 oral dose of TMZ for 5 days, and fotemustine in a dose of 100 mg/m^2 was given intravenously on day 15, one week after TMZ, for six months. Complete and partial response rates were evaluated for six months both with MRI and with neurological evaluation. Time to tumor progression, Ki67 %, Karnofsky performance status, and survival rates for each group were calculated. Combination therapy of two agents were well tolerated in 80% of the patients without any significant side effects. Significant although statistically not proven better prognostic results, even a complete response, were achieved in the sequential administration of TMZ and fotemustine. Other ways of administration that are discussed, such as i.v. TMZ or locoregional administration, have yet to be established.

148. DO WE NEED A MORE DIFFERENTIATED APPROACH FOR THE TREATMENT OF NEUROCYTOMAS?

D. Rades,[1] F. Fehlauer,[1] K. Lamszus,[2] C. Hagel,[3] and S.E. Schild[4]; [1]Radiation Oncology, [2]Neurosurgery, and [3]Neuropathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [4]Radiation Oncology, Mayo Clinic Scottsdale, Scottsdale, Arizona, USA

Reports on neurocytoma became more frequent after 1995, which reflects the increasing importance of this entity. This analysis aims to give treatment recommendations for well-differentiated neurocytomas, atypical neurocytomas (MIB-1 index >3%, atypical histology), and neurocytomas in children. All reported neurocytoma cases and our previous papers were reviewed for age, gender, histology, MIB-1-labeling index, extent of surgery, radiotherapy (RT), local control (LC), and survival (OS). More data were obtained directly from the authors. This approach provided more detailed information and a longer follow-up period than the data from the literature alone. Complete resection (CTR), CTR + RT, incomplete resection (ITR), and ITR + RT were compared for LC and OS at 5 years and at 10 years. Comparisons were performed for the whole series, for well-differentiated lesions, for atypical lesions, and for neurocytomas in children ⩽18 years. In the ITR + RT group, radiation doses ⩽54Gy and 54 Gy were compared. Data were complete in 427 patients (70 children, 357 adults); 321 patients had well-differentiated lesions, and 86 had atypical lesions. Well-differentiated lesions were associated with better LC and OS than atypical lesions ($P < 0.001$). CTR was significantly superior to ITR ($P < 0.001$). After CTR, outcome was not significantly improved by RT. After ITR, RT improved OS in the whole series ($P = 0.04$), in patients with well-differentiated lesions ($P = 0.03$), and in patients with atypical lesions ($P = 0.05$), but not in children ($P = 0.19$). LC was significantly improved in all groups ($P < 0.001$, children $P = 0.01$). A dose >54 Gy appeared beneficial only after ITR of atypical lesions ($P = 0.05$ for LC). In children, ⩽50 Gy and >50Gy appeared comparably effective for LC and OS. CTR should be performed whenever possible and does not require postoperative RT. After ITR, RT improves outcome. The 54-Gy dose appears sufficient for long-term control of well-differentiated lesions; 50–54 Gy appears sufficient for children; 56–60 Gy is required for atypical lesions, depending on the treatment volume and the expected toxicity.

149. CONCURRENT ADMINISTRATION OF PHENYTOIN DOES NOT AFFECT THE PHARMACOKINETICS OF CELECOXIB IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

S. Grossman, J. Olson, T. Batchelor, D. Peereboom, G. Lesser, S. Desideri, X. Ye, T. Hammour, and J. Supko; NABTT CNS Consortium, Baltimore, Maryland, USA

Evidence implicating cyclooxygenase-2 (Cox-2) in tumor angiogenesis has generated interest in evaluating the role of inhibitors of this enzyme, such as celecoxib, in cancer treatment. Hepatic metabolism by cytochrome P450 2C9 (CYP2C9) is a major route of elimination for celecoxib. Antiepileptic drugs that are frequently used in glioblastoma patients induce a number of CYP450 enzymes, including CYP2C9. This study was conducted to determine the effects of enzyme-inducing antiepileptic drugs (EIAEDs) on the pharmacokinetics of celecoxib. Secondary objectives were to determine the safety of celecoxib in this setting and estimate the duration of survival when celecoxib was administered concurrently with radiation therapy in patients with newly diagnosed glioblastoma multiforme. Patients were divided into 2 groups (+EIAED and –EIAED) based on anticonvulsant use. Celecoxib administration began one week before conventional radiation therapy was started. A single 400-mg dose of celecoxib was taken on the first day of treatment. Twice-daily dosing (400 mg every 12 h) was begun the following day and continued until tumor progression, dose-limiting toxicity, or study withdrawal. No adjuvant chemotherapy was permitted. Pharmacokinetic blood samples were obtained serially for 24 h after the first dose of celecoxib and prior to a morning dose once a week during weeks 2 to 6. A validated LC/MS assay was used to determine the concentration of celecoxib in plasma. A total of 35 patients (22 +EIAED and 13 –EIAED) were accrued from October 2003 to September 2004. Fourteen patients (40%) were women, and 5 (14%) were African American. All patients in the +EIAED group were receiving phenytoin. The study was closed before reaching the stated goal of 22 patients in each cohort in light of the positive results of the EORTC adjuvant temozolomide study. Celecoxib therapy was very well tolerated, and no gastrointestinal bleeding or renal toxicities were noted. Only one patient complained of grade 3–4 epigastric distress and this responded to a dose reduction. Pharmacokinetic data is currently available for 14 +EIAED patients and 10 –EIAED patients. There was no significant difference ($P = 0.66$) between the maximum concentration of drug in plasma following administration of the first dose in the +EIAED (1.85 ± 0.71 μg/ml) and –EIAED (1.98 ± 0.48 μg/ml) groups. Similarly, the area under the plasma concentration-time profile from time zero to 24 h was similar ($P = 0.79$) for the two groups (+EIAED, 14.9 ± 5.7 μg·h/ml; –EIAED, 13.9 ± 9.1 μg·h/ml). Survival figures remain too premature to report, as 31 of the 35 patients remain alive. These preliminary results strongly suggest that the plasma pharmacokinetics of celecoxib is not significantly affected by the concomitant administration of EIAEDs. Furthermore, plasma levels in this study are similar to data in published reports on patients with arthritis who were not receiving concomitant glucocorticoids. This drug was well tolerated in this clinical setting. Survival results will be presented as the data matures.

150. A MONO-INSTITUTIONAL, PHASE 2, DOSE-ESCALATION STUDY OF SAFETY/EFFICACY OF NEWER, RECENTLY MARKETED, ANTICONVULSANT DRUGS FOR TUMOR-ASSOCIATED EPILEPSY (TAE)

M. Riva, A. Gatti, F. Imbesi, G. D'Aliberti, A. La Camera, C.A. Defanti, and M. Collice; Ospedale Niguarda, NeuroSciences, Milano, Italy

Seizures are a common symptom of brain tumors and have a significant impact on neurobehavioral functioning and quality of life (QoL). Patients with seizures are often fearful of having them and may become socially isolated. Old anticonvulsant drugs (AEDs) - such as phenytoin (PHT), phenobarbital (PB), and carbamazepine (CBZ) – induce the CYP450 enzyme system (interfering with other commonly used drugs and increasing chemotherapeutic agent clearance) and produce, in these pts, more idiosyncratic and side effects than in a general epilepsy-pt population. Furthermore, one third of these pts could be defined as "drug-resistant," and only one third achieves successful seizure control. At present, physician practice in TAE prophylaxis is characterized by significant behavioral heterogeneity, and literature lacks data concerning efficacy and toxicity of new, recently marketed, AEDs. A monoinstitutional phase 2 study has been carried out to compare monotherapy efficacy (topiramate, lamotrigine, and oxcarbazepine) in a dose-escalation study for TAE. The characteristics of the three groups are as follows: (1) lamotrigine group: 33 primary and 7 metastatic brain tumor patients (P-MBT); 52% epilepsy as symptomatic onset, 48% in the follow-up; treatment (median) 6 months; efficacy in 62%; toxicity 4 pts: 1 Stevens-Johnson, 3 intense skin reaction; (2) topiramate group: 38 PBT and 14 MBT; 44% epilepsy as symptomatic onset, 56% in the follow-up; treatment (median) 8 months; efficacy in 76%; no toxicity; (3) oxcarbazepine group: 49 PBT, 11 MBT; 57% epilepsy as symptomatic onset, 43% in the follow-up; treatment (median) 8 months; efficacy in 71%; toxicity 7 pts:

5 hypo-Na^+, 2 intense skin reaction. We conclude that in the present study, LTG, TPM and Ox-CBZ have shown the following, even in monotherapy: (1) a clinical efficacy in seizure control similar to the one obtained with "old-AEDs": 30.3% of pts suffered from one or more Ps and/or GTCS in the whole follow-up, requiring polytherapy in about 10% (better for TPM and OxCBZ than LMT); (2) fewer side effects (especially for TPM); (3) no significant pharmacological interferences; and (4) no need for hematological controls. Some general considerations are possible: (1) Br Mets-TAE is more responsive to treatment than high-grade glioma-TAE (75 vs. 64%); (2) plasma levels of the new AEDs do not correlate with clinical control; (3) maximum tolerated doses seem to be advisable and dose-increase often results in a satisfactory efficacy after a seizure. However, the most relevant practical problems in neurooncological pts treated with 2nd-generation AEDs are (1) the necessity of a long period of titration, to avoid side effects and (2) the only possibility of an oral administration. In conclusion, new AEDs have proved to be useful and handy drugs in the management of a "difficult" epilepsy such as TAE.

151. TEMOZOLOMIDE IN GLIOMATOSIS CEREBRI

E. Laguzzi,[1] R. Rudà,[1] F. Giunta,[1] A. Pace,[1] M. Salvati,[1] M. Scerrati,[1] A. Silvani,[1] C. Carapella, [1]and R. Soffietti[1]; for the Italian Association of Neuro-Oncology (AINO, Italy)

Gliomatosis cerebri is a diffusely growing neuroepithelial tumor whose optimal treatment is unclear. Few data are available about response to radiotherapy and chemotherapy. The objective of this prospective study was to assess the efficacy of temozolomide in patients with gliomatosis cerebri. Since 1999, 28 patients with histologically confirmed (biopsy or partial resection) gliomatosis cerebri were treated with temozolomide either at progression after prior radiotherapy/chemotherapy or upfront. Tissue specimens were diagnostic for glioblastoma in 2 cases, malignant glioma in 5, anaplastic astrocytoma in 4, gemistocytic astrocytoma in 2, astrocytoma in 9, oligoastrocytoma in 1, oligodendroglioma in 2, glial proliferation typical of gliomatosis cerebri in 3. Patient characteristics were as follows: median age, 46 years (range, 14–70 years); 13 males and 15 females; median KPS at diagnosis 70 (range, 50–90). Presenting symptoms were as follows: seizures (11 patients), intracranial hypertension (7), motor deficits (5), mental status changes (2), drowsiness and diplopia (2), dizziness and vomiting (1). Twelve out of 28 pretreatment MRI scans demonstrated some contrast enhancement. Eleven of 28 patients had received radiation therapy, and 4 had received chemotherapy (BCNU) prior to temozolomide. All patients were treated with temozolomide, 200 mg/m^2 per day for 5 days every 4 weeks until progression or unacceptable toxicity. Response was evaluated, according to Macdonald criteria, on MRI using both T1-weighted with gadolinium and FLAIR images. The median number of cycles was 7 (range, 1–20). One patient (4%) showed a CR of the contrast-enhancing area, 2 patients (7%) a PR of the Flair hyperintense area, 14 (50%) an SD, and 11 (39%) a PD. Among patients with SD, 2 had a reduction of tumor volume of 20% to 40% ("minor response"). Overall response rate (CR + PR + "minor response") was 18%. Median time to tumor progression (TTP) was 6 months (range, 1–27), with a median survival of 12 months (range, 3–119). A clinical benefit, consisting in a reduction of seizures or improvement of intracranial hypertension, was observed in 9/28 patients (32%). PFS at 6 months was 57%, at 12 months 25%. Three patients showed grade III–IV hematological toxicity. Temozolomide seems to be effective in gliomatosis cerebri. The study is ongoing.

152. TEMOZOLOMIDE (TMZ) WITH O6-BENZYLGUANINE (BG) PLUS CPT-11 IN THE TREATMENT OF RECURRENT MALIGNANT GLIOMA: A PHASE I TRIAL

A. Desjardins, J. Quinn, D. Reardon, J. Rich, S. Sathornsumetee, J. Vredenburgh, A. Walker, S. Tourt-Uhlig, D. Bigner, and H. Friedman; Duke University Medical Center, Durham, North Carolina, USA

The combination of TMZ and CPT-11 reveals a marked increase in activity compared with either agent used alone. The addition of BG to this combination dramatically increased the growth delay of the O6-alkylguanine-DNA alkyltransferase (AGT)-positive malignant glioma D-456MG. Those results have prompted the initiation of a phase 1 study of TMZ with BG plus CPT-11 in patients with malignant glioma. Eligibility included adult patients with recurrent malignant gliomas. Patients were divided in two strata: those receiving enzyme-inducing anticonvulsant (EIAC) and those not receiving EIAC. Each patient was treated with a 1-h BG infusion at 120 mg/m^2, followed by a single dose of TMZ at 355 mg/m^2 and a 90-min infusion of CPT-11 in dose escalation (starting at 60 and 40 mg/m^2, respectively), and thereafter a 48-h continuous BG infusion at 30 mg/m^2/day. Repeat dosing of TMZ and CPT-11 occurs on day 1 of a 21-day cycle. Responses were assessed by functional and imaging criteria after two cycles (6 weeks). The primary end points of this study were to determine the maximum tolerated dose (MTD) of CPT-11 when administered following TMZ plus BG and to determine the activity and toxicity of this combination. Fifty patients have been treated, 47 with glioblastoma multiforme (GBM), two with anaplastic astrocytoma (AA), and one with anaplastic oligodendroglioma (AO). Twenty-two patients have been enrolled to the EIAC stratum at CPT-11 doses of 60, 90, 120, and 150 mg/m^2. Twenty-eight patients have been accrued to the non-EIAC stratum at CPT-11 doses of 40, 60, and 80 mg/m^2. In the non-EIAC stratum, dose de-escalation for TMZ was required from 355 to 267 and then to the present dose of 200 mg/m^2. Dose-limiting toxicities observed thus far have been limited to hematologic toxicities, including neutropenia (9 grade 4), leukopenia (1 grade 4), and thrombocytopenia (2 grade 4). Forty-one patients are assessable for response. One patient continues to show a partial response after 10 cycles, 14 showed a stable disease for at least 4 cycles, and 25 patients progressed after either the first or second cycle. The MTD of this drug combination for the non-EIAC stratum has been defined at a dose of TMZ of 200 mg/m^2 and a dose of CPT-11 of 60 mg/m^2 with DLT limited to hematologic events. The MTD has yet to be identified for the EIAC stratum. Partial response has been observed in one patient, which remains on treatment after 10 cycles.

153. A COMPARISON OF RECOVERY FOLLOWING REMIFENTANIL-DESFLURANE AND REMIFENTANIL-SEVOFLURANE ANESTHESIA FOR PATIENTS UNDERGOING CRANIOTOMY FOR TUMOR

M. Varma,[1] S. Williamson,[1] and P. Kane[2]; Departments of [1]Anaesthesia and [2]Neurosurgery, James Cook University Hospital, Middlesborough, UK

Craniotomy for tumor carries the risk of postoperative complications such as bleeding, cerebral edema, and cerebral ischemia. If not diagnosed early and treated promptly, these can lead to neurological deficit. Therefore, rapid recovery and early neurological assessment are useful goals in the anesthetic management of patients undergoing craniotomy for tumor. The anesthetic technique should enable a rapid and predictable recovery. The pharmacology of remifentanil and desflurane suggests that recovery will be faster if used in combination compared to remifentanil/sevoflurane anesthesia. We compared emergence from remifentanil/desflurane versus remifentanil/sevoflurane anesthesia in patients undergoing craniotomy for tumor. Forty patients undergoing anesthesia for elective craniotomy for tumor were randomly assigned to receive remifentanil/desflurane or remifentanil/sevoflurane anesthesia. Following induction with remifentanil, propofol and rocuronium, anesthesia was maintained with study vapor and remifentanil in oxygen and air. All treatment was standardized. Recovery staff blinded to the study recorded early recovery parameters. The times required for spontaneous ventilation, eye opening, extubation, stating name, stating date of birth and achieving post anesthesia recovery score (Aldrete) >9 were 50% shorter after remifentanil/desflurane compared to remifentanil/sevoflurane anesthesia. In patients undergoing craniotomy for tumor surgery, recovery is significantly faster and more predictable after remifentanil/desflurane compared to remifentanil/sevoflurane anesthesia allowing an earlier neurological examination.

154. A PHASE 1 TRIAL OF OSI-774 (TARCEVA) IN PATIENTS (PTS) WITH RECURRENT MALIGNANT GLIOMAS (MG) ON ENZYME INDUCING ANTI-CONVULSANTS: A NORTH AMERICAN BRAIN TUMOR CONSORTIUM TRIAL

J. Raizer,[1] L. Abrey,[2] P. Wen,[3] T. Cloughesy,[4] I.A. Robins,[5] H.A. Fine,[6] F. Lieberman,[7] V.K. Puduvalli,[8] K.L. Fink,[9] and M. Prados[10]; [1]Northwestern University, Feinberg School of Medicine, Chicago, Illinois; [2]Memorial Sloan-Kettering Cancer Center, New York, New York; [3]Dana-Farber Cancer Institute, Boston, Massachusetts; [4]University of California, Los Angeles, Los Angeles, California; [5]University of Wisconsin Hospital & Clinics, Madison, Wisconsin; [6]Neuro-Oncology Branch, NCI, NIH, Bethesda, Maryland; [7]University of Pittsburgh, Pittsburgh, Pennsylvania; [8]The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [9]The University of Texas Southwestern Medical Center, Dallas, Texas; [10]University of California San Francisco, San Francisco, California; USA

To determine the MTD of OSI-774, a small molecule tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR), in patients with recurrent malignant gliomas on enzyme inducing anti-convulsant drugs (EIAEDs). Pts with recurrent MG or meningioma were treated in a standard phase 1 design with 3 pts per cohort until the DLT was reached. DLT was defined as grade 3 thrombocytopenia, grade 3 or 4 anemia or neutropenia, any grade 3 nonhematologic toxicity, or failure to recover from toxicities within 2 weeks of the last dose of OSI-774 treatment first cycle. The starting dose was 150 mg/day continuously and increased by 50 mg for cohort 2, 75 mg for cohort 3, and then in 125-mg increments for all

subsequent cohorts until the DLT was reached. Patients could not have had more than 3 prior relapses and 2 prior chemotherapies. Patients were evaluated for response with MRI every 56 days. PK studies and AGP levels were done in most patients. Thirty-two pts were enrolled: 21 GBM (14M:7F), 8 AA (4M:4F), 2 AO (Male), and 1 atypical meningioma (Female). Median age was 44 (19–76), and median KPS was 90 (60–100). Number of prior chemotherapies was as follows: 2 pts had none, 11 pts had 1, 12 pts had 2, and 3 pts had 3. The DLT occurred at 775 mg/day and consisted of one each of the following: grade 3 rash, grade 3 hypophosphatemia, and grade 3 thrombocytopenia. The MTD was determined to be 650 mg/day; 1 patient out of 6 patients had DLT at that level (grade 3 deep vein thrombosis/pulmonary embolism). The maximal tolerated dose of OSI-774 for patients on EIAEDs is 650 mg/day. Pharmacokinetic data and outcome data will be presented.

155. CONTRIBUTION OF RADIOSURGERY IN THE SURGICAL TREATMENT OF SKULL BASE MENINGIOMA

Y. Sonoda, S. Takemura, K. Sakurada, M. Saino, S. Sato, S. Saito, H. Jokura, and T. Kayama; Department of Neurosurgery, Yamagata University School of Medicine, Yamagata, Japan

Management of skull base meningiomas has been challenging, even though they are benign tumors. Since tumors are located deep in the skull and can involve important neurovascular structures, surgical treatment has been associated with high morbidity and mortality. However, advances in surgical instrumentation and newly developed approaches to the cranial base were introduced. Simultaneously, radiosurgery was introduced to treat skull base tumors. With this combination of modern techniques, we would like to discuss how radiosurgery contributes to the treatment of skull base meningiomas. During 1995 to 2004, 50 patients with skull base meningiomas underwent surgical treatment in our department. The mean patient age was 56 years (range, 22–72 years). Sixteen patients (32%) were men, and 34 (68%) were women. The tumors were located at anterior cranial base (n = 26: 3 olfactory groove, 2 planum sphenoidale, 10 tuberculum sellae, and 10 anterior clinoidal process), middle cranial base (n = 4: 4 cavernous sinus), and posterior cranial base (n = 20: 12 petroclival, 7 petrous apex, 1 foramen magnum). Nineteen patients (38%) had progressive visual impairment. Cranial nerve palsy was found in 9 cases (18%). Nine patients (18%) showed conscious disturbance due to increased intracranial pressure. Our treatment strategies are (1) the tumor should be left undetached in case there is no CSF space between the tumor and the surrounding structures to avoid new deficit, (2) the residual volume should be reduced to less than 20 ml, which is small enough for radiosurgery (3) the distance between residual tumor and optic nerve should be less than 3 mm. Total removal was achieved in 33 cases (66%). In the remaining 17 (34%), a small amount of tumor was intentionally left because of invasion of the cavernous sinus (8 cases), brain stem (4 cases), the perforators from carotid artery or middle cerebral artery (3 cases), the lower cranial nerves (one case), or the optic nerve (one case). Postoperative mortality was none and morbidity was found in 6 cases (12%). Visual function was improved in 16 patients (89%). The radiosurgery was followed within three months after the open surgery in 14 patients. During the follow-up period (5–112 months; mean, 59 months), tumor regression was observed in 7 patients (50%), and the tumor was unchanged or decreased in 7 patients. The tumor growth control rate was 100%. No patients experienced deterioration of their clinical symptoms after radiosurgery. In the majority of the cases, we could totally remove the tumors safely. On the other hand, we left a small part of the tumors invading important neurovasucular structures and treated residual tumors by radiosurgery. Radiosurgery was a safe and effective treatment for residual tumors. Our result indicated radiosurgery could change surgical strategy of skull base meningiomas invading important neurovasucular structures.

156. PHASE 1/2 TRIAL OF A TWICE-DAILY REGIMEN OF TEMOZOLOMIDE AND CELECOXIB FOR TREATMENT OF RELAPSED/REFRACTORY GLIOBLASTOMA MULTIFORME AND ANAPLASTIC ASTROCYTOMA

S. Pannullo,[1] J. Burton,[2] J. Serventi,[1] P. Stieg,[1] S. Hariharan,[3] R. Elsoueidi,[2] I. El-Jassous,[2] and C. Balmaceda[4]; [1]Neurological Surgery, New York Presbyterian Hospital-Weill Cornell, New York, New York; [2]Medicine, Staten Island University Hospital, Staten Island, New York; [3]Neuroscience, JFK Medical Center, Edison, New York; [4]Neurology, New York Presbyterian Hospital-Columbia University, New York, New York; USA

The COX-2 enzyme is overexpressed in anaplastic astrocytoma (AA) and glioblastoma (GBM), and COX-2 inhibitors have preclinical efficacy in glioma models. Since this drug class has nonoverlapping toxicity with chemotherapy, a phase 1/2 clinical trial was initiated for patients with recurrent or progressive AA or GBM with the combination of temozolomide (TMZ) and celecoxib (CEL). For phase 1, a modified Fibonacci design was used with 3 patients per cohort. Chemotherapy was fixed, with TMZ being given with an oral loading dose of 200 mg/m^2 followed by 9 doses of 90 mg/m^2 BID for 5 days. CEL was given in 5 escalating dose levels starting at 60 mg/m^2 BID up to 240 mg/m^2 BID (maximum of 400 mg BID) for 10 days. Cycles were repeated every 28 days. Forty-six patients (28 M, 18 F) received 235 cycles of therapy, with 37 of the patients carrying a diagnosis of GBM and 9 with AA. Prior treatment consisted of radiation (N = 46) and chemotherapy (N = 12). Median age was 54 years (range, 34–74). This BID regimen of TMZ plus CEL was well tolerated by most patients, and no dose-limiting toxicity was observed at any of the 5 CEL dose levels. Hematologic toxicity was mild with grade 4 neutropenia occurring in 1/235 (0.4%) cycles, grade 3 neutropenia in 2/235 (.85%), and grade 3 thrombocytopenia in 3/235 (1.3%). Grade 3/4 toxicity did not recur following TMZ dose reduction. Grade 1/2 constipation was common, occurring in 13/46 (28%) patients. Tumor responses were evaluated every 8 weeks. In the 41 patients evaluable for response, after 2 cycles, 3/41 pts (7.4%) had a partial response (PR), 32/41 (78%) had stable disease (SD), and 6/41 (14.6%) had progressive disease (PD), resulting in an overall response rate (OR) of 85%. After 6 cycles, the responses were as follows: 1/18 (5.6%) CR, 5/18 (27.8%) PR, 5/18 (27.8%) SD, and 7/18 (38.9%) PD, for an OR of 61%. One patient had a PR in the brain for 13 cycles, but developed tumor in the cervical spinal cord. The average duration of response was 5.6 months (range, 2–15). The 6-month progression-free survival rate for the 41 evaluable patients was 13/41 (31.7%), with a 6-month overall survival rate of 31/41 (75.6%). A regimen of twice-daily TMZ and CEL is safe and potentially effective for the treatment of recurrent high-grade gliomas. Further study of TMZ plus CEL-based regimens is warranted.

157. LONG-TERM SURVIVAL WITH BLOOD-BRAIN BARRIER DISRUPTION THERAPY IN PATIENTS WITH GLIOBLASTOMA MULTIFORME: A REPORT OF THREE CASES

H. Ulrich-Pur,[1] S. Hofmann,[2] M. Hassler,[1] K. Heimberger,[3] B. Fazeny-Dörner,[1] K. Dieckmann,[4] C. Dittrich,[6] E. Suess,[5] and E. Marosi[1]; [1]Clinical Oncology/Med I and [2]Departments of Internal Medicine IV, [3]Neuroradiology, [4]Radio-oncology, and [5]Neurology, Medical University Vienna, Vienna; [6]Clinical Division of Oncology, Department of Internal Medicine III, Kaiser Franz Josef-Hospital Vienna, Vienna; Austria

Long-term survival is rare in patients with glioblastoma multiforme (GBM). The 5-year survival rate has remained at 2% for the last 30 years. One of the problems of glioblastoma chemotherapy is the penetration of drugs through the blood-brain barrier (BBB). BBB disruption therapy protocols have been established and are mainly used in the therapy of primary central nervous system lymphoma. We present three cases of highly malignant gliomas who became long-term survivors (LTGBMS) after blood-brain barrier disruption therapy (BBBD). After debulking surgery, three patients with high-grade gliomas underwent a blood-brain barrier disruption (BBBD) therapy consisting of a 30-s intracarotid infusion of mannitol followed by both intraarterial and intravenous chemotherapy. Cytotoxic regimen consisted of intraarterial methotrexate 1200 mg on days 1 and 2, intravenous cyclophosphamide 15 mg/kg on days 1 and 2, and intravenous etoposide 150 mg/m^2 on days 1 and 2. Treatment courses were repeated every 4 weeks for a total of 4 courses. Noteworthy, two of the patients had no radiotherapy after surgery. Cranial magnetic resonance imaging follow-up was performed every 3 to 6 months until progression occurred, 6 years, 10 years, and 11 years and 11 months after initial diagnosis, respectively. At progression, patients received a second-line intravenous chemotherapy consisting of fotemustine in combination with dacarbacine. Despite the use of cytotoxic drugs that were of almost marginal benefit when used intravenously in patients with high-grade gliomas, after BBBD, patients survived for 8 years, 12.9 years, and 13.4 years, respectively. These patients had the benefit of periods of disease-free survival lasting 6 years, 10.9 years, and 11.9 years. Our data suggest that in selected cases of high-grade glioma patients, BBBD therapy is effective and leads to long periods of disease-free survival. Unfortunately, even more than ten years after therapy, patients relapsed near the initial tumor site, highlighting the need for further therapeutic progress in GBM therapy.

158. TEN CASES OF SYMPTOMATIC SINGLE PARENCHYMAL CNS METASTASES TO THE PINEAL GLAND FROM SYSTEMIC MALIGNANCIES

B. Lassman,[1] J.N. Bruce,[2] and M.R. Fetell[3]; [1]Neurology, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Neurological Surgery and [3]Neurology, Columbia University Medical Center, New York, New York; USA

Metastases to the pineal gland are typically noted as incidental findings at autopsy. In contrast, we identified 10 cases of symptomatic single parenchymal metastases to the pineal gland. This is the largest antemortem series reported. Lung cancer was the primary tumor in 3 cases (2 non-small cell cases, 1 small cell case). Breast, renal, cervical, esophageal, gastric, and colon cancers were the primary tumor type for 1 case each, as was a cancer of unknown primary. In 4 cases, the neurologic symptoms antedated the discovery of a systemic malignancy; in the other 6 cases, the primary tumor was in remission when neurologic symptoms developed. There were 6 women and 4 men with a median age of 56 years (range, 36–70). The clinical and radiographic findings were fairly uniform: In all cases, a contrast-enhancing pineal lesion with obstructive hydrocephalus was discovered by cranial CT or MRI, and the pineal metastasis produced the first clinical manifestation of CNS disease. The metastasis or associated hydrocephalus induced mental status changes ranging from confusion to coma in 8 patients. Oculomotor or pupillary abnormalities were observed in 6 patients including features of Parinaud's or Sylvian aqueduct syndromes. Documented leptomeningeal tumor spread was present at the time of diagnosis in 6 patients. Treatment of hydrocephalus by CSF diversion (with ventriculoperitoneal shunting or third ventriculostomy in 4 cases each) led to transient clinical improvement in 7 patients and no change in 1. Radiotherapy was given to 6 patients with at least partial response in 4. However, the overall prognosis was poor, with median survival of 4.5 months (range, 1–92+) from the time of first diagnosis of pineal metastasis. Only 2 patients survived beyond 1 year (21 months and 92 months), neither of whom had leptomeningeal spread. Metastatic disease should be considered in the differential diagnosis of pineal tumors even in the absence of a history of systemic cancer. Leptomeningeal spread is an important feature that should be investigated in cases of metastases to the pineal gland.

159. PRODIGE: A PHASE 3 RANDOMIZED PLACEBO-CONTROLLED TRIAL OF THROMBOPROPHYLAXIS USING DALTEPARIN LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) IN PATIENTS WITH NEWLY DIAGNOSED MALIGNANT GLIOMA

J. Perry, L. Rogers, N. Laperriere, J. Julian, W. Geerts, G. Agnelli, M. Malkin, R. Sawaya, R. Baker, T. Finch, and M. Levine; Ontario Clinical Oncology Group and PRODIGE Investigators. Hamilton, Ontario, Canada

Venous thromboembolism (VTE) is common in patients with malignant glioma, occurring in 20% to 30% of patients per year of survival. Clinical risk factors may include tumor grade, leg weakness, the use of chemotherapy, and surgery. Recently it has been shown that Tissue Factor (TF), the principal initiator of coagulation, is overexpressed in malignant glioma cell lines and human tumor samples. TF may induce a thrombogenic state in patients and may be a therapeutic target. In addition to anticoagulant properties, LMWH has been associated with prolonged survival in patients with nonmetastatic solid tumors through an anticancer effect. We are conducting a large phase 3 RCT testing the efficacy and safety of chronic daily administration of dalteparin in patients with newly diagnosed malignant glioma. Patients are randomized 1:1 to receive dalteparin 5000 anti-Xa units s.c. daily versus daily s.c. placebo. The primary outcome is VTE-free survival at 6 months, and progression-free survival, overall survival, toxicity, and neurocognitive performance are secondary outcome measures. The expected cumulative risk of VTE in the control group is 13% at 6 months. At least 40 events will be needed in order to detect a 60% relative risk reduction in the dalteparin group with 80% power. Allowing for dropouts, the projected sample size is 512 patients. Clinical risk factors, concurrent therapies, tumor response, and survival data are being collected. A companion molecular study examining the role of TF and other regulators of coagulation is underway. Patients must be adults with newly diagnosed glioblastoma multiforme or anaplastic glioma, with no history of prior VTE, and not on chronic anticoagulation. As of November 2004, 124 were patients randomized across 15 active study centers. Of 281 eligible patients, 157 (56%) were excluded for reasons including conflicting trials, needle aversion, concomitant illness, and possible VTE. An independent DSMB is supervising this trial and an update on trial progress will be presented. A placebo-controlled trial testing the role of thromboprophylaxis in patients with malignant glioma is feasible and is accruing patients at an increasing number of clinical trial sites worldwide. Of particular interest to neuro-oncologists is not only the prevention of symptomatic VTE, but the potential survival advantage conferred by LMWH in other solid tumors. This study is supported in part by a grant in aid from Pfizer Inc. and coordinated by the Ontario Clinical Oncology Group.

160. LONG SURVIVAL RESULTS WITH REPEATED BIOCHEMOTHERAPY (BUCLADESINE + FOTEMUSTINE) IN DE NOVO GBM PATIENTS

N. Oktar, T. Dalbasti, S. Islekel, and N. Ozdamar; Faculty of Medicine, Department of Neurosurgery, Ege University, Izmir, Turkey

This clinical study was designed to evaluate the impact of biochemotherapy (bucladesine-polymer locoregional therapy and systemic fotemustine chemotherapy) at the time of recurrence of the de novo glioblastoma multiforme (GBM) patients. In a randomized prospective manner, 50 patients who were diagnosed as de novo GBM were included in this study. Five different therapy protocols were used. The first group of 10 patients had tumor resection only. The second group assessed having only systemic chemotherapy as six i.v. infusions of fotemustine after tumor resection. The third group had implantation of bucladesine-loaded biodegradable polymeric sustained-release (bcl-SR) pellets. The fourth group received six i.v. infusions of systemic fotemustine as in the second group, in addition to local implantation of bcl-SR pellets. Finally, the fifth group assessed having stereotactic biopsy and implantation of bcl-SR rods under local anesthesia repeated in six-month intervals. In this trial of local interstitial biologic therapy with long acting (4 to 5 months of release time) bcl-SR did show a statistically significant delay of recurrence on the treatment of GBM patients. The best treatment results were obtained from the local bcl-SR + systemic fotemustine–treated group, in which the survival rate estimated by Kaplan-Meier method was 100% in de novo GBM at 12 months. Moreover, in the last group, the mean survival rate was 85.1 ± 25.9 weeks, and 1-year, 2-year, and 3-year survival rates were 100%, 40%, and 10% respectively. Mean survival times of 28, 32, 37, 63, and 85 weeks for the control, fotemustine, bcl-SR, fotemustine+bcl-SR and repeated stereotactic bcl-SR + fotemustine groups were achieved, respectively. In this prospective clinical study conducted on primary GBM cases, although the numbers of patients are small, there is a significant benefit of repeated local bcl-SR when used in combination with systemic fotemustine therapy (biochemotherapy) with no adverse effects.

161. SYSTEMIC TEMOZOLOMIDE COMBINED WITH LOCOREGIONAL MITOXANTRONE IN TREATING RECURRENT GLIOBLASTOMA PATIENTS

A. Silvani,[1] M. Eoli,[1] A. Salmaggi,[1] E. Lamperti,[1] A. Botturi,[1] G. Broggi,[3] L. Bissola,[2] G. Finocchiaro,[2] and A. Boiardi[1]; [1]Neuro-oncology; [2]Unit of Experimental Neuro-oncology; [3]Neurosurgery, Istituto Nazionale Neurologico Carlo Besta, Milan, Italy

The purpose of this study was to evaluate in recurrent glioblastoma patients the feasibility and effectiveness of a combination treatment with systemic temozolomide and locoregional mitoxantrone. The first end point of the study was the 6-month PFS; the secondary end points were response rate and OS. Twenty-two recurrent GBM patients were enrolled for second tumor debulking, with local positioning of a Rickam reservoir in order to locally deliver chemotherapy into the tumor created resection cavity, with the aim of controlling local tumor recurrence. We designed a protocol using systemic temozolomide (200 mg/m^2, days 1–5 every 28) in association with mitoxantrone, delivered through the reservoir (4 mg, days 1–5 every 28) positioned into the area of tumor exeresis. After reoperation, a residual tumor mass no larger than 2 cm was identified in 18/22 patients. The patients were treated with monthly cycles of chemotherapy until evolution of the tumor but in no case for more than 10 cycles. Responses were evaluated by MRI scans performed every two months and images assessed according to McDonald's criteria. Response rates were as follows: no complete responses [CRs], 5 partial responses [PRs], 13 cases of stable disease [SD], and 4 cases of progressive disease [PD]. The median progression-free survival [PFS] and survival time [ST] of the whole group of treated patients was 7 and 11 months, respectively, and more than ¼ of the patients survived over 18 months. During the study the patients' compliance was complete, and no dropouts occurred. Hematological toxicity was mild, and after repeated local injections only minor neurological side effects occurred. We conclude that although some bias in patient selection is not excluded in this pilot study, results are interesting: The PFS was as long as survival of recurrent GBM reported in the literature.

162. MANAGEMENT OF GLIOBLASTOMA MULTIFORME IN ELDERLY PATIENTS: SHOULD WE BE AGEIST?

D. Rodrigues[1], G. Hendry[1], D. Scoones[2], and P. Kane[1]; [1]Neurosurgery; [2]Neuropathology, James Cook University Hospital, Middlesbrough, UK

Past studies have suggested that aggressive treatment for glioblastoma multiforme (GBM) in the elderly is not appropriate because of apparent poor outcome. Global application of this view may deny some patients beneficial and "state of the art" treatment. Does this ageist view still hold true? We conducted a retrospective audit of outcome in all elderly patients

(age >60 years) diagnosed with GBM in our institution between 2001 and 2003. The records were audited with respect to surgical treatment (biopsy, resective surgery) and adjuvant treatment (radiotherapy, chemotherapy). Outcome was analyzed on the basis of pre- and post-treatment Karnofsky performance scale (KPS) and on survival. Statistical analysis performed using Student's *t* test and chi-squared test. Fifty eligible patients were identified from the neuropathology database (34 males, 16 females, mean age of 68.9 ± 6.3 years). For the purpose of analysis, patients receiving biopsy alone, resective surgery alone, or biopsy plus radiotherapy were grouped as a single group (Group I) and compared with outcome in patients receiving resective surgery plus radiotherapy ± chemotherapy (Group II). There were 18 patients in Group I and 32 patients in Group II. The age in Group I was 72.9 ± 6.5 versus 66.5 ± 5 in Group II ($P < 0.001$). There was no statistically significant difference in the pre- and post-treatment KPS in the 2 groups. The mean survival in months in Group I was 3.61 ± 2.9 and in Group II was 9.7 ± 5.5 ($P < 0.001$). The mean time to last follow-up in months was 6.6 ± 5.2. Outcome in elderly patients with GBM in general remains poor. However, elderly patients should not be treated as a homogenous group; as appropriately selected patients can have a good functional and survival outcome. Further work is needed to determine the selection criteria for treatment of elderly patients with GBM.

163. FACTORS PREDICTING SURVIVAL IN PATIENTS WITH OLIGODENDROGLIAL TUMORS: EVALUATION OF HISTOPATHOLOGY, MOLECULAR GENETICS, NEUROIMAGING, AND THERAPY IN 210 PATIENTS

W. Shapiro, L. Ashby, J. Pueschel, A. Scheck, and S. Coons; Barrow Neurological Institute, Neurology, Phoenix, Arizona, USA

Understanding outcome for oligodendroglial tumors—oligodendroglioma (O), oligoastrocytoma (OA), and their anaplastic counterparts (AO, AOA)—requires attention to patient-, tumor-, and treatment-related variables. We present a large series of such patients. Two hundred ten newly diagnosed patients were identified. Histopathological features and proliferative activity (MIB-1) were determined, along with deletion of chromosomes 1p and 19q (FISH). Preoperative MRIs were reviewed for enhancement. Survival data and treatment information were obtained from the clinical records. There were 139 low-grade tumors (95 O, 44 AO) and 71 high-grade tumors (31 AO, 40 AOA). Median age was 39 years (range, 19–83) without age difference among the 4 subgroups. Patients aged ⩾40 (MST 440 vs. 196 weeks; $P = 0.0002$). Patients with low-grade tumors lived longer than those with high-grade tumors (MST 446 vs. 138 weeks; $P \geqslant 5\%$ ($P < 0.001$). Overall, patients with low-grade O or OA, and with 1p del lived longest. Patients whose tumors enhanced on MRI or had an MIB-1 ⩾5% fared less well. Treatment did not influence survival, but treatment was not randomized prospectively. Multivariate analysis is currently being performed.

164. HUMAN GLIAL TUMORS CONTAIN CELLS WITH STEM CELL-LIKE PROPERTIES

C. Watts,[1,2] and S.L.R. Ball[2]; [1]Neurosurgery and [2]Cambridge Centre for Brain Repair, Cambridge University, Cambridge, UK

Isolation of a population of multipotent stem-like cells that have subsequently been shown to maintain glial tumors in vivo has recently been reported. This suggests that despite the heterogeneity inherent in many brain tumors a single cell type may be responsible for tumor growth and maintenance. Understanding the relationship between brain tumors and stem cells may help in the development of more effective therapies. Protocols developed for the culture of adult neural stem cells were applied and modified for use with WHO IV glial tumor material from 5 patients. Spheroid aggregates of tissue were derived. After serial passage in defined propagation medium, clonal populations were transferred into differentiation medium for 4 days, and phenotypic characterization was performed by using standard immunohistochemical techniques. Multiple spheroid colonies of slowly proliferating cells were identified from multiple tumor samples. Serial passage of individual spheres yielded further expansion in the number of spheres with prolonged cell cycling and slow proliferation compared to typical tumor cells and normal mammalian adult neural stem cells. Differentiation and characterization of single spheres identified cells expressing neuronal filament B-tubulin consistent with a neuronal phenotype. Glial tumors appear to contain cells with characteristics of neural stem cells that make them ideal candidates for malignant transformation. Understanding stem cell signaling pathways and developing ways to manipulate them will be critical in developing new treatments.

165. EPHA2 RECEPTOR AND ITS LIGAND, EPHRINA1, ARE DIFFERENTIALLY EXPRESSED IN MALIGNANT GLIOMAS AND NORMAL BRAIN

J. Wykosky, D. Gibo, and W. Debinski; Department of Neurosurgery, Brain Tumor Center of Excellence, Wake Forest University School of Medicine, Winston-Salem, North Carolina, USA

EphA2 is a member of the largest family of tyrosine kinase receptors, the Eph receptors. These proteins play an important role in neural development and are involved in mediating contact-dependent processes between cells. EphA2 is expressed at low levels on the surface of adult epithelial cells, where it interacts with its membrane-bound ligand, ephrinA1. The expression of EphA2 has been found elevated in a number of cancers, including breast, colon, prostate, and ovarian carcinomas. In addition, the overexpression of this receptor has been linked to tumor neovascularization as well as invasiveness. In our previous analysis using cDNA microarrays, we found EphA2 to be one of the most uniformly overexpressed genes in glioblastoma multiforme (GBM). We were therefore interested in examining the expression of the EphA2 receptor in GBM vs. that of normal brain. The level of EphA2 was determined by Western blotting and immunohistochemistry (IH) using both monoclonal and polyclonal EphA2 antibodies. In Western blots, an immunoreactive band corresponding to the size of the EphA2 receptor (130 kDa) was detected in all established human GBM cell lines studied, including A-172, SNB-19, U-251 MG, U-87 MG, and U-373 MG, as well as in human GBM tissue lysates. Notably, EphA2 Western blots of normal brain protein medleys obtained from Clontech or Chemicon and lysates from the frontal lobe tissue of a normal human brain showed a very faint EphA2 immunoreactive band at 130 kDa or no band at all. IH performed on GBM cell lines revealed a honeycomb pattern of staining for EphA2 that is characteristic of the expected membrane-localized expression. Furthermore, paraffin-embedded and frozen sections of human GBM display specific and abundant staining for EphA2 that was nearly absent in normal brain. In addition, IH performed on a tissue microarray of 60 sections of various types and grades of brain malignancies demonstrated intense EphA2 staining among the higher-grade (III and IV), more invasive astrocytomas, but markedly less, if any, in the lower grade tumors. IH also revealed that, unlike EphA2, ephrinA1 was present in paraffin-embedded sections of normal brain. Interestingly, ephrinA1 was localized specifically to vascular endothelial cells in normal brain, but present at a uniformly low level throughout human GBM paraffin-embedded specimens. Thus, EphA2 is specifically overexpressed not only in malignancies of epithelial origin, but also in malignant gliomas. Furthermore, EphA2 is expressed differentially with respect to its ligand, ephrinA1, in both malignant gliomas and normal brain. Overall, EphA2 represents a novel target for the development of molecular therapeutics for the treatment of patients with high-grade gliomas. We are currently investigating the role of EphA2 and its interaction with ephrinA1 as a useful means for diagnosis and therapeutic approaches such as targeted drug delivery.

166. CHROMOSOME 10q LOSS IN MEDULLOBLASTOMA: ASSOCIATION WITH THE LARGE CELL/ANAPLASTIC VARIANT

C. Fuller,[1] J. Dalton,[1] and R. Gilbertson[2]; [1]Pathology; [2]Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA

Medulloblastoma is by far the most common CNS embryonal tumor. Although patient stratification is based primarily on clinical variables, distinctive histologic variants and molecular alterations have also been found to correlate with prognosis. In particular, large cell/anaplastic (LC/A) medulloblastoma characteristically presents with CSF dissemination and follows a highly aggressive clinical course. Subsets of aggressive medulloblastomas have been found to harbor amplifications of the *c-myc* oncogene, many with concomitant LC/A morphology. Several studies have suggested that deletions involving chromosome 10q may also play a role in medulloblastoma tumorigenesis, though no correlation has thus far been established between these alterations and histologic subtype. Fluorescence in situ hybridization (FISH) was performed on formalin-fixed paraffin-embedded tissue sections from tissue microarrays containing 79 medulloblastomas to determine c-myc and 10q status. Each case was designated as one of the following histologic variants: classic, nodular/desmoplastic (N/D), or large cell/anaplastic. Dual color hybridizations included the following probe pairs: *c-myc* (8q24)/8p23.1, *TNKS2* (10q23.32)/10p15.2, *PTEN* (10q23.31)/*DMBT1* (10q25.3-26.1). The cohort included 40 classic, 25 N/D, and 14 LC/A medulloblastomas. Overall, detectable amplifications of *c-myc* and losses involving 10q were present in 6 cases (8%) and 9 cases (11%), respectively. In all cases with losses involving 10q, all three of the targeted loci were deleted indicating a large region of chromosomal loss, likely the entire long arm. Fifty percent of tumors with *c-myc* amplification and 67% with 10q loss were of LC/A morphology. As a group, 43% of LC/A medulloblastomas had 10q loss while 21% had amplification *c-myc*; 14% harbored both abnormalities. Only 5% of classic and 4% of N/D cases

had either alteration, and 2 of these cases had focal anaplasia. Our findings demonstrate that losses involving chromosome 10q are encountered in a subset of medulloblastomas and involve a large region of that chromosomal arm. Both *c-myc* amplification and 10q loss appear to correlate with the LC/A morphology, the later alteration encountered twice as often as the former in this series.

167. METHYLATION PROFILE OF PRIMARY CNS LYMPHOMAS

L. Chi-Hang Chu, *S. Grossman*, and J. Herman; Johns Hopkins University, Baltimore, Maryland, USA

Patients with systemic malignancies have been shown to have substantial quantities of tumor-specific DNA in their plasma. Studies in systemic malignancies are underway to determine if this can serve as a plasma marker of tumor burden. We recently used methylation specific polymerase chain reaction (MSP) to identify the methylation status of four gene promoters (p16, p73, RARb, and MGMT) within resected brain tumor tissue and in the plasma of patients with low- and high-grade gliomas. (Proc. Am. Soc. Clin. Oncol. 2004). The presence of tumor-specific DNA in the plasma was defined as identification of the same methylated promoter (MP) in the primary brain tumor and the plasma. The gliomas contained methylation of at least one promoter in 9 of the 10 (90%) patients studied, and 6 pts (67%) had methylation of at least one of the same promoters in the plasma DNA. A plasma marker would be of particular importance in patients with primary CNS lymphomas (PCNSL), where genuine improvements in outcomes are being realized. In this study, we sought to characterize the methylation status of CNS lymphoma patients using a panel of 14 genes ($p16^{INK4a}$, $p15^{INK4b}$, $p14^{ARF}$, *p73, Rb, hMLH1*, *GSTP1*, *MGMT, RARb, CRBP-1, TIMP2*, *TIMP3*, and *DAPK)*. With IRB approval, we isolated DNA from paraffin-embedded samples from 31 CNS lymphoma patients and studied the methylation patterns in the CpG islands of the genes using a nested PCR approach. PCR product was diluted and amplified in the methylation-specific polymerase chain reaction (MS-PCR), which was performed with primers specific for either methylated or modified unmethylated DNA using positive and negative controls. The results showed a high degree of aberrant methylation in PCNSL for *DAPK* (21/28 = 75%), $p16^{INK4a}$ (19/28 = 68%), *CRBP-1* (16/25 = 64%), *THBS-1* (17/28 = 61%), and $p14^{ARF}$ (13/24 = 54%); *RARb* (13/26 = 50%), *MGMT* (13/27 = 48%), *TIMP2* (11/26 = 42%), *TIMP3* (11/28 = 39%), and $p15^{INK4b}$ (12/31 = 39%). This methylation profile provides an important first step in developing a quantitative tumor-specific plasma biomarker that could be used to monitor tumor status in patients with PCNSL.

168. C-KIT, A POWERFUL TUMOR MARKER IN GERMINOMA, IS MUTATED, AND TACE REGULATES C-KIT SHEDDING IN GERMINOMAS

H. Nakamura,[1] K. Makino,[1] Y. Ushio,[2] and J. Kuratsu[1]; [1]Department of Neurosurgery, Kumamoto University, Kumamoto; [2]Department of Neurosurgery, Otemae Hospital, Osaka, Japan

The proto-oncogene c-kit encodes a transmembane tyrosine kinase receptor for stem cell factor (SCF). C-kit is thought to have a crucial role for tumor formation such as testicular seminomas, gastrointestinal stromal tumors (GISTs), and intracranial germinomas. The soluble isoform of c-kit (s-kit) is reportedly detectable in cerebral spinal fluid of patients with germinomas and germinomas with STGC. TACE (ADAM17), which is a member of matrix-metalloproteinases, has been indicated to be a candidate for shedding of c-kit and generating a soluble isoform of c-kit (s-kit). S-kit in CSF is now thought to be a powerful indicator for existence of intracranial germinomas. In this study we performed immunohistochemical study on 25 human primary intracranial germinomas and germinomas with STGC, staining the same sections for c-kit and placental alkaline phosphatase (PLAP). Moreover, immunohistochemical staining for TACE was performed in primary intracranial germinomas. The genomic DNA was extracted from the germinomas and the mutation of c-kit gene was examined. Immunohistochemical expression was graded by using a semiquantitative scoring system where – = 0%, 1+ = 1–25%, 2+ = 26–50%, 3+ = 51–75%, and 4+ = 76–100%. Of the 25 cases, 7 (28%) were graded 3+ and 18 (72%) 4+ for c-kit; 8 (32%) were 3+ or 4+ for PLAP. All 3 cases negative for PLAP-staining were strongly positive for c-kit, and all embryonal carcinomas, immature teratomas, and yolk sac tumors were negative for c-kit staining. The positive reaction for TACE was confirmed in germinomas and germinomas with STGC. However, it was negative in other nongerminomatous germ cell tumors. C-kit gene mutation was found in several cases of the patients with germinomas. We conclude that C-kit has an important role for germinoma formation, and the mutation of c-kit gene was confirmed in several cases. Both c-kit and s-kit may be powerful tumor markers for germinomas with or without STGC. Moreover, TACE is specifically expressed on the surface of the tumor cells and generates s-kit by shedding of c-kit in germinomas and germinoma STGC.

169. SURVIVAL OF ANAPLASTIC GLIOMAS CORRELATES WITH GENETIC PROFILE

M. Sanson,[1] F. Laigle-Donadey,[1] J. Lejeune,[1] E. Criniere,[2] Y. Marie,[2] K. Mokhtari,[3] A. Carpentier,[1] K. Hoang-Xuan,[1] and J. Delattre[1]; [1]Neurology, [2]INSERM U495, and [3]Neuropathology, CHU Pitié-Salpêtrière, Paris, France

Based on WHO classification, anaplastic gliomas are divided into grade III oligodendrogliomas (OIII), oligoastrocytomas (OAIII) and astrocytomas (AIII). Known prognostic factors include age, performance status, and genetic alterations such as 1p and 19q chromosome loss, 10q loss, and EGFR amplification. Our aim was to investigate the impact of the most common genetic alterations on survival, based on 739 anaplastic gliomas collected in our database, including 445 males, 294 females (sex ratio = 1.51) ranging from 10 to 98 years (median = 48 years). Genetic analysis included search for loss of heterozygosity (LOH) on chromosome 1p and 19q, 9p, 10q, *EGFR* and *MDM2* amplification, *P53* mutation and expression, *PTEN* mutation: Partial or complete screening was available for 261 anaplastic gliomas. Overall survival (OS) was 26.2 months and was correlated to age (15.5 months for patients > 48 years, vs. 47.9 months for patients <48 years; $P < 0.0001$) but not to the histological subtype. Loss of 1p and 19q (both linked P <10-11) were associated with OIII ($P < 0.001$), 10q loss was related to *EGFR* amplification ($P <$ 10-14), and to *PTEN* mutation ($P <$ 0.05). On univariate analysis, survival correlated with 1p loss (66.5 vs. 26.2 months, $P < 0.0001$), 19q loss (65.3 vs. 26.2 months, $P < 0.005$), 10q loss (19.4 vs. 41.6 months, $P < 0.0001$), *PTEN* mutation (11.1 vs. 36.1 months, $P < 0.0004$), *EGFR* amplification (16.6 vs. 37.1 months; $P < 0.0001$), *MDM2* amplification (7.3 vs. 31.5 months, $P < 0.0025$), *P16/CDKN2A* deletion (18.9 vs. 33.1 months, $P = 0.013$). The difference did not reach significance for LOH 9p ($P = 0.064$), p53 expression ($P = 0.11$), or mutation ($P = 0.9$). LOH 10q and *EGFR* amplification were the only genetic alterations correlated to age ($P < 0.0001$), being more frequent in older patients (51 vs. 43 years and 55.5 vs. 43 years, respectively). In conclusion, genetic analysis of anaplastic gliomas provides useful prognostic information for clinical practice and for stratifying clinical studies.

170. IMMUNOHISTOCHEMICAL EXPRESSION OF IMATINIB TARGETS IN GLIOBLASTOMA: AN APPROPRIATE METHOD TO SELECT PATIENTS FOR TARGETED THERAPY?

C. Haberler[1,2,3]; [1]Klin Institut of Neurologie, [2]Department of Internal Medicine I, [3]Department of Neurosurgery, Medical University of Vienna, Vienna, Austria

The tyrosine kinase (TK) inhibitor imatinib (Gleevec) selectively targets PDGFR-a, -β, c-kit, c-abl, and arg and has proven successful in the treatment of chronic myeloid leukemia. In recurrent glioblastoma, phase 2 therapy trials using imatinib have been initiated. As only a fraction of patients seems to benefit from imatinib therapy, and because of potential side effects and high costs of imatinib therapy, selection of the right patients is important. The goal of our study was to assess systematically immunohistochemical expression of the major TKs targeted by imatinib in glioblastoma, as expression of these factors could be used to select patients for imatinib therapy. In a cohort of 101 glioblastoma patients, anti-PDGFR-a, -β, c-kit, c-abl, and arg protein immunohistochemistry was performed, and expression of these proteins was assessed semiquantitatively. PDGFR-a and arg expression in tumor cells was widespread in 1/101 cases, respectively. Focal PDGFR-a, -β, c-kit, c-abl, and arg immunolabeling was detected in 25/101, 19/101, 4/101, 7/101 and 31/101 cases, respectively. We show here for the first time in a large series of glioblastomas that PDGFR-a, -β, c-kit, c-abl, and arg expression is immunohistochemically detectable in a fraction of cases. The value of anti-tyrosine kinase immunolabeling as predictive factor for patient selection remains to be clarified by comparative analysis of tumor tissue of therapy-responders versus nonresponders.

171. GAMMA-CATENIN EXPRESSION CORRELATES WITH GOOD PROGNOSIS IN MEDULLOBLASTOMAS

K. Misaki,[1] H. Fujisawa,[2] M. Hasegawa,[2] and J. Yamashita[2]; Toyama Rosai Hospital, Neurosurgery, Uozu, Japan; Kanazawa University, Neurosurgery, Kanazawa, Japan

In medulloblastoma, gene amplification and mRNA overexpression of the c-myc gene are reported to be adverse prognostic indicators. However, the frequency of mRNA overexpression (50%) cannot be explained by gene amplification (4%) alone, and therefore, other mechanisms independent of gene amplification may exist. Because *c-myc* is located downstream of the *Wnt* signal pathway, we examined associated molecules in primary tumors by immunohistochemical and cytogenetic analyses and have discussed their clinical relevance. Twenty-four medulloblastomas were studied. Immunohistochemistry of c-myc, beta- and gamma-catenins, and cyclin D1 was performed. Differential PCR was conducted for the gene amplification of c-myc, N-myc, and cyclin D1. Mutations of beta- and gamma-catenins were examined by PCR-SSCP analysis and direct DNA sequencing. Western blot analysis was available in 5 cases. The clinical significance of the results was statistically analyzed by the Kaplan-Meier method. Cytoplasmic/membranous staining of beta- and gamma-catenin was detected in 19 (79%) and 9 (37%) cases, and nuclear expression of cyclin D1 and c-myc was detected in 6 (25%) and 21 (83%) cases, respectively. The expression of gamma-catenin in immunohistochemistry was confirmed by Western blotting, and the expression levels were well correlated between the two. *c-myc* and *N-myc* amplification was detected separately in two cases. Mutations of beta- and gamma-catenins were not found. Statistically, patients without CSF dissemination (Chang M0) showed significantly better outcome than those with dissemination (Chang M1~3) ($P = 0.0002$), and only gamma-catenin expression correlated with good prognosis ($P = 0.003$) among the molecules analyzed. Furthermore, gamma-catenin expression was also significant in the M0 group ($P = 0.022$). Although insignificant ($P = 0.057$), cyclin D1 expression showed a trend of adverse outcome, and all patients with cyclin D1 expression expired. The expression of beta-catenin and c-myc did not correlate with prognosis ($P = 0.31$ and 0.53, respectively). The immunohistochemistry of gamma-catenin is useful for further stratification or individualization in medulloblastoma treatment. It was also found that cyclin D1 expression has the potential to be an adverse prognostic indicator.

172. VH GENE ANALYSIS OF PRIMARY CNS LYMPHOMAS

H. Pels,[1] M. Montesinos-Rongen,[4] C. Schaller,[2] U. Schlegel,[1] I. Schmidt-Wolf,[3] O. Wiestler,[1] and M. Deckert[2]; [1]Departments of Neurology, [2]Neurosurgery, and [3]Internal Medicine, University of Bonn, Bonn; [4]Department of Neuropathology, University of Cologne, Cologne; [5]German Cancer Research Center, Heidelberg; Germany

Primary CNS lymphomas are highly malignant non-Hodgkin's lymphomas of B-cell origin associated with a poor prognosis. These neoplasms show variable sensitivity to radio- and chemotherapy. A molecular basis for these differences in treatment responses has not yet been established for primary CNS lymphomas in a comprehensive series of patients. Here, we performed PCR analyses of the immunoglobulin gene rearrangements of 18 PCNSL including nine patients who responded well to therapy and nine patients who showed resistance to treatment. Variable gene segment distribution, mutation frequency of variable region genes, and clinical course were analyzed. Our data suggest a tendency toward a higher mean mutation frequency of variable region genes in patients responding to treatment (17.2%) and a lower mutation frequency (11.8%) in patients exhibiting a poor response to therapy, respectively. Furthermore, a restricted usage of the VH4 gene family was observed in the majority of nonresponding patients. To further validate the prognostic impact of these molecular parameters, studies in a larger cohort of patients will be required.

173. PERINUCLEAR EXPRESSION OF LRIG PROTEINS IS A GOOD PROGNOSTIC INDICATOR IN ASTROCYTIC TUMORS

H. Hedman,[1] D. Guo,[1] J. Nilsson,[1] H. Haapasalo,[3] O. Raheem,[3] U. Andersson,[1] T. Bergenheim,[2] and R. Henriksson[1]; [1]Oncology and [2]Neurosurgery, Umeå University Hospital, Umeå, Sweden; [3]Tampere University Hospital, Pathology, Tampere, Finland

The LRIG protein family has three members, LRIG1–3, which are widely expressed integral membrane proteins. LRIG1 antagonizes the activity of epidermal growth factor receptor (EGFR) family tyrosine kinase receptors by enhancing receptor ubiquitylation and degradation. In this study, we evaluated the mRNA expression level of LRIG1–3 in human glioma cell lines and control-matched tumor tissues, characterized the subcellular localization of a synthetic LRIG1-GFP fusion protein, and analyzed the immunohistochemical staining patterns of LRIG1–3 in 404 astrocytic tumors. LRIG1–3 mRNA was detected in all human glioma cell lines and matched tumor samples. Ectopically expressed LRIG1-GFP localized to nuclear, perinuclear, and cytoplasmic compartments in a cell line-specific manner. Immunoreactivity of LRIG1–3 was seen in different patterns in the astrocytic tumors. Perinuclear staining of LRIG1–3 inversely correlated with WHO grade and survival of the patients. Positive LRIG3 perinuclear and cytoplasmic staining correlated with a lower proliferation index. LRIG3 perinuclear staining was in addition to tumor grade an independent prognostic factor. Thus, expression of LRIG1–3 might be of importance in the pathogenesis and prognosis of astrocytic tumors.

174. DEVELOPMENT AND APPLICATION RT-PCR SYSTEMS TO DETERMINE HERV EXPRESSION IN ASTROCYTOMA CELL LINES

P. Nelson, R. Smith, G. Conde, D. Roden, and J. Darling; University of Wolverhampton, RIHS, Wolverhampton, UK

Human endogenous retroviruses (HERVs) belong to the family of transposable elements that make up 8% of the human genome. Unlike exogenous retrovirus (e.g., HIV and HTLV), HERVs are inherited in a Mendelian manner. More than 22 families of HERVs have been identified over the past two decades. Importantly, some HERVs have been found to possess large open reading frames and produce viral like particles. More latterly, these viruses have been linked with certain autoimmune diseases and cancers. Indeed, HERVs may contribute toward carcinogenesis through retrotransposition, promoter insertion, immunomodulation, disruption of normal HERV-related functions, recombination, or by the production of fusion proteins. Of importance, HERV-K, HERV-W, and HERV-H have the potential to be transcriptionally active in the brain. We have developed robust RT-PCR systems using primers/probes specific to HERV-K and HERV-W to assess mRNA expression in conjunction with the house keeping gene, histidyl tRNA synthetase. In employing a gel-documentation system, we are able to provide semiquantitative levels of HERV expression in cell lines. Pilot data shows markedly enhanced expression of HERV-K in the cell line U251-MG (derived from a glioblastoma multiforme; WHO grade IV astrocytoma) as compared to a control cell line SW480 (colon adenocarcinoma): RT-PCR values; 1.0 and 0.42, respectively. This observation raises an intriguing possibility that HERV-K expression may be elevated in malignant brain tumors. In addition, this approach provides a useful approach to optimize primers and probes prior to using real-time quantitative PCR.

175. MOLECULAR GENETICS AND RESPONSE TO PCV CHEMOTHERAPY IN OLIGODENDROGLIAL NEOPLASMS: A SINGLE CENTRE PROSPECTIVE STUDY

C. Walker[1], B. Haylock[2], D. Husband[2], K. Joyce[1], D. Fildes[1], M. Jenkinson[3], T. Smith[4], J. Broome[4], D. du Plessis[3], and P. Warnke[3]; [1]JK Douglas Research Laboratory, Clatterbridge Cancer Research Trust, Bebington; [2]Clatterbridge Centre for Oncology, Bebington; [3]Department of Neurological Science, University of Liverpool, Liverpool; [4]Walton Centre for Neurology and Neurosurgery, Liverpool, UK

Recent research suggests that the -1p/-19q genotype is associated in oligodendroglial neoplasms with prolonged survival and chemosensitivity. However, the role of genetic analyses to guide patient management remains controversial. In this prospective study, the impact of genotype on the response of oligodendroglial neoplasms to procarbazine, lomustine, and vincristine (PCV) chemotherapy was investigated in a routine clinical setting. Seventy-six patients treated with PCV chemotherapy at a single center between 2000 and 2003 were investigated. PCV was given as first therapy (50 cases) or at recurrence following radiotherapy (26 cases). Response was assessed by using Macdonald criteria and T1-weighted MR or CT images taken with contrast agents for enhancing tumors or T2-weighted MR images for nonenhancing tumors. Allelic imbalance in 1p36, 19q13, 17p13, 10p12-15 and 10q22-26 and *p53* mutation (exons 5-8) were investigated by using tumor samples enriched by laser capture microdissection. The -1p/-19q genotype was found in 12/19 oligodendroglioma WHO grade II, 10/23 oligoastrocytoma WHO grade II, 11/13 oligodendroglioma WHO grade III, and 6/21 oligoastrocytoma WHO grade III. In enhancing tumors, response was associated with the -1p/-19q genotype and inversely related to loss of 17p13, chromosome 10 loss, and *p53* mutation in the series as a whole, or when Grade II and III or primary and recurrent cases were analyzed separately. The -1p/-19q genotype was associated with longer progression-free survival (PFS) and overall survival, while 17p13 loss, *p53* mutation, and chromosome 10 loss indicated poor prognosis. Genotype had greater impact in recurrent cases. Genetic factors remained independent prognostic factors in multivariate analysis. In nonenhancing grade II tumors, 4/6 with -1p/-19q responded to PCV as initial therapy compared with 2/11 with intact 1p/19q. Nonenhancing cases with -1p/-19q showed a

trend toward longer PFS and significantly greater time from first to second therapy. In the study overall, 26% of cases with intact 1p/19q responded to PCV; 5 had *p53* mutation, 3 had no detectable genetic alterations, and 1 had loss of 1p36 and chromosome 10. In addition to anaplastic tumors, enhancing and nonenhancing grade II tumors with the -1p/-19q genotype responded to PCV chemotherapy. This prospective study supports previous retrospective observations that the -1p/-19q genotype is highly predictive of chemosensitivity in oligodendroglial neoplasms, but some tumors with intact 1p/19q also benefit from PCV.

176. THERAPEUTIC IMPLICATIONS OF INTERFERON REGULATORY FACTOR (IRF)-1 AND IRF-2 IN DIFFUSELY INFILTRATING ASTROCYTOMAS (DIA): RESPONSE TO INTERFERON (IFN)-BETA IN GLIOBLASTOMA CELLS AND PROGNOSTIC VALUE FOR DIA

A. Yoshino, Y. Katayama, T. Yokoyama, T. Watanabe, A. Ogino, T. Ota, C. Komine, and T. Fukushima; Neurological Surgery, Nihon University School of Medicine, Tokyo, Japan

The precise mechanisms governing the direct effect of IFN-b, including apoptosis induction, are not yet fully understood. To gain a better insight into these mechanisms, we investigated the signaling pathways focusing particularly on interferon regulatory factor 1 (IRF-1) and IRF-2 in glioblastoma cell lines. Furthermore, we attempted to determine whether or not IRF-1 and IRF-2 act as additional prognostic indicators in diffusely infiltrating astrocytomas (DIA). We first assessed the cytotoxic effects of IFN-b based on a cell growth study and modified MTT assay, and then quantified the apoptosis using a sandwich enzyme immunoassay following IFN-b treatment in the cell lines, U-87MG, T98G, and A-172. Subsequently, we carried out an analysis of apoptosis-related molecules as evaluated by densitometric analysis of Western blots, focusing on IRF-1 and IRF-2, and 2 major initiator caspases, caspase-8 and caspase-9. Furthermore, we assessed the expression of type I IFN receptor, IRF-1, and IRF-2 using immunohistochemical techniques in 63 DIA (15 of WHO grade II, 18 of grade III, and 30 of grade IV), and analyzed their impact on prognosis. An increase in apoptosis was apparent after 48 h of IFN-b treatment (1×10^4 IU/ml) in T98G but not in U-87MG or A-172. IFN-b treatment for 6 h significantly enhanced the expression of IRF-1 in all 3 cell lines. However, an enhanced expression of IRF-2 was observed only in the not-most-sensitive, non-apoptosis-induced U-87MG and A-172. While minimal processing of caspase-8 was noted in the 3 cell lines throughout the experiment, caspase-9 activation was observed in the apoptosis-detected T98G after 48 h of treatment, as indicated by a 1.33-fold increase ($P = 0.037$). On the other hand, the IRF-1 LI and IRF-1/IRF-2 LI ratios were greater in low-grade DAI and were negatively correlated with the histopathological grade in DIA ($P = 0.017$ and $P = 0.001$, respectively). Furthermore, the IRF-1/IRF-2 LI ratio was negatively correlated with the MIB-1 LI in DIA ($P = 0.004$) and represented an independent and most powerful determinant of overall survival compared to other conventional prognostic factors ($P = 0.018$). However, the relation was not statistically significant when only patients with high-grade DIA were assessed. Our findings suggest that upregulation of IRF-1 and IRF-2 might be an important determinant of susceptibility to IFN-b-mediated cytotoxicity including apoptosis. Furthermore, the IRF-1/IRF-2 LI ratio may reflect the proliferative state of DIA and constitute an important prognostic marker in DIA. Thus, IRF-1 and IRF-2 could represent one of the therapeutic target sites for the regulation of cell growth in DIA.

177. PROGNOSTIC SIGNIFICANCE OF O6-METHYLGUANINE-DNA METHYLTRANSFERASE (MGMT) PROTEIN EXPRESSION IN ADULT ANAPLASTIC GLIOMAS

M. Brell,[1] *A. Tortosa*,[2] J.J. Acebes,[1] J.M. Gil,[3] T. Ribalta,[6] E. Verger,[7] S. Villà,[4] N. Viñolas,[8] I. Ferrer,[5] and F. Graus[9]; [1]Neurosurgery and [2]Nursery Department, Bellvitge Hospital, [3]Oncology and [4]Radiation Therapy Department, Institut Català d'Oncologia, [5]Bellvitge Hospital, Institut of Neuropathology, Universitat Barcelona, L'Hospitalet de Llobregat, Barcelona; [6]Pathology, [7]Radiation Therapy, [8]Oncology, and [9]Neurology Departments, Hospital Clinic of Barcelona; Spain

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that removes mutagenic and cytotoxic adducts from the O6 position of guanine. MGMT activity in glioma cells could be related to resistance to alkylating agents. The aim of this study was to analyze MGMT protein expression in anaplastic gliomas and to establish their relationship among tumor characteristics, patient survival, and MGMT methylation status of the promoter. Ninety-three patients with anaplastic glioma (WHO grade III) were analyzed for MGMT protein expression by immunohistochemistry on paraffin-embedded sections. *MGMT* promoter methylation profile was analyzed in 40 specimens by methylation specific PCR. We evaluate the prognostic implication of patients' epidemiological data (including age and gender), MRI scan characteristics (diffuse, ring, and no enhancement), extent of resection, histopathology, postoperative KPS, proliferation index (Ki-67 expression), and MGMT immunohistochemical expression. Overall survival was calculated according to the Kaplan-Meier method and compared with the log-rank test. A multivariate analysis was carried out according to proportional-hazard analysis (Cox's model). Sixty (64.5%) patients were men, and the median age of the patients was 48 years. Median overall survival was 18 months. Fifty-one tumors (54.8%) showed nuclear staining of MGMT. No correlation between MGMT expression and promoter methylation was observed. Cox's regression analysis revealed that postoperative KPS $\geqslant 80$, proliferative index $\leqslant 4.8\%$, absence of contrast enhancement on MRI and gross total resection were independent predictors of longer overall survival. There was a trend toward longer overall survival for those patients with negative MGMT immunohistochemistry (RR = 1.66, $P = 0.066$). In a subset analysis of patients who received chemotherapy (n = 72), absence of MGMT expression was independently associated with better survival (RR = 2.12, $P = 0.027$). Unlike previous investigators, we did not find a correlation between *MGMT* promoter methylation and survival. However, we observed a correlation between MGMT protein expression and survival in those patients who received chemotherapy. This previously reported feature will need to be validated in future studies.

178. ESTHESIONEUROBLASTOMA: A STUDY OF PROGNOSIS

V.G. Khurana, *C. Giannini*, M. Piccirilli, D.M. Bernold, W.R. Bamlet, R. Foote, J. Buckner, R.L. McClelland, and M.J. Link; Pathology, Mayo Clinic Rochester, Rochester, Minnesota, USA

Esthesioneuroblastoma is a rare tumor, for which experience at individual institutions has been limited. At present, there is no consensus regarding its systematic treatment. In order to determine prognostic factors for outcome in the management of this tumor, we retrospectively studied the records of 78 patients treated for esthesioneuroblastoma at the Mayo Clinic between 1976 and 2003. Histologic slides from original biopsy/surgery were reviewed in 60 (of 78) patients, and Hyams pathologic grade (1–2, low; 3–4, high) was determined. Statistical analysis included Kaplan-Meier overall and disease-free survival and Cox proportional hazards modeling, uni- and multivariate, to assess the strength of association of the following variables with survival: age and gender, Hyams grade, modified Kadish stage (A to D), extent of surgery, and adjuvant treatment. There were 35 females (45%). The average patient age at diagnosis was 51 years and average follow-up was 6.4 years (Hyams grade, n = low, 31; high, 29; modified Kadish stage, n = A, 4; B,15; C,42; D,15). Fifty-four patients underwent gross total resection, 16 subtotal, and 8 biopsy only, while 51 patients received radiotherapy and 31 chemotherapy. The 5-year survival (68% overall) was: 84% for low and 50% for high grade tumors ($P = 0.004$); 88% for stages A and B, 72% for stage C, and 34% for stage D patients ($P < 0.05$). Controlling for age and extent of resection, stage D exhibited significantly higher mortality than either stage A + B ($P = 0.0005$) or stage C ($P = 0.006$), while high-grade tumors had significantly higher mortality than low grade ($P = 0.007$). In total, 53 patients were at risk for recurrent disease, and 32 (60%) of these went on to develop recurrence: 9% at 1 year, 36% at 5 years, and 77% at 10 years. Our data suggest that both modified Kadish stage and Hyams grade at the time of diagnosis have a significant impact on prognosis.

179. GENETIC ANALYSIS OF MENINGIOMAS REVEALS CHROMOSOME-SPECIFIC CORRELATIONS WITH GRADE AND RECURRENCE

E.J.M. Speel,[1] S. Claessen,[1] J. Stavast,[2] E. Meulemans,[2] G. Blaauw,[3] J. Teepen,[4] R. Janzer,[5] and A. Twijnstra[3]; [1]University of Maastricht, Molecular Cell Biology, Maastricht, The Netherlands; [2]Pathology and [3]Neurology, Academic Hospital Maastricht, Maastricht, The Netherlands; [4]St. Elisabeth Hospital, Neurology, Tilburg, The Netherlands; [5]University of Lausanne, Neuro-Pathology, Lausanne, Switzerland

Meningiomas are common central nervous system neoplasms that exhibit a remarkably diverse histopathology and biological behavior. Apart from the involvement of the NF2 gene, the molecular pathology of meningioma development and progression is poorly understood. We examined 25 benign (WHO grade I; MI), 10 atypical (WHO grade II; MII) and 6 anaplastic, malignant (WHO grade III; MIII) meningiomas, including 14 tumors of 5 patients showing recurrence (n = 3) and progression (n = 2), for DNA copy number gains and losses by comparative genomic hybridization (CGH) and for chromosome 1, 7, and 22 alterations by fluorescence in situ hybridization (FISH) using centromere- and *NF2* gene–specific probes. In addition, 44 tumors of 12 patients showing malignant progression were

studied by microsatellite analysis using 7 markers for loci on chromosomes 1p and 22q. Results were statistically correlated with available clinical data. CGH analysis identified (1) genomic alterations in all but 6 MI tumors; (2) a marked accumulation of the average number of alterations per tumor with increasing WHO grade, i.e., 5.2 for MI, 10.0 for MII, and 11.8 for MIII ($P = 0.001$; M1 vs. MII + III); (3) besides 22q losses most frequently 17pq and 20q gains (36%–40%) in MI, losses of 1p, 14q (both 50%), 6q (40%), and gains of 17q (70%), 20q (60%), 17p (50%), and 11q (40%) in MII, and a further increase of most of these alterations (1p losses and 17q gains; 83%), as well as amplifications on 5p15, 5q35, 8q24, and 20q13 in MIII tumors; (4) a significant association of 1p and 6q losses and 15q gains with higher WHO grade (MI vs. MII/MIII; $P = 0.05$); and (5) a strong correlation of tumor recurrence with 1p and 10p losses ($P = 0.057$ and 0.006, respectively) and 1q gains ($P = 0.032$), as well as with an increase in total number of alterations and losses per tumor, i.e., 5.7 vs. 11.5 ($P = 0.007$) and 2.4 vs. 6.0 ($P = 0.003$), respectively. FISH analysis in these tumors showed chromosome 1 and 7 copy numbers indicating a diploid DNA content, while 1 copy of the *NF2* locus was lost in 18/23 (78%) MI, 8/9 (89%) MII and 5/5 (100%) MIII tumors, predominantly together with a chromosome 22 centromere. Microsatellite analysis revealed LOH for at least 1 marker at 22q in 8/10 and at 1p in 7/11 additional patient cases showing progression, which was already detected in the primary tumor. Our study indicates that meningiomas are mainly diploid tumors showing high frequencies of chromosome 22q loss including the *NF2* gene as well as 17pq and 20q gains already early in tumorigenesis. Tumor recurrence proved to be associated with 1p and 10p losses and 15q gains, as did 1p and 6q losses and 15q gains with tumor progression. The identified chromosomal regions may lead to the discovery of novel genes that play a role in meningioma tumorigenesis and may help to predict their biological behavior. This study was supported by the Royal Netherlands Academy of Sciences (KNAW), Van Leersum Foundation, and the Foundation Neurosurgery Heerlen.

180. PROSPECTIVE ANALYSIS OF MATRIX METALLOPROTEINASE-9 (MMP-9) IN THE SERUM OF PATIENTS WITH HIGH-GRADE GLIOMAS

A. Hormigo,[1] B. Gu,[2] E. Riedel,[3] J. Rao,[5] M. Fleisher,[2] K. Panageas,[3] L. DeAngelis,[1] and E. Holland[4]; [1]Neurology, [2]Clinical Laboratories, [3]Biostatistics, and [4]Neurosurgical Service of the Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York; [5]Biomedical and Therapeutics Sciences, University of Illinois College of Medicine, Peoria, Illinois, USA

The objective of the study is to determine if matrix metalloproteinase-9 (MMP-9) can be used as a serum marker in patients with malignant gliomas. Our previous work has shown that the level of YKL-40 protein can predict survival of patients with high-grade gliomas. However, the YKL-40 does not significantly correlate with response to therapy. We have now identified MMP-9 as a potential marker for response to therapy in gliomas. MMPs are proteases that degrade extracellular matrix proteins, and MMP-9 is expressed by human gliomas and associated with tumor infiltration and angiogenesis. Serum MMP-9 levels were determined by ELISA assay prospectively and correlated with magnetic resonance imaging (MRI) scans in a prospective longitudinal study. The tumor response was determined by standard criteria: complete response (CR), partial response (PR), stable disease (SD) and progression of disease (POD) for each MRI. We analyzed serum samples from 57 patients with anaplastic gliomas (AG) and 48 patients with glioblastoma multiforme (GBM) (range, 1–12 samples; median of 4 and 3 per patient, respectively). For AG the mean value of MMP-9 was 323 ng/ml for patients who had a radiographic CR, 196 ng/ml for PR, 372 ng/ml for SD, and 519 ng/ml for POD. For GBM, the mean value of MMP-9 was 190 ng/ml for CR, 356 ng/ml for PR, 425 ng/ml for SD, and 526 ng/ml for POD. To determine if MMP-9 levels predict the response categories, we used a cumulative logit model that corrected for multiple measurements per patient. This analysis showed a significant difference of MMP-9 values per response type for AG ($P = 0.002$) and a marginally significant difference for GBM ($P = 0.08$). Similar to what is seen with YKL40, the MMP-9 levels transiently increase immediately following surgery reaching the maximum level at 48 h after tumor resection (mean 1174 ng/ml and 1070 ng/ml, respectively) and decreased over the next two weeks. Therefore, reliable levels must be obtained at least two weeks post-surgery. In conclusion, serum levels of MMP-9 correlate with response type at least for AG. Ongoing longitudinal studies are designed to (1) assess correlation with survival and (2) determine whether MMP-9 can complement YKL-40 as a reliable serum marker for disease status in patients with high-grade gliomas and be used to guide clinical decisions during a patient's disease course.

181. CLINICAL SIGNIFICANCE OF EGFR AMPLIFICATION AND THE EGFRVIII TRANSCRIPT IN CONVENTIONALLY TREATED ASTROCYTIC GLIOMAS

L.M. Backlund,[1] L. Liu,[2] B.R. Nilsson,[1] D. Grander,[1] K. Ichimura,[2] and V.P. Collins[2]; [1]Oncology-Pathology, Karolinska Institute, Stockholm, Sweden; [2]Department of Pathology, Division of Molecular Histopathology, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK

The aim of this study was to evaluate the clinical value of assessing amplification and the common 5' rearrangement of the EGFR gene (EGFRvIII) in 223 astrocytic tumors by correlating the data with patient survival. Previous studies have evaluated amplification alone and provided contradictory results. Amplification was analyzed by densitometry of Southern blots or quantitative PCR, and the EGFR transcripts were examined by RT-PCR and sequenced to identify aberrations. In addition, RNase protection assay was carried out on a subgroup of the tumors to confirm the PCR results. We found no significant association between EGFR amplification or rearrangement and survival in a series of 160 glioblastoma patients. There was no association between EGFR status and age in this patient group. We noted a tendency toward decreased survival in the 42 patients with anaplastic astrocytomas with any EGFR abnormality. This was most marked for the EGFRvIII rearrangement ($P = 0.061$). The latter anaplastic astrocytoma patients were significantly older than those without 5' rearrangements ($P = 0.020$). No EGFR abnormalities were identified in the astrocytoma patients. We conclude from this large set of astrocytic tumors that neither EGFR amplification nor the presence of the EGFRvIII transcript predict patient outcome in conventionally treated glioblastomas. In anaplastic astrocytomas, however, although uncommon, EGFR aberrations are associated with shorter survival.

182. MGMT PROMOTER HYPERMETHYLATION IS MORE FREQUENT IN SECONDARY GLIOBLASTOMAS AND IS INDEPENDENT FROM OTHER PROGNOSTIC FACTORS

M. Eoli, L. Bissola, F. Menghi, B. Pollo, A. Silvani, L. Valletta, G. Broggi, A. Boiardi, and G. Finocchiaro; Neurological Institute C. Besta, Clinical Neuro-oncology, Milan, Italy

O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that specifically removes mutagenic, carcinogenic, and cytotoxic O^6-alkylguanine DNA adducts induced by alkylating agents like nitrosoureas. Repair of cytotoxic DNA damage by MGMT is a potentially important factor of resistance to alkylating agents, commonly used in the treatment of glioblastoma multiforme (GBM). Using methylation-specific PCR (MSP) we investigated the inactivation of the DNA-repair gene MGMT by promoter hypermethylation in 67 GBMs obtained from patients subsequently treated by conventional radiotherapy and CDDP + BCNU. We observed that the MGMT gene was methylated in 24 patients (36%). This finding was associated with prolonged overall survival (24 vs. 14 months; log-rank $P = 0.0002$) and with a longer progression-free survival (PFS) (11 vs. 8 months; log-rank $P = 0.003$). Among these 67 GBMs, secondary GBMs had prolonged overall survival (26 vs. 14 months; log-rank $P = 0.01$) than de novo tumors, whereas other prognostic factors were not statistically associated with ST or PFS. Moreover, the frequency of MGMT methylation was higher in secondary than in primary GBMs (78% vs. 22%, $P = 0.01$), but was not associated with age, KPS, and RTOG class risk. Other genetic markers (LOH on chromosome 19q and chromosome 17p, EGFR amplification and p53 mutations) are under study to assess their influence on the treatment response and overall survival of patients with GBM. These findings indicate the relevance of epigenetic and genetic factors in the prognosis of glioblastomas.

183. HYPOXIA INDUCIBLE FACTOR-1 ALPHA EXPRESSION CORRERATES WITH HISTOLOGICAL GRADING IN HUMAN GLIOMAS

M. Saino, K. Sakurada, Y. Sonoda, S. Sato, and T. Kayama; Department of Neurosurgery, Yamagata University School of Medicine, Yamagata, Japan

Hypoxia inducible factor-1 (HIF-1) is considered to play an important role in the adaptation of cells to hypoxia and angiogenesis via regulation of vascular endothelial growth factor (VEGF) in various tumors. Astrocytomas, especially glioblastoma and anaplastic astrocytoma, are known to be hypervascular tumor. We investigated the relationship between HIF-1 alpha expression and several factors such as VEGF expression, Ki-67 labeling index, and histological grading in human gliomas. Expression of HIF-1 alpha, VEGF, and Ki-67 antigen were investigated by immunohistochemistry in 35 specimens of supratentorial astrocytomas (15 glioblastomas, 11 anaplastic astrocytomas, and 9 low-grade astrocytomas). Histological

grading was determined according to WHO criteria. Strong and moderate expression of HIF-1 alpha was observed in 6 (40%) of 15 glioblastomas, in 7 (63.7%) of 11 anaplastic astrocytomas, and 2 (22.2%) of 9 low-grade astrocytomas. The case did not show HIF-1 alpha expression was observed in 4 (26.7%) of 15 glioblastomas, 3 (27.3%) of 11 anaplastic astrocytomas, and 5 (45.5%) of 9 low-grade astrocytomas. The cases with strong and moderate expression of HIF-1 alpha tend to show strong VEGF expression. The mean Ki-67 labeling index was 20.4% in the strong and moderate HIF-1 alpha expression group and 8.5% in the no HIF-1 alpha expression group. There was correlation of HIF-1 alpha expression with VEGF expression, Ki-67 labeling index, and histological grading. Malignant astrocytomas widely expressed HIF-1 alpha, which contributes to angiogenesis via VEGF. Furthermore, HIF-1 alpha would correlate with tumor cell proliferation. These results suggest that neovascularization due to upregulation of HIF-1 prevents hypoxic damage and permit tumor cell progress.

184. MICROGLIA IN GEMISTOCYTIC ASTROCYTOMAS

F. Geranmayeh,[1] B.W. Scheithauer,[2] and M.B. Graeber[1]; [1]University Department of Neuropathology, Imperial College London and Hammersmith Hospitals Trust, London, UK; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

Gemistocytic astrocytomas behave more aggressively than other diffusely growing astrocytic tumors. They are characterized by a high mutation rate of the p53 gene and cytological as well as immunological abnormalities including frequent perivascular mononuclear infiltrates. Microglia are resident brain macrophage precursor cells that form a network of immune competent cells within the normal central nervous system. They are of increasing interest in the context of glioma growth. We selected 23 tumor samples from among 201 samples obtained from patients with gemistocytic astrocytomas operated on at the Mayo Clinic between 1985 and 1998. These tumors have been previously analyzed for p53 mutations, p53 protein, and proliferative activity (Neurosurgery 48, 187 2001). Immunolabeling for three established microglial markers, CR3/43, Ki-M1P, and iba1 was carried out on adjacent tissue sections. A high number of microglial cells were detected in gemistocytic astrocytomas. More microglia were present when the fraction of gemistocytes was high. Varying numbers of gemistocytic tumor cells expressed MHC class II molecules. Importantly, the more class II immunoreactive gemistocytes were present, the fewer class II positive microglial cells could be detected. Our findings support the view that gemistocytic astrocytomas contain unusually high numbers of microglial cells. The finding of aberrant MHC class II expression by gemistocytic tumor cells correlating with a loss of immune competent, MHC class II–expressing microglia sheds new light on the immunology of these tumors. We suggest that the findings reported here may be related to the especially poor prognosis of gemistocytic astrocytomas. It is well known that the proliferative potential of neoplastic gemistocytes is very low, and it has remained an intriguing question as to why these tumors are so "successful" biologically. The fact that aberrant expression of MHC class II molecules by nonprofessional antigen presenters may induce T cell anergy could provide one explanation.

185. PROGNOSTIC VALUE OF MGMT METHYLATION, TP53 MUTATION, AND MDM2, EGFR, OR CDK4 AMPLIFICATION IN MALIGNANT GLIOMA PATIENTS TREATED WITH ADJUVANT TEMOZOLOMIDE CHEMOTHERAPY

C. Luyken,[1] S. Loeser,[2] B. Blaschke,[2] S. Köhler,[1] A. Kreuzmann,[1] S. Seghrouchni,[1] G. Reifenberger,[2] and M. Sabel[1]; [1]Clinic of Neurosurgery; [2]Department of Neuropathology, Heinrich-Heine-University, Düsseldorf, Germany

The response of malignant gliomas to chemotherapy with alkylating drugs such as temozolomide (T) varies from patient to patient. The present study investigates the significance of tumor-associated genetic aberrations in the TP53, MDM2, EGFR, and CDK4 genes, as well as the methylation of the MGMT promoter for response to T and overall survival (OS) of patients with malignant gliomas. Fifty-one patients with malignant gliomas (anaplastic WHO grade III [AG], n = 14, WHO grade IV [GBM], n = 37) were treated with T as first-line chemotherapy after tumor resection and radiation therapy. From each patient, clinical data were systematically recorded, including age at diagnosis, gender, tumor location, Karnofsky performance score (KPS), extent of resection, tumor volume (determined before and after resection, before chemotherapy, and every 3 months), OS, and response to treatment. The tumor tissue was investigated for mutations in the TP53 gene (exons 5–9); amplification of the EGFR, CDK4 and MDM2 genes; and hypermethylation of the MGMT promoter. Molecular findings were correlated to OS and response to T. Fifty-nine percent of the patients were males, and median age at surgery was 54.3 years. The mean preoperative KPS was 80%. OS was 277.4 weeks (AG) and 87 weeks (GBM). A median number of 8 cycles of T was administered. Grade 3 or 4 toxicity occurred in 6 patients. T was terminated because of tumor progression in 31 patients (4 AG, 29%; 27 GBM, 73%). Thirty-three percent of the tumors demonstrated EGFR amplification (17% AG, 38% GBM), while MDM2 amplification was detected in 7% (8% AG, 6% GBM), CDK4 amplification in 16 % (8% AG, 18% GBM), and TP53 mutation in 20% (12,5% AG, 22% GBM). MGMT hypermethylation could be demonstrated by PCR in 54% of the cases (82% AG, 46% GBM). Statistical analyses revealed that MGMT hypermethylation was significantly associated with longer OS ($P = 0.0013$, log-rank test) and better response to T ($P = 0.034$, log-rank test). Neither EGFR, MDM2, and CDK4 amplification nor TP53 mutation was significantly correlated to OS or response to T. In line with recent publications from other groups, our study clearly indicates MGMT hypermethylation as a clinically important molecular marker that is significantly associated with longer overall survival and better response to temozolomide treatment in patients with malignant gliomas. In contrast, genetic alterations in the TP53, MDM2, EGFR, and CDK4 genes appear to be less important as prognostic factors. This study was supported by grants (70-3088-Sa I) from Deutsche Krebshilfe.

186. SECRETORY MENINGIOMAS AS MORPHOLOGICALLY DISTINCT MENINGIOMA ENTITY

H.D. Mennel,[1] T. Riegel,[2] W. Tirakotai,[2] and I. Celik[3]; [1]Department of Neuropathology, [2]Clinic of Neurosurgery, and [3]Institute of Theoretical Surgery, Philipps University, Marburg, Germany

The unique features, both concerning clinical and pathological aspects, of secretory meningioma have not been completely understood so far. We present a hitherto not noticed pathological finding that could shed new light upon this rare entity. Fourteen cases of secretory meningiomas could be identified out of more than 1300 meningiomas from our institutions during the last 25 years. Tumors were retrospectively analyzed regarding their clinical and pathological features with special emphasis upon mechanisms that might explain the clinically observed common edema. The main morphological finding was the occurrence of a significantly higher proportion of mast cells in secretary meningiomas compared to other unselected meningioma subtypes: mean 2.43 (range, 0.020–5.60) versus 0.27 (range, 0.00–2.00; $P = 0.0001$, Mann-Whitney-U-test). These mast cells can be visualized and counted with the CD-117 immunoreaction, an antibody directed against the product of the c-kit oncogene. Mast cells were identified in close connection to vessel wall (pericytic) proliferations. Electron microscopy of such proliferated vessels revealed increasing vacuolization in the peripheral layers of proliferated vessels, constantly adjacent to small edematous areas, in which the mast cells often were freely floating. Yet, we could not establish a positive correlation between microscopic mast cell density and macroscopic edema as found by CT scans in respective cases. Furthermore, the pseudopsammoma bodies that characterize those tumors are surrounded by cells that react vividly with cytokeratins. Cytokeratin subgroups such as CK 7, CK 8, and CK 5/6 are equally found at random and in combinations; only CK 20 was regularly absent from those cells. Further attempts to characterize mast cells and secretory products by a panel of tissue hormones (serotonin and others) failed to give clues as to the action of mast cells and to the pathogenesis of pseudopasmmomam bodies (secretory products) within these tumors. Pericytic vessel proliferation, edema formation of secretory meningiomas, and the newly described occurrence of mast cells may well be correlated. The further analysis of these features and of their connection to the formation of pseudopsammoma bodies is difficult because these tumors are as rare, only approximately 1% of all meningiomas.

187. DOES MR SPECTROSCOPY PREDICT SURVIVAL IN GLIOMAS?

J. Crossman,[1] D. Birchall,[2] P. Mitchell,[1] and P. Crawford[1]; [1]Departments of Neurosurgery and [2]Neuroradiology, Newcastle General Hospital, Newcastle Upon Tyne, UK

The WHO grading of a glioma is based on observed histopathological features. While the grade predicts the behavior of the tumor, it is only approximately related to length of patient survival following diagnosis. MR spectroscopy of gliomas is well correlated with the histopathological grade. However, the use of MR spectroscopy data to predict duration of survival following diagnosis is not widespread. We have investigated the use of a Cox proportional hazards model using MR spectroscopy data obtained from a prospective series of histopathologically proven gliomas to predict duration of survival following diagnosis. We have compared the accuracy of this model with four other models based on WHO grade, tumor histopathology, and the MRI characteristics of the tumor. MR spectroscopy data was prospectively acquired with a Philips 1.5T scanner using multivoxel chemical shift spectroscopy. Standard post-acquisition processing of the MR spec-

troscopy data was performed, and the following ratios were calculated: (i) choline/creatine, (ii) choline/naa, (iii) choline/creatine$_{normal}$, (iv) lactate/creatine$_{normal}$, (v) choline/choline$_{normal}$, (vi) choline$_{normal}$/creatine$_{normal}$, (vii) choline$_{normal}$/naa. Five Cox proportional hazards models were constructed, (i) WHO grade, (ii) histopathological features of the tumor, (iii) MR spectroscopy, (iv) MRI characteristics of the tumor, and (v) combined MRI/MR spectroscopy. Each model also included covariates for age at diagnosis and information on whether surgical debulking and/or further oncological treatment occurred. Because some of the patients remained alive at the time of analysis, the Kaplan-Meier algorithm was used to calculate the survival function. The survival predicted by each model was compared to the actual survival at three months, one year, and two years. Thirty patients (19 males) were studied. The age range at diagnosis was 26 to 79 years (mean, 57 years). Eighteen patients were WHO grade IV, 8 patients WHO grade III, and 4 patients WHO grade II. Actual survival at 3 months was 87%, at one year 50%, and at two years 43%. The histopathology and MR spectroscopy models most closely predicted the actual survival (MR spectroscopy: 3 months 94%, one year 59%, two years 45%; histopathology: 3 months 92%, one year 58%, two years 48%). All of the models overpredicted survival, particularly in the first year. This investigation demonstrates that MR spectroscopy is a useful tool to help estimate survival following diagnosis of a glioma and suggests its use in conjunction with other data known to be of prognostic value.

188. 1p/19q STATUS IN RECURRENT OLIGODENDROGLIAL TUMORS: WHAT DOES IT TELL US?

I. Lavon,[1] B. Zelikovitsh,[1] D. Fuchs,[1] Y. Fellig,[2] D. Soffer,[2] and *T. Siegal*[1]; [1]Leslie and Michael Gaffin Center for Neuro-Oncology and [2]Department of Pathology, Hadassah Hebrew University Hospital, Jerusalem, Israel

Oligodendrogliomas frequently show allelic loss of chromosomal arms 1p and 19q. These deletions are associated with an improved response to chemotherapy and longer overall survival when compared to tumors with no such allelic loss. The assessment of the genetic status of 1p/19q is routinely performed by analyzing the primary neoplasm, while the implication of the re-evaluation of the recurrent tumor is unclear. Our objective was to evaluate the status of 1p and 19q in both the primary and the recurrent oligodendroglial tumors. The status of 1p/19q was evaluated from paired tumor-blood DNA samples by using PCR-based microsatellite analysis. Ten tumors of 5 patients were evaluated and included the primary and recurrent neoplasm. For 3 patients, initial diagnosis was oligodendroglioma (WHO II, 2 pts; WHO III, 1 pt). Two of these tumors had codeletions at 1p and 19q chromosomes, and one had no loss. At recurrence, the two low-grade tumors transformed to a higher grade and were now diagnosed as anaplastic oligodendroglioma (WHO III). The repeat evaluation of 1p/19q status showed again, a combined allelic loss. The anaplastic tumor with no initial loss remained +1p/+19q. Two other patients had an initial diagnosis of an oligoastrocytoma, one low grade and the other anaplastic (WHO III). The low-grade tumor had codeletions at 1p/19q, and the anaplastic tumor had an intact 1p with a deletion at 19q. At recurrence, both tumors were rediagnosed by a stereotactic biopsy, obtained from a part of the tumors that demonstrated radiographic changes but were located at sites of the residual tumors that were not resected on initial surgery. The pathology of the low-grade tumor has not changed, but no deletions were found in the recurrent neoplasm. The anaplastic tumor transformed to WHO IV grade, and this recurrent tumor presented no deletions. Our findings may indicate that pure oligodendrogliomas are of monoclonal origin, while mixed oligoastrocytomas probably contain a biclonal population. It is possible that different parts of the heterogenous tumors represent different clonal expansion. Alternatively, it can not be excluded that over time and following treatment, the more resistant clones that do not manifest deletions overtake and become dominant.

189. ANAPLASTIC ASTROCYTOMA PATIENTS WITH GLIOBLASTOMA-LIKE TUMOR GENOTYPE HAVE A POOR OUTCOME

L.M. Backlund,[1] B.R. Nilsson,[1] L. Liu[2], K. Ichimura,[2] and V.P. Collins[2]; [1]Oncology-Pathology, Karolinska Institute, Stockholm, Sweden; [2]Department of Pathology, Division of Molecular Histopathology, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK

Anaplastic astrocytoma (AA, WHO grade III) is, second to glioblastoma, the most common and most malignant type of adult CNS tumor. Since survival for patients with AA varies markedly and there are no known useful prognostic or therapy response indicators, the primary purpose of this study was to examine whether the knowledge of genetic abnormalities in AA had any value in this regard. The survival data on 37 carefully sampled AA was correlated with the results of a detailed analysis of the status of 9 genes involved in the development of astrocytic tumors. These included 3 genes coding for proteins in the p53 pathway (i.e., TP53, p14ARF, and MDM2), 4 in the Rb1 pathway (i.e. CDKN2A, CDKN2B, RB1 and CDK4) and PTEN and EGFR. We found that abnormalities in at least one of the four genes (CDKN2A, CDKN2B, RB1, CDK4) coding for components of the Rb1 pathway were associated with shorter survival ($P = 0.009$). This finding was consistent in multivariate analysis, including adjustment for age ($P = 0.013$). The findings suggest that analysis of the genes coding for Rb1 pathway components provides additional prognostic information in AA patients.

190. PRIMITVE NEUROECTODERMAL TUMOR ARISING WITHIN LOW-GRADE ASTROCYTOMA. A REPORT OF THREE CASES

D. Pal,[1] G. Hall,[1] A. Chakrabarty,[2] P. Roberts,[3] and P. Chumas[1]; [1]Neurosurgery and [2]Neuropathology, Leeds General Infirmary; [3]Cytogenetics, St. James's University Hospital, Leeds, UK

We present three cases of primitive neuroectodermal tumor (PNET) arising in low-grade astrocytomas treated with radiotherapy. One case was identified at clinical presentation, two from a review of our histopathology database. All three patients had histologically proven low-grade astrocytomas and had received radiotherapy following biopsy. Two patients had partial resection for recurrence, one at five years post-surgery and the other at ten years with histological confirmation of low-grade astrocytoma. At subsequent recurrence at the same site, nine and 29 years following original presentation and nine years later for the third patient, further tumor debulking was performed. Histology now showed PNET (WHO grade 4). Two patients died within one year of final surgery. The third patient is still alive, but with evidence of clinical progression. Cytogenetics showed a complex karyotype with multiple chromosome abnormalities in all three patients; recurrent abnormalities included deletions of 1q32, 2p13, 13q14, and 19q13. PNET arising in a previous low-grade astrocytoma has not been described previously. Two reports in the literature describe PNET arising at a distant site following radiotherapy for low-grade astrocytoma. Whether these cases represent transformation of low-grade astrocytoma into PNET or PNET arising de novo in the previous radiotherapy field will be discussed.

191. MOLECULAR CHARACTERIZATION OF PDGF SUBFAMILY OF RECEPTOR TYROSINE KINASES PATHWAY IN GLIOSARCOMAS

R.M. Reis,[1] A. Martins,[1] S.A. Ribeiro,[1] D. Basto,[1] A. Longatto-Filho,[1] F. Schmitt,[1] and J.M. Lopes[2]; [1]Life and Health Sciences Research Institute (ICVS), Health Sciences School, University of Minho, Braga; [2]Institute of Molecular Pathology and Immunology of University of Porto, Porto, Portugal

Deregulation of PDGF subfamily of receptor tyrosine kinases, such as PDGFR-a and c-Kit, and anomalous activation of RAS-RAF-MAPK intracellular signaling pathway have a crucial role in the development and progression of several neoplasms. GISTs are characterized by frequent *c-Kit* oncogenic mutations and less commonly by *PDGFR-a* mutations. Activating mutations of *B-RAF*, a member of RAF kinase family, are frequently found in several tumors, mainly melanomas. The evaluation of this pathway is of great importance, owing to the fact that signal transduction mediated by c-Kit and PDGF receptors can be efficiently blocked by specific tyrosine kinase inhibitors such as Imatinib (Gleevec, Novartis). Gliosarcoma is a rare and poorly characterized malignant brain tumor that exhibits a biphasic tissue pattern with areas of glial and mesenchymal differentiation. Molecular studies showed that gliosarcomas display a genetic profile similar to glioblastomas and support the concept of monoclonal origin of both components. The aim of this study was to characterize the molecular alterations of PDGF subfamily of receptor tyrosine kinases pathway in gliosarcomas. Immunohistochemistry for PDGF-A, PDGFR-a and c-Kit were analyzed in both components of six gliosarcomas. Activating mutations in *PDGFR-a* (exons 12 and 18) and *c-Kit* (exons 9, 11, 13, and 17) genes were studied by PCR followed by direct sequencing. *B-RAF* gene (exons 11 and 15) was screened by using PCR-SSCP followed by direct sequencing. Expression of PDGF-A was found in all cases: three with similar moderate to intense immunoexpression in both glial and mesenchymal components, two with predominant intense expression in the glial component, and one case with predominant moderate mesenchymal expression. Co-expression of PDGFR-a was observed in three cases, mainly in glial component. c-Kit immunopositivity was observed in both components of one case. The mutational analysis of *PDGFR-a* showed the presence of a 2440-49_50insA intronic insertion in two cases, and a 2472C>T silent mutation in two cases. No mutations were detected in *c-Kit* and *B-RAF* genes. PDGF-A was overexpressed in gliosarcomas. PDGFR-a was overexpressed in 50% of cases, predominantly in the glial component supporting an autocrine loop

gliosarcomas; overexpression of c-Kit was not a frequent event. Activating mutations of *PDGFR-a* and *c-Kit* were not found, suggesting that other mechanisms are responsible for overexpression of these receptor tyrosine kinases in gliosarcomas.

192. EXPRESSION OF CD15 ON NEOPLASTIC CELLS OF INTRINSIC BRAIN TUMORS: A MODULATORY ROLE IN METASTASIS?

G. Pilkington,[1] A.J. Bolteus,[2] J. Rees,[3] R. Folkerth,[4] K. Asano,[5] and P. Forsyth[6]; [1]School of Pharmacy & Biomedical Sciences, University of Portsmouth, UK; [2]Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut, USA; [3]Institute of Neurology, University College, London, UK; [4]Harvard Medical School, Boston, Massachusetts, USA; [5]Department of Neurosurgery, Hirosaki University, Hirosaki-shi, Japan; [6]Tom Baker Cancer Centre, University of Calgary, Alberta, Canada

CD15, the adhesive sialyl LewisX oligosaccharide, acts as the cellular ligand for E-, L- and P- selectins as well as binding homophilically. It is widely distributed in epithelial cells and granulocytes. Within the developing normal brain neurones, astrocytes, oligodendrocytes, and Bergmann glia have also been reported to express this epitope. CD15 is also strongly expressed in many highly metastatic somatic cancers and is thought to mediate tumor cell adhesion to vascular endothelium prior to extravasation, enabling entry to, and colonization of, new sites for metastatic spread. We have previously shown that CD15 is expressed by experimental rat and mouse gliomas but that human glioma cells in vitro are invariably negative (Martin et al, Anticancer Res. 15, 1159, 1995). The ability of such glioma cells to adhere to non-brain vascular endothelial cells was also seen to be poor. In the present study we have examined archival histological sections from 19 cases of intrinsic brain tumor (including astrocytoma, anaplastic astrocytoma, oligodendroglioma, and glioblastoma multiforme) from which extraneural metastatic spread was apparent. In each of these cases, taken from five different hospitals, sections of the original tumor plus the secondary deposit (in chest wall, lymph nodes, scalp, bone marrow and lung) were examined by immunohistochemical staining using the DAKO anti-CD15 antibody (MO733). While CD15 expression was seen in infiltrating hematogenous cells, and discounted, expression on neoplastic cells within both primary and secondary sites was seen. Corresponding primary site "control" cases for the various histological types of tumor that had not been seen to metastasize showed CD15 positivity for neoplastic cells in only one case (glioblastoma multiforme). We conclude and postulate that expression of CD15 in primary brain tumors may indicate a possible propensity to spread outside the nervous system by virtue of facilitating adhesion of neoplastic glia to non-brain vascular endothelium. The Samantha Dickson Research Trust is gratefully acknowledged for their support of this research.

193. YKL-40 EXPRESSION IS ASSOCIATED WITH ACTIVATED PI3K AND MAP-KINASE PATHWAYS IN GLIOBLASTOMA: IMPLICATIONS ON AGE AND SURVIVAL

C. Pelloski, A. Mahajan, S. Woo, and K. Aldape; Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

We have previously shown that YKL-40 is overexpressed in glioblastoma (GBM) and its overexpression is associated with poorer response to radiation and reduced survival time. YKL-40 has been shown to activate the AKT and MAP kinase (MAPK) pathways in vitro. Therefore in this study, we test for an association of YKL-40 with activated intermediates of these pathways in GBM tumor specimens. The interaction between age and molecular marker status, with respect to overall survival (OS), is analyzed as well. The sample set comprised 294 patients with newly diagnosed GBM who underwent resection from 1994 to 2003. Immunohistochemical staining was performed on patient tissue for YKL-40, activated (phospho-) species of the AKT pathway (p-AKT, p-mTOR, and p-70S6K) and p-MAPK. Staining was scored as positive or negative. Clinical factors associated with decreased OS included older age and lower KPS (all $P < 0.003$, Kaplan-Meier). Expression of YKL-40, p-mTOR, p-70S6K and p-MAPK were highly correlated with each other (all $P < 0.009$, Spearman). There was a trend in correlation between YKL-40 and p-AKT. Expression of YKL-40, p-AKT, and p-MAPK correlated with advanced age (all $P < 0.01$). YKL-40, p-mTOR, p-70S6K, and p-MAPK expression levels were associated with decreased overall survival within the entire patient set (all $P < 0.02$, Kaplan-Meier). However, nearly all of the survival variation explained by the markers was observed within the young patient population. Specifically, among patients younger than the median age (59 years), univariate analyses showed YKL-40, p-mTOR, p-70S6K, and p-MAP to be prognostic factors (all $P < 0.01$). Multivariate analysis (Cox regression) revealed that lower KPS (HR 3.8, $P = 0.03$) and YKL-40 positivity (HR 1.8, $P = 0.03$) were independent prognostic factors after adjusting for extent of surgery, and p-mTOR, p-70S6K, and p-MAPK staining scores. None of these factors were significant in univariate analysis within the older patient group. The in vitro observation that YKL-40 activates the AKT and MAPK pathways is supported by the demonstrated correlation with these activated species in patient tumor specimens. While these markers are in general more prevalent in older patients, the survival impact of these markers on younger patients is far greater than on older patients. These findings may explain the survival differences between older and younger patients with GBM. The presence of these markers in patients younger than the median age for GBM (55–60) should be considered in risk stratification.

194. INVESTIGATION OF THE EGFR-GENE AMPLIFICATION STATUS DETERMINED BY FISH AS A PREDICTIVE OR PROGNOSTIC MARKER FOR PATIENTS WITH RECURRENT HIGH-GRADE GLIOMA TREATED WITH TEMOZOLOMIDE

B. Neyns,[1] J. Sadones,[1] B. Van Mierlo,[1] C. Chaskis,[2] A. Michotte,[3] J. Menten,[6] E. Joosens,[7] T. Strauven,[5] G. Storme,[4] and J. De Greve[1]; [1]Medische Oncologie, [2]Neurochirurgie, [3]Anatomopathologie/ Neurologie, and [4]Radiotherapie, Academisch Ziekenhuis Vrije Universiteit Brussels, Brussels; [5]Neurologie, AZ St-Augustinus, Antwerpen; [6]Radiation-Oncology, Universitair Ziekenhuis Gasthuisberg Katholieke Universiteit Leuven, Leuven; [7]AZ-Middelheim, Medische Oncologie, Antwerpen; Belgium

The epidermal growth factor receptor (EGFR) gene is frequently amplified and mutated in high-grade glioma (HGG). Immunohistochemical staining for the EGFRvIII mutant is a negative prognostic factor for patients with anaplastic glioma (Buckner et al., J. Clin. Oncol. 22 (14S), 1508, 2004). We investigated the prognostic and predictive value of EGFR-gene amplification determined with fluorescence in situ hybridization (FISH) in HGG treated with temozolomide (Temodal, TMZ) at recurrence following surgery and radiotherapy. Clinical data and tumor material were collected from a retrospective cohort of patients treated at 4 Belgian hospitals. All patients were treated with TMZ at recurrence. In addition to conventional diagnostic histopathology, HGG were characterized by FISH for the amplification status of the EGFR-gene (Vysis, LSI EGFR/CEP 7 Dual Color Probe). Our patient population consisted of 36 men and 22 women (N = 58) with a median age of 58 years (range, 16–80 years). At the initiation of TMZ treatment, tumors consisted of 14 AA/AOA and 44 GBM. Eighteen tumors were found to have an amplification of the EGFR gene (4/14 AA/AOA, 14/44 GBM). No significant association was found between EGFR-gene amplification and sex, age (at diagnosis or at initiation of TMZ), glioma localization, type of surgery (resection versus biopsy), histology (at diagnosis or at initiation of TMZ), the interval between diagnosis of HGG and the initiation of TMZ, or response to TMZ (objective response or disease control). We found no significant correlation between EGFR-gene amplification and time to progression or overall survival following the initiation of TMZ treatment (Kaplan-Meier survival statistics, log-rank test, $P > 0.3$). In a subgroup analysis of the 17 patients with an AA/AOA or secondary GBM, EGFR-gene amplification was associated with an inferior overall survival (log-rank test, $P = .04$). We could not demonstrate a predictive or prognostic value of EGFR-gene amplification in recurrent HGG treated with TMZ. Our observations support the concept that EGFR-amplification is a negative prognostic factor for OS in patients with an AA/AOA or secondary GBM.

195. LOW SERUM S100β LEVELS IN PATIENTS WITH NEWLY DIAGNOSED LUNG CANCER CORRELATE WITH AN ABSENCE OF BRAIN METASTASES ON MRI

M.A. Vogelbaum, P. Mazzone, T. Masaryk, T. Mekhail, V. Fazio, S. McCartney, N. Marchi, A. Kanner, and D. Janigro; Cleveland Clinic Foundation, Brain Tumor Institute, Cleveland, Ohio, USA

Early detection of metastatic brain tumors in patients with a known systemic cancer may provide these patients with less invasive treatment options. We have previously observed that disruption of the blood-brain-barrier (BBB), which occurs in the setting of metastatic brain tumors, is accompanied by a release of the astrocytic protein S100β into the serum where it can be detected by use of a simple, relatively low cost assay. We hypothesized that measurement of S100β in the serum may serve as a screening test for cerebral metastases in individuals with lung cancer. Individuals with newly diagnosed non-small cell lung cancer were enrolled in the study if there were no symptoms or signs of neurologic dysfunction suggestive of the presence of metastatic brain tumors. Each participant underwent MRI of the brain and had serum drawn with measurement of the S100β level. The level of S100β was compared to the results from MRI imaging.

Thirty-eight individuals agreed to participate in the trial. Seven of the 38 had evidence of metastatic disease on MRI of the brain, and 22 had normal MRI scans. The remaining 9 patients had no brain metastases but had T2 changes consistent with chronic microvascular disease. The S100β level was lower in those with normal MRIs than those with chronic microvascular disease or metastatic disease (0.07, 0.49, and 0.28 μg/liter respectively; $P < 0.002$ for patients with normal MRIs compared to those with chronic microvascular disease, and $P < 0.03$ for patients with normal MRIs compared to those with metastatic disease). Utilizing a cutoff of 0.12 mg/L the sensitivity of S100β for cerebral metastatic disease was 100%, specificity was –13%, negative predictive value was 1, and positive predictive value (PPV) was 0.47. If patients with chronic microvascular disease were excluded, the specificity was 86% and the PPV was 0.88. Low serum levels of S100β (< 0.12 μg/liter) accurately identified lung cancer patients who do not have metastatic brain tumors. However, an elevated serum S100β in this group of patients does not specifically indicate the presence of brain metastases. Nevertheless, the results of this study suggest that assessment of serum S100β can be used to narrow the population of lung cancer patients who may benefit from periodic surveillance imaging.

196. LOSS OF HETEROZYGOSITY (LOH) OF 1p IN OLIGODENDROGLIAL TUMORS IS RELATED TO REDUCED EXPRESSION OF NUCLEAR FACTOR KAPPA B (NFKB)

I. Lavon,[1] D. Fuchs[1], Y. Fellig,[2] Z.B.B. Zelikovitsh,[1] *T. Siegal*[1]; [1]Leslie and Michael Gaffin Center for Neuro-Oncology; [2]Department of Pathology, Hadassah Hebrew University Hospital, Jerusalem, Israel

In oligodendrogliomas, LOH on chromosome 1p is associated with tumor chemosensitivity. The molecular mechanism for this association is unknown. Nuclear factor kappa B (NFkB) is a transcription factor found in the cytoplasm as hetero- or homodimer that exerts its effects through binding to nuclear DNA and activating transcription of target genes. While the ultimate gene targets of NFkB are diverse, its activation can block apoptosis in numerous tumor cell lines. Moreover, NFkB inactivation is associated with increased susceptibility of tumor cells (including glioma cells) to antineoplastic therapy, probably secondary to facilitated apoptosis. Many tumors exhibit constitutive activity of NFkB, which possibly contributes an antiapoptotic effect associated with chemoresistance. We postulated that the chemosensitivity of oligodendroglioma may be related to reduced NFkB activation. Our objective was to evaluate NFkB activation in oligodendrogliomas and its relationship to 1p status. We evaluated 41 oligodendroglial tumors (WHO II, 26; WHO III, 15) for their LOH status by PCR. Activated NFkB which undergoes nuclear translocation was assessed by immunohistochemistry (IHC) of the p65 subunit. The level of NFkB activation was defined as high (50%–100% nuclear staining), intermediate (10%–50%) or low (<10% nuclear staining). All tumors (100%) with an intact 1p (n = 17) had high activation of NFkB as opposed to 43% (10/23) of tumors with 1p LOH. NFkB level of activation differed significantly ($P < 0.02$) between tumors with or without 1p LOH. This difference was more pronounced in the WHO II group, where high activation was found in all 11 tumors with intact 1p but in only 30% of those with 1p loss ($P = 0.0004$). We suggest that low NFkB activation plays a role in the chemosensitivity of oligodendrogliomas with LOH at 1p. Recent publications demonstrated that TNFa has a central role in NFkB activation and in protection against apoptosis. The deleted region on 1p (1p36) contains some of the tumor necrosis factor receptor super family (TNFRSF) such as TNFR2 that was shown to mediate NFkB activation. Further investigations are needed in order to conclude whether a deletion of TNFRSF at 1p site can induce chemosensitivity as a result of reduced NFkB activation.

197. DETERMINATION AND PROGNOSTIC SIGNIFICANCE OF MITOTIC INDEX IN GRADE II AND III INFILTRATING ASTROCYTOMAS USING THE MITOSIS MARKER PHOSPHO-HISTONE H3

K. Aldape,[1] G.C.H. Fuller,[1] L. Huang,[1] C. Giannini,[2] and P. Burger[3]; [1]The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [2]Mayo Clinic, Rochester; [3]Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Histologic classification of infiltrating astrocytomas into malignancy grades II or III depends primarily on the presence of mitotic activity. The accuracy in determining mitotic activity can be affected by the amount of available tumor for study as well as cellularity of the tumor. Methods to better quantify mitotic activity may improve the ability to stratify patients into prognostic groups. Phospho-histone H3 (pHH3) is specifically upregulated in mitosis, and anti-pHH3 immunohistochemistry facilitates the detection of mitotic figures in paraffin sections. Cases of newly diagnosed supratentorial astrocytomas in adults (>18 years) were identified in the pathology records. Archival paraffin material (total = 123) was obtained for cases of grade II astrocytoma (A, n = 20) or grade III (anaplastic) astrocytoma (AA, n = 103). A representative section from each case was stained for pHH3. Mitoses were identified by positive staining as well as morphologic confirmation of features of mitosis. The area of tumor with the highest mitotic activity on low power examination was selected for mitotic index determination. The mitotic index was determined by counting the number of positively stained mitoses per 1000 tumor cells. The mitotic index ranged from 0 to 20 mitoses per 1000 tumor cells, with a median of 2. In univariate analysis, higher mitotic index was a significant predictor of poorer survival, and this remained significant ($P < 0.01$) in multivariate Cox analysis after adjustment for age and tumor grade. After condensing the mitotic index data into 2 groups with low and high mitotic activity, Kaplan-Meier analysis indicated that cases with a mitotic index of 0, 1, or 2 (n = 66) had a significantly improved ($P = 0.04$) median survival (434 weeks) compared with cases with an index of 3 or above (n = 57, 177 weeks). We conclude that pHH3 staining provides a means to rapidly identify mitotic figures in order to obtain a true mitotic index. Determination of mitotic index offers theoretical advantages in the quantification of mitotic activity compared with current practices. Higher mitotic index is a significant independent predictor of survival in grade II and grade III astrocytomas. An index of 2 mitoses or less per 1000 cells in the most mitotically active area appears to define a group of tumors with survival time typical of grade II tumors, while an index of 3 or greater portends a prognosis typical of grade III anaplastic astrocytoma.

198. NESTIN, CXCL12/SDF1, PDGFR-BETA AND VEGF EXPRESSION IN ENDOTHELIAL PROLIFERATION ON 80 GLIOMAS: CLINICOPATHOLOGICAL CORRELATIONS AND SUGGESTIONS FOR ANGIOGENESIS

B. Pollo,[1] E. Maderna,[1] C. Calatozzolo,[2] and *A. Salmaggi*[2]; [1]Neuropathology and [2]Neuro-oncology, Istituto Nazionale Neurologico C. Besta, Milan, Italy

Nestin is a class VI intermediate filament (IF), highly expressed in embryonic progenitor cells and is a marker for multipotential neuroepithelial stem cells, involved in early stages of lineage commitment, in proliferation and in differentiation. Nestin is detected in neuroepithelial tumors and in endothelial cells in active proliferation. The VEGF expression upregulates CXCR4 on endothelial cells, binding the proangiogenic chemokine SDF1/CXCL12 (Stromal Derived Factor). CXCL12 have a role on angiogenesis and chemotaxis of endothelial cells throughout the activation of his receptor. PDGF has a role in glial tumorigenesis and modulates angiogenesis, and its receptor PDGFR-beta may be overexpressed in gliomas on angiogenic endothelial cells. To investigate the amount and the meaning of endothelial proliferating cell–expressed nestin related to the presence of VEGF, SDF1/CXCL12, and PDGFR-beta in gliomas, we performed an immunohistochemical study, with their specific antibodies, on 80 patients with gliomas of different phenotype and malignancy grade. The patients were categorized by disease: 20 glioblastomas, 12 anaplastic astrocytomas, 12 anaplastic oligoastrocytomas, 4 anaplastic oligodendrogliomas, 10 astrocytomas, 12 oligoastrocytomas, and 10 oligodendrogliomas. Moreover, all the cases were immunostained for GFAP, vimentin, Ki67/MIB1, and CD34. Nestin was variably expressed in neoplastic cells and in endothelial proliferations, suggesting a correlation to malignancy grade and clinical outcome. The immunodetection of nestin and CXCL12 in some endothelial cells in low-grade gliomas with shorter time to tumor progression suggests a role in early angiogenesis. A correlation was found also to expression of VEGF and PDGFR-beta. These results suggest that some cells expressing nestin, a marker of neural progenitor cells, stimulated by growth factors and chemokines, may be involved in angiogenesis as proliferating endothelial cells. Our data, especially in low-grade gliomas, might provide useful information related to angiogenesis and might add prognostic information in patients with variable life expectancy.

199. LOSS OF HETEROZYGOZITY ON CHROMOSOME 1p ± 19q IS A MAJOR FAVORABLE PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL IN LOW-GRADE GLIOMAS

F. Laigle-Donadey,[1] J. Lejeune,[1] E. Criniere,[2] Y. Marie,[2] M. Kujas,[3] L. Capelle,[4] M. Sanson,[1] K. Hoang-Xuan,[1] and J. Delattre[1]; [1]Neurologie, [2]INSERM U495, [3]Neuropathologie, and [4]Neurochirurgie, CHU Pitié Salpêtrière, Paris, France

The morphologic classification of low-grade gliomas remains controversial and insufficient. To investigate whether commonly described genetic alterations have prognostic significance, search for loss of heterozygosity (LOH) on chromosome 1p, 9p, 10q, 19q, *EGFR* amplification, and p53 expression was performed in a series of 131 low-grade gliomas. The profile of molecular alterations was then correlated with clinical parameters and

course of the disease, mainly progression-free survival (PFS). There was a correlation between an oligodendroglioma phenotype and LOH 1p ± 19q (P = 1.4. 10-8), and between an astrocytoma phenotype and P53 expression (P = 3. 10-7). On multivariate analysis, only LOH on chromosome 1p was associated with increased PFS (RR = 0.52), while histology and clinical parameters were not significant. This study indicates that LOH 1p is the single most important prognostic factor for PFS in low-grade gliomas.

200. A GENETIC POLYMORPHISM OF THE CHEK2 GENE AS A PROGNOSTIC FACTOR IN GLIOBLASTOMA MULTIFORME

M. Simon,[1] R. Fimmers,[2] M. Ludwig,[3] P. Süssmann,[1] J. Oldenburg,[4] and J. Schramm[1]; [1]Department of Neurosurgery, [2]Institute of Medical Statistics and Informatics, and [3]Institute of Clinical Biochemistry, University Hospital Bonn, Bonn; [4]Institute for Transfusion Medicine, University Hospital Frankfurt/Main, Frankfurt/Main, Germany

CHEK2 gene germline mutations have been identified in some families with Li-Fraumeni syndrome. Li-Fraumeni patients suffer from various malignancies including glioblastoma multiforme (GBM). The CHEK2 gene is located on chromosome 22q12.2. Up to 30% of sporadic GBMs may harbor LOH (loss of heterozygosity) for markers from chromosome 22q. The CHEK2 gene product is a negative cell cycle regulator and important effector of the DNA damage control pathways. We tested the hypothesis that a genetic polymorphism of the CHEK2 gene might modify the incidence and the clinical course of sporadic human GBM. DNA was isolated from peripheral blood of 213 patients with primary GBMs and 192 control subjects. Samples were genotyped with respect to a biallelic SNP (single nucleotide polymorphism), CHEK2 IVS1, -711 A/T, using PCR and restriction digests. For all patients the following clinical parameters were recorded: age, sex, histology (GBM NOS, giant cell GBM, gliosarcoma), localization, degree of resection (biopsy, partial, subtotal and gross total resection), postoperative radiotherapy, postoperative chemotherapy, postoperative Karnofsky performance score. No association between the CHEK2 SNP and GBM incidence was found. Interestingly, the CHEK2 IVS1, -711 genotype correlated significantly with survival (P = 0.034, log-rank test), most clearly among patients receiving chemotherapy (n = 28, P = 0.0008). Highly significant clinical prognostic factors were age, degree of resection (biopsy vs. cytoreductive surgery), postoperative radiation and chemotherapy, and postoperative Karnofsky score (all $P < 0.0001$, log-rank test). This study confirms the important role of well-known clinical prognostic factors such as age, Karnofsky performance index, and postoperative radiotherapy. Our data support the use of cytoreductive surgery and chemotherapy for GBMs. Most interestingly, this investigation suggests a genetic polymorphism as a prognostic marker for GBMs. Since CHEK2 is involved in the detection and repair of DNA damage (e.g. after chemotherapy), possible explanations include differential chemosensitivity mediated by different CHEK2 IVS1, -711 alleles.

201. MORPHOLOGICAL AND MOLECULAR HETEROGENEITY WITHIN TUMORS WITH OLIGODENDROGLIAL CHARACTERISTICS

M.C.M. Kouwenhoven, B. de Jong, P.A. Sillevis Smitt, M.J. van den Bent, and J.M. Kros; Neuro-oncology, Erasmus University Medical Center, Rotterdam, The Netherlands

Gliomas are known for their morphologic heterogeneity. Generally, gliomas are typed according to areas with classic histological parts, but within many tumors areas with less typical histology exist. The classic histology of oligodendrogliomas is the honeycomb architecture with chicken wire-like vasculature, the tumor cells having a perinuclear shrinkage artifact. However, tumor parts showing astrocytic differentiation, mixed histology, predominant gemistocytic parts, pseudoependymal or neurocytic differentiation, rhabdoid changes, or mucoid degeneration are variably encountered in biopsy material as well. In approximately 60% of oligodendroglial tumors losses of 1p and 19q are found. This genotype has been linked with a favorable response to both radio- and chemotherapy, and patients show better survival as compared to those suffering from oligodendroglial tumors without these characteristics. It is unclear whether histological heterogeneous tumors are also genetically heterogeneous, and whether tumor parts other than those with classic histology will carry the oligodendroglioma-specific genotype. This information may be important when considering the (high) probability of sampling error and treatment of these tumors, and glioma classification in general. For this study a selected group of 14 gliomas, consisting of 6 anaplastic oligodendrogliomas, 6 mixed anaplastic oligoastrocytomas, and 2 glioblastomas, all with partly classical oligodendroglial and other histology, were used. Within each tumor, up to 8 regions (total number of regions studied was 60) were characterized morphologically and genotyped by fluorescent in situ hybridization (FISH) for 1p and 19q. Loss of 1p was found in 8/14 tumors and loss of 19q in 7/12 tumors. Fifty percent of the losses of 1p were detected in all tumor areas, while the other 50% were only seen in part of the tumors. In 57% of tumors, loss of 19q was found in all tumor areas, and in the other 43% only in parts of the tumors. Loss of 1p was detected in tumor areas other than those with classic oligodendroglial histology in 47% of cases, and loss of 19q in 45% of cases. The results illustrate that, besides phenotypical heterogeneity, there is also genotypical heterogeneity and that genotyping of gliomas without classic oligodendroglial histology may result in positive identification of 1p/19q losses. This may be relevant for small (stereotactic) biopsies. Alternatively, these findings may reflect limitations of the tests used; therefore, further research is indicated.

202. A PROSPECTIVE, BLINDED ANALYSIS OF A-PROTEIN LEVELS IN PEDIATRIC PATIENTS WITH CENTRAL NERVOUS SYSTEM TUMORS

P. Manley,[1] C. Turner,[1] S. Chi[1], M. Zimmerman,[1] C. Chordas,[1] A. Gordon,[1] A. Thomas,[1] K. Hoffman,[2] and M. Kieran[1]; [1]Pediatric Oncology, Dana Farber Cancer Institute, Boston, Massachusetts; [2]Cytra Corporation, Wrentham, Massachusetts; USA

The purpose of this prospective blinded study was to evaluate the sensitivity and specificity of A-PROTEIN levels in pediatric patients with brain tumors. A-PROTEIN is a 23 kD molecule that is highly conserved among mammals and non-mammalian species and was initially named for its presumed role in adenosine nucleotide exchange. While the exact function of this protein remains controversial, it does appear to be involved in cell division and signal transduction through both G-protein and Ca^{2+} binding protein activity. A-PROTEIN is normally localized to the intracellular plasma membrane. It is located in both normal and neoplastic tissue. Abnormal expression of A-PROTEIN has been identified in a number of tumors including small cell carcinoma of the lung, carcinoma of the breast, colon, prostate, and cervix. Brain tumors have been reported to express exceedingly high plasma levels of A-PROTEIN and thus may be of significant diagnostic and prognostic value. Pediatric neuro-oncology patients were prospectively identified from the neuro-oncology program at Children's Hospital Boston and the Dana Farber Cancer Institute. Patients included those with newly diagnosed disease pre- and post-surgery, during treatment, and during routine follow-up while maintaining remission (based on concurrent MRI scans), as well as at the time of recurrence or progression. A total of 154 A-PROTEIN levels in both CSF and blood from 54 pediatric patients were evaluated. No correlation of A-PROTEIN level was detected based on patient age (within the pediatric population) or clinical status. Forty-nine samples from patients with no evidence of disease based on MRI scans were evaluated. Of these samples, 46% were negative, while 29% were positive and 25% were equivocal. For patients with stable disease, 73 samples were obtained, and 51% demonstrated a negative A-PROTEIN level; 19% of these samples were positive and 30% were equivocal. By contrast, 20 samples from patients with progressive disease were evaluated. Of these, 55% had normal A-PROTEIN levels, 35% were positive, and 10% were equivocal. Based on these results, the sensitivity of the assay was 39% and the specificity was 62%. Based on our data, A-PROTEIN levels were not predicative of disease status in children with brain tumors. Whether this results from the variability of A-PROTEIN levels during growth and development needs to be further evaluated.

203. RESPONSE TO RADIOTHERAPY IS NOT ASSOCIATED WITH LOSS OF HETEROZYGOSITY (LOH) FOR CHROMOSOMES 1p36 AND 19q13 IN WHO GRADE II ASTROCYTIC GLIOMAS

A. Fathi,[1] C. Murtin,[3] E. Vassella,[3] M. Arnold,[3] J. Curschmann,[2] M. Reinert,[1] P. Siegenthaler,[1] J. Weis,[3] A. Kappeler,[3] and *L. Mariani*[1]; [1]Universitätsklinik für Neurochirurgie and [2]Universitätsklinik für Radioonkologie, Inselspital, Bern, Switzerland; [3]Institut für Pathologie, Universität Bern, Switzerland; [4]Universitätsklinik der RWTH, Institut für Neuropathologie, Aachen, Germany

Loss of heterozygosity (LOH) of 1p36 and 19q13 is probably both prognostic for survival and a predictive marker of chemosensitivity in patients with WHO grade II gliomas. A high proportion of patients with WHO grade II oligoastrocytomas (OA) and astrocytomas (A) respond to radiotherapy. We investigated whether or not the presence of these deletions might be related to response to radiotherapy in those tumors. From our database of patients with gliomas WHO grade II (OA, A) treated between 1991 and 2000, we selected patients who received radiotherapy either at primary diagnosis or after tumor progression. The tumor volume was measured and monitored after radiation therapy. Tumor response was assessed according to standard criteria and graded as complete response (CR), partial response (PR, >50%), minor response (MR, ±25%), and progressive disease (PD, >25%). The presence of LOH for chromosome 1p (4 loci) and 19q (3 loci) was assessed on archival, paraffin-embedded tumor specimens

by using a PCR-technique. Thirty-eight patients received radiotherapy. There were 31 A and 7 OA. The tumor volume was available for 27 patients (71%), 12 having received radiotherapy at primary diagnosis and 15 at tumor progression. There was no CR. Five patients had a PR (18.5%), 8 patients MR (29.6%), 12 patients a SD (44.4%), and 2 had PD. The LOH status could be assessed in 17 and 16 patients for 1p and 19q, respectively. Of 12 patients with intact chromosome 1p36, 6 had either PR or MR, and 6 had either SD or PD. Approximately 50% of patients with astrocytic WHO grade II tumors show objective tumor response after radiotherapy. Tumor response is not associated with LOH for 1p36 and 19q13.

204. PROGNOSTIC VALUE OF 1p/19q REARRANGEMENT IN OLIGODENDROGLIOMAS: RETROSPECTIVE STUDY WITH 86 PATIENTS

Y. Guegan,[1] N. Auger,[2] S. Saikali,[3] A. Hamlat,[1] B. Laviolle,[4] and M. Le Calve[2]; [1]Clinique Neurochirurgicale, [2]Laboratoire de Cytogénétique, [3]Laboratoire d'Anatomopathologie, and [4]Service de biostatistiques, CHU Pontchaillou, Rennes, France

Oligodendrogliomas represent up to 25% of diffuse gliomas, the most frequent primary tumors of the central nervous system. The tumors, which present a 1p/19q co-deletion, have a better prognosis, with a higher chemoradiosensitivity and longer survival. A retrospective study of 1p/19q rearrangements in primary oligodendrogliomas treated between 1990 and 2001 at the Rennes Hospital was done. A confrontation between clinical data and factors influencing the prognosis was made. The tumors were graded by two neuropathologists. FISH (fluorescent in situ hybridization) was performed on 5-mm-thick sections from archival formalin-fixed paraffin-embedded tumors. The probes used, located in 1p36 and 19q13 regions, were developed from BAC and were directly labeled by nick translation. For each probe, hybridization signals were scored from a minimum of 200 nuclei. A univariate and a multivariate statistical analysis was performed. The prognosis was correlated with the age of the patients at diagnosis (odds ratio = 1.1), with the presence of necrosis (odds ratio = 13.7), and with the chromosomal status (odds ratio = 0.026). We found 58% (n = 59) of tumors with a deleted status, 22% (n = 19) with a disomic status, and 20% (n = 17) with a polysomic status. Tumors with a deleted status had a better prognosis and a better response to chemotherapy than the 1p/19q disomic tumors. A third population of tumors had a polysomic status, which cannot be detected by LOH search and is hardly identified by CGH (comparative genomic hybridization). This population of patients had a poor prognosis whatever the treatment was. This study reveals the coexistence of three independent prognostic factors: age, necrosis presence, and mainly chromosomal status. Even though the deleted population is known, we reveal for the first time a new population, the polysomic status tumors population, which has a very poor prognosis.

205. IMMUNOHISTOCHEMICAL SCREENING BASED ON GENE EXPRESSION PROFILES PREDICTIVE OF MENINGIOMA RECURRENCE

W. Hendricks,[1,2] S. Coons,[3] A. Scheck,[1] K.E. Ramsey,[4] and M. Preul[2]; [1]Neuro-Oncology Research, [2]Neurosurgery Research, and [3]Neuropathology, Barrow Neurological Institute, Phoenix, Arizona; [4]The NINDS/NIMH Microarray Consortium, Translational Genomics, Phoenix, Arizona; USA

Current clinicopathological models used in the diagnosis, prognostication, and treatment of meningiomas do not always reliably predict tumor behavior and patient survival. A pathological tool that better distinguishes the more aggressive subset of these tumors will allow a more accurate prediction of their behavior, leading to earlier therapeutic intervention and improved patient outcome. Differential gene expression in 54 meningiomas was analyzed by using Affymetrix U133 Plus 2.0 GeneChip Human Genome microarrays. Expression profiles based on histologic grade, primary/recurrent identity, invasion, and whether the tumor recurred within a two-year follow-up period were used to identify genes likely to provide clinically useful diagnostic/prognostic information. Class prediction and clustering analyses were then performed to evaluate the ability of these profiles to predict clinical parameters associated with the aggressive phenotype. Genes in profiles were subsequently filtered based on the availability of antibodies to their protein products. The diagnostic and prognostic value of each antibody is being evaluated in up to 1200 tumors on 20 tissue microarrays. Neither the profiles made according to the lone parameters of grade, primary/recurrent status, and invasion nor the profiles made according to a combination of these parameters predicted tumor recurrence better than WHO grade alone. However, a profile based on recurrence on follow-up reveals distinct molecular signatures for these tumors. These data show that clinically aggressive meningiomas (as defined by clinical recurrence within 2 years) share common molecular features to allow for expression profiling for diagnosis and prognostication. Searches of publicly available antibody databases based on genes whose expression can predict aggressive behavior reveal candidates for further screening with antibodies on tissue microarrays. Current candidates include CD40, CD96, and SFRP4. These findings are being further correlated with histologic grade, subtype, invasion, and recurrence. The use of microarray-based expression profiling to identify prognostically significant genes that can then be analyzed by immunohistochemical screening, a technique readily available in clinical laboratories, provides a novel tool to significantly enhance clinical management of meningiomas and improve overall patient outcome.

206. TEMOZOLOMIDE (TMZ) AS INITIAL TREATMENT FOR PROGRESSIVE LOW-GRADE OLIGODENDROGLIOMAS: TREATMENT RESULTS AND CORRELATION BETWEEN GENETIC PROFILE AND MGMT PROTEIN EXPRESSION

N. Levin,[1] I. Lavon,[1] B. Zelikovitsh,[1] D. Fuchs,[1] Y. Fellig,[2] F. Bokstein,[1] and *T. Siegal*[1]; [1]Leslie and Michael Gaffin Center for Neuro-Oncology and [2]Department of Pathology, Hadassah Hebrew University Hospital, Jerusalem, Israel

Loss of heterozygosity (LOH) on chromosomes 1p and 19q has been associated with favorable response to chemotherapy and good prognosis in oligodendrogliomas. The DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) may induce resistance to DNA-alkylating drugs. Recent studies show that TMZ, an oral alkylating agent, has efficacy in progressive low-grade oligodendrogliomas (LGO). Yet, limited data is available regarding the association between 1p/19q profile and MGMT protein expression in these tumors. The objective of this study was to evaluate the response of progressive LGO to TMZ and to assess the association between 1p/19q profile and MGMT protein expression. Adult patients whose MRI findings and/or clinical deterioration were compatible with progressive LGO were eligible for the study if they were radiotherapy naïve. TMZ dose was 200 mg/m^2/d for 5 days repeated every 28 days. Clinical and MRI data served for evaluation of outcome, and Kaplan-Meier estimates were used to assess median time to tumor progression (TTP) and progression-free survival (PFS). 1p/19q status was evaluated from paired tumor-blood DNA samples by using PCR-based microsatellite analysis. MGMT protein expression was studied in paraffin-embedded tumor sections by immunohistochemistry. Twenty-eight patients (median age, 38; range, 17–77) were enrolled. Median time between tumor diagnosis and TMZ treatment was 33.5 months (range, 1–133). Median number of TMZ cycles/pt was 12 (range, 2–24). Marked clinical improvement was recorded in 15 pts (54%), an objective response in 17(61%) with 7 minor responses, stable disease in 10 (36%), and progressive disease in one patient. Median TTP is 31 months with PFS of 70% at 24 months. LOH of 1p and/or 19q was found in 14/16 tumors of whom, 11 improved on TMZ, and 3 had stable disease. Of the 2 tumors with no LOH, one had stable disease and the other progressed. MGMT protein expression was evaluated in 16 LGO, whose LOH status showed 13 codeletions at 1p/19q and 3 had an intact 1p chromosome. LGO with an intact 1p demonstrated high expression of MGMT (>50% nuclear staining) as opposed to tumors with 1p loss that exhibited a relatively low MGMT (0–50% nuclear staining). MGMT expression was significantly associated with 1p loss ($P < 0.0004$). TMZ is active in progressive LGO. 1p/19q deletions are associated with response to TMZ, and MGMT protein expression significantly correlates with the allelic loss on 1p chromosome.

207. HISTOPATHOLOGIC GRADING OF ADULT MEDULLOBLASTOMAS IS NOT PREDICTIVE OF SURVIVAL

M.T. Giordana,[1] B. Pollo,[2] R. Ferracini,[3] A. Silvani,[2] C. D'Alessandro,[1] G. Cavallo,[4] A. Paioli,[4] P. Ghiglione,[1] and A. Chio'[1]; [1]Department of Neuroscience, University of Turin, Torino; [2]Departments of Neuropathology and Neuro-oncology, C. Besta Institute, Milano; [3]Department of Pathology, University of Bologna, Bologna; [4]Department of Oncology, Bellaria Hospital, Bologna; Italy

Histopathologic grading of pediatric medulloblastomas based upon increasing grade of anaplasia predicts clinical behavior. The present study was aimed to grading medulloblastoma of adult patients (>18 years old) by evaluating 83 samples for pathologic features of malignancy and anaplasia. Nodularity, desmoplasia, nuclear size, nuclear pleomorphism, necrosis, endothelial proliferations, and MIB-1 labeling index (LI) value have been evaluated by multivariate analysis with age, tumor site, and total survival. Morphometric analysis of nuclear size was performed by using the Eclipse Net program. Patients treated with standard postoperative radiotherapy (35 Gy to the craniospinal axis and 50 Gy to the posterior fossa) were considered for correlation with survival. Pathologic data and total survival were compared by Kaplan-Meier and log-rank analysis. Nodular/desmoplastic features were present in 50% of cases; however, none of them qualified

for medulloblastoma with extensive nodularity. Small isomorphic nuclei characterized 70% of tumors, while others had a moderate nuclear pleomorphism without significantly enlarged nuclei. Isolated large cells with pleomorphic nuclei were found in two cases only. MIB-1 LIs ranged from 9.6% to 55.5% (median 30.2%) and were not significantly different in nodular versus classic medulloblastomas. No correlation was found between total survival duration and the evaluated pathologic features. Assessment of anaplasia grade based either on coexistence or increasing degree of the analyzed features did not predict outcome. Severe degrees of anaplasia were not found. Based on our data, the histologic evaluation of medulloblastomas from adults with respect to features of anaplasia does not give prognostic information. For a satisfactory stratification of adult patients with medulloblastoma, we have to turn to biological and molecular factors and analyze their prognostic role.

208. RANDOMIZED CONTROLLED TRIAL OF A PATIENT SPECIFIC REMINDER OF ROYAL COLLEGE OF PHYSICIANS GUIDELINES FOR COMMUNICATION IN PATIENTS WITH MALIGNANT GLIOMA

R. Grant,[1] I. Whittle,[1] A. Gregor,[1] G. Kaar,[2] R. Swingler,[3] and P. Mathew[3]; [1]Edinburgh Centre for Neuro-Oncology, Western General Hospital, Edinburgh, Scotland; [2]Neurosurgery, Aberdeen Royal Infirmary, Aberdeen, Scotland; [3]Neurosciences, Ninewells Hospital, Dundee; Scotland; UK

Some studies have shown that Patient Specific Reminders (PSR) can improve the standard communication between treating doctors and patients or colleagues. We developed PSR cards to remind treating doctors of the Royal College of Physician guidelines on communication, for example, breaking the news of diagnosis of brain tumor, diagnosis, and prognosis. We were interested to see if a PSR attached to the patient notes improved communication. This was a randomized controlled trial involving 163 patients with intrinsic brain tumors treated in three centers (Edinburgh, Aberdeen, and Dundee), randomized to PSR card to inform the neurosurgeon about RCP guidelines about communication with patient and colleagues or no PSR card (standard care). Quality of communication with (a) patient, (b) GP, and (c) radiation oncologist was assessed after discharge by confidential questionnaires. Completed questionnaires returned by patients were scored by 3 blinded independent assessors of a brain tumor charity. The GP questionnaires returned by GPs were sent to 3 independent GPs for scoring; the Radiation Oncology questionnaires were sent to 3 independent hospital doctors for scoring. The assessors rated the individual questionnaires for overall communication as very good, good, poor, very poor. Assessors only knew the age and the presumed diagnosis. The median score of the 3 assessors was taken as the overall grade for each questionnaire. Statistical analysis was performed by using the chi squared test. Quality of communication in patient questionnaires was considered good or very good in 55% to 74% and very poor in 9%. There was no statistical difference between those allocated a PSR and those with "standard care" (DF = 6, $P = 0.365$). GPs considered communication good or very good in 36% to 48% of cases and very poor in 13% to 24% (PSR 13%: standard care 24%), no statistical difference (DF = 6, $P = 0.53$). Hospital doctors considered rated communication with radiation oncologists as good or very good in 55% to 62% of cases and very poor in 2.5% to 5%, no statistical difference (DF = 6, $P = 0.965$). This is the only RCT of the effect of a patient-specific reminder regarding guidelines on communication for neurosurgeons dealing with brain tumor patients. The PSR alone did not dramatically or statistically significantly improve the quality of information given to patients, GPs, or radiotherapists by the neurosurgical teams in 3 centers in this study.

209. THE COLLECTIVE CARE PATHWAY OF THE NEURO-ONCOLOGY NURSE PRACTITIONER AND THE ADVANCED PRACTITIONER IN RADIOTHERAPY

L. Muffett and K. Burton; Oncology, Addenbrooke's Hospital, Cambridge, UK

Primary brain tumors (gliomas) represent a small proportion of all cancers, yet the impact of the disease is catastrophic, as the illness causes both physical and cognitive disabilities. For the client and family, this presents many challenges, as the journey is unpredictable. It is this uniqueness that requires specialist input, ranging from advanced technical treatments to palliative care. The concept of the above roles evolved from The Calman-Hine Report (DOH 1995) and The NHS Plan (DOH 2000 A), which subsequently initiated The NHS Cancer Plan (2000 B). It was this report that explicitly identified recommendations for staff investment and patient care and acknowledged the need for site-specific multidisciplinary teams. It proposed that core members of the team should include a specialist nurse and a therapy radiographer. Since their implementation, both roles have evolved, and the current designations replace the previous titles of Specialist Radiographer and named nurse. Both roles are diverse. The Advanced Practitioner role focuses on providing expert knowledge in the planning and delivery of radiotherapy, coordinating the pathway of radiotherapy, and providing support for the patient and family. Whereas the Nurse Practitioner role is very subjective, it focuses on providing support to both clients and family members at all stages of the journey. The role encompasses a holistic approach, centering on providing palliative care. To work together autonomously, without overlapping, has required setting foundations. Defining roles, setting boundaries, undertaking clinical supervision, and reflecting on the illness trajectories have assisted us in planning the care pathways. All patients are discussed within the team's multidisciplinary meeting prior to their first consultation. It is at this assessment that planning of the patient journey begins. Depending on treatment intervention, a determination is made of which professional takes the lead. Fortunately, due to our professional flexible working relationship, when situations change or remits of roles are stretched, we are able to adapt accordingly to focus on service delivery rather than egos! We believe that this unique combined approach enhances patient care. Both roles provide expert knowledge, which is pivotal to the patient journey, the team, and each other. It is this awareness of roles, competencies, boundaries, and patient preference that allows us to identify phases of the patient journey which require greater input. In order to evaluate the impact of our roles on the service, we aim to audit the patient experience in the very near future.

210. IMPACT OF A NEUROONCOLOGIST-COORDINATED BRAIN TUMOR MANAGEMENT TEAM ON SURVIVAL OF PATIENTS WITH GLIOBLASTOMA MULTIFORME: THE HARTFORD HOSPITAL EXPERIENCE

A. Flowers; Neurology, Hartford Hospital, Hartford, Connecticut, USA

In the United States it is estimated that 18,400 patients are diagnosed per year with primary malignant brain tumors (BT) (ACS, 2004). Malignant gliomas account for 77% of malignant primary BT in adults. Over 50% of patients with malignant glioma have glioblastoma multiforme (GBM). Most of these patients are cared for in a community setting on a "one way street" pathway, from the diagnosing physician to a neurosurgeon, then a radiation oncologist, then a medical oncologist. In centers where the care of BT patients is provided by a multidisciplinary team, with either a neurooncologist or a medical oncologist assuming a coordinator role, patients are more likely to become active participants in their care and to enroll in clinical studies, and the overall patient satisfaction is higher. The present study evaluates outcomes of patients with GBM cared for at Hartford Hospital, in a multidisciplinary setting. From 1996 to 2003, 100 patients with GBM were evaluated at Hartford Hospital by a multidisciplinary team coordinated by a neurooncologist. Treatment and survival data are available for 76 patients; 24 patients were lost for follow-up. Median age was 59 years (24–87 years). There were 43 males and 33 females. Seventy-six patients had surgery, 10 had second and 3 had third resection, 76 patients had external beam RT, 8 had radiosurgery, and 54 had chemotherapy (11 in clinical studies). Survival data for these patients was analyzed and compared with survival of a group of 35 GBM patients (median age 71; 37–88) treated at Hartford Hospital with RT alone or palliative care, either due to patients' age, or poor prognostic factors. Survival was also compared with national SEER reported data. Survival for patients treated by the multidisciplinary team (MDT) was significantly longer than for patients treated conservatively. Median survival was 1 year versus 5.5 months. Comparative 1-, 2-, 5-, and 10-year survival rates are for HH MDT 52.6%, 19.7%, 7.89%, and 1.3%, respectively, versus SEER 29.1%, 8.8%, 3.4%, and 2.4%, respectively. Only 6% of patients treated conservatively were alive at 1 year, none at 2 years. Functional outcome was also analyzed for MDT-managed patients. Fifty percent of patients with GBM were still working at 1 year. Patients who survived beyond 2 years were able to maintain a good level of functioning up to 4 years. The main predictor for survival was patients' age, with longest survival for patients under 55 years of age. Multidisciplinary management of patients with GBM, coordinated by a neurooncologist, has a positive impact on survival by providing comprehensive care; close follow-up, early intervention at recurrence, more treatment options, and a strong support system. The conventional pathway is appropriate for patients with poor performance status, those with rapidly growing tumors, or those who do not wish to be treated aggressively if at all.

211. THE PRESENTATION OF CHILDHOOD BRAIN TUMORS: A META-ANALYSIS

S. Wilne,[1] J. Collier,[2] D. Walker,[1] R. Grundy,[3] C. Kennedy,[4] and J. Punt[1]; [1]Academic Division of Child Health and [2]School of Nursing, University of Nottingham, Nottingham; [3]Institute of Child Health, University of Birmingham, Birmingham; [4]Department of Child Health, University of Southampton, Southampton; UK

Brain tumors can be difficult to diagnose. Many of the initial symptoms and signs are also seen in other more common and less serious childhood illnesses. The challenge for health care professionals is to identify children who may have a CNS tumor from the majority that do not. A meta-analysis of the presenting signs and symptoms of childhood brain tumors was conducted as the initial stage in a project to devise guidelines for the referral and imaging of children who may have a CNS tumor. The objective of this study was to conduct a meta-analysis of the presenting symptoms and signs of childhood brain tumors. The English-language literature was searched from 1996 to 2004 by using Medline, Embase, and the Cochrane library. Case-series and cohort studies detailing the presenting symptoms and/or signs of children with brain tumors and including at least 10 children were included in a meta-analysis of presenting symptoms and signs. Thirty-two papers describing 2200 patients were identified as satisfying the inclusion criteria. Sixty-eight symptoms and signs were identified as occurring in children with brain tumors, although not every paper contained data on every symptom and sign. Meta-analyses of the data revealed that the most frequent presenting signs and symptoms were headache (43%; 41%–45%); nausea and vomiting (41%; 39%–43%); abnormalities of gait and co-ordination (29%; 27%–31%); papilloedema (17%; 16%–19%); changes in behavior and school performance (13%; 12%–15%); seizures (13%; 12%–15%); signs of raised intracranial pressure (11%; 10%–13%); squint (11%; 10%–13%); ataxia (10%; 9%–11%); and anorexia or weight loss (8%; 7%–9%). Thirty-one additional symptoms and signs occurred at lower frequencies. This analysis emphasizes the diverse ways in which childhood brain tumors present. While the classical symptoms of raised intracranial pressure, headache, and vomiting are identified as the commonest presenting symptoms of childhood brain tumors, they occurred in fewer than 50%. Childhood CNS tumors may present in multiple ways and thus to many branches of medicine. Brain tumors have a long symptom interval in comparison to other childhood tumors. Families and patients find this distressing, and it may adversely affect outcome. Awareness among health care professionals that the presentation of brain tumors may mimic that of more common childhood illnesses may facilitate diagnosis and reduce their symptom interval.

212. THE IMPACT OF THE NURSE PRACTITIONER ON LENGTH OF STAY OF NEURO-ONCOLOGY PATIENTS IN AN ACUTE CARE SETTING IN A LARGE TEACHING HOSPITAL

G.C. Kolak,[1] J.W. Henson,[1] N.M. Leahy,[1] E. Stephen,[2] and C. Pappas; [1]Massachusetts General Hospital; [2]Center for Neuro-Oncology, Boston, Massachusetts, USA

The value of the nurse practitioner (NP) as part of a multidisciplinary team has been well documented in both nursing and medical literature. The Massachusetts General Hospital (MGH) established a full-time nurse practitioner position to decrease the length of stay (LOS) of neuro-oncology patients, with the additional goals of improving patient education and outpatient transition. The neuro-oncology NP program was initiated in 1999. A retrospective analysis was performed of the average LOS of 627 patients admitted to the neuro-oncology service for three periods: 8 months prior to the addition of the NP (period 1) and the two 8 month periods following addition of the NP (periods 2 and 3). We also analyzed the number of LOS outliers between the first two periods. Data were obtained from the hospital's Clinical Performance Management (CPM) database. The neuro-oncology team consisted of a staff neuro-oncologist, neuro-oncology fellow, and NP. The NP assisted in the medical management and discharge planning of general neuro-oncology patients hospitalized due to complications of their disease or side effects of treatment. The NP also managed all elective chemotherapy patients by coordinating pre-admission laboratory evaluation, writing chemotherapy and admission orders, performing daily medical care, and planning patient discharges. Average LOS was 10.7 days in period 1, prior to the addition of the NP. There was a decrease in average LOS of 3.3 days (from 10.7 to 7.4 days) at the end of period 2, and 4.5 days (from 10.7 to 6.2 days) at the end of period 3 ($P = 0.05$). There were 10 non-chemotherapy patients who had LOS greater than 2 SD of average LOS. Of the 10 patients, 7 were admitted in period 1, prior to the addition of the NP, whereas 3 were admitted in period 2. Informal interviews with staff neuro-oncologists, nursing administration, and staff nurses revealed an improvement in patient education, smoother transitions to the outpatient setting, and better coordination of patient care in general. This subsequently resulted in an overall improvement in patient satisfaction. Nurse practitioners are effective in the acute care setting in decreasing LOS of neuro-oncology patients. The addition of the nurse practitioner improved the care of neuro-oncology patients both by decreasing average LOS and increasing patient satisfaction.

213. QUALITY OF LIFE IN BRAIN CANCER PATIENTS AND COPING STRATEGIES

C. Lucchiari, R. Lovati, A. Silvani, M. Eoli, E. Lamperti, A. Salmaggi, A. Boiardi, and G. Filippini; Istituto Nazionale Neurologico C. Besta, Neuro-oncology, Milan, Italy

The aim of our study was to measure the quality of life (QoL) in brain cancer patients using objective and subjective measures, in order to assess QoL and problematic areas to briefly fit patients' needs in a hospital setting. We studied 111 consecutive subjects with high-grade gliomas between 23 and 79 years old (mean age, 48.9) during their treatment at the National Neurological Istitute C. Besta of Milan (Italy). Each patient completed the following scales: the Fact-G scale 4.0 (Italian version) and the Fact-Br brain specific module for the evaluation of QoL in brain cancer patients, the HAD scale for depression and anxiety, and the half-structured interview SeiQol-Dw for subjective evaluation of QoL and patient's experience. For each patient we first measured the functional status by the Karnofsky index (KPS) and the cognitive status through the Mini Mental State Examination. The mean ($\pm$ SD) score of the Fact-G was 73.4 $\pm$ 13.7 and the Fact-Br was 123.1 $\pm$ 23.7. The HAD depression sub-scale showed that 19% of patients had a light to moderate depression and 3% a severe depression state. Concerning the SeiQol-Dw interview, we performed a content analysis of the SeiQol descriptions, and we categorized answers in eight main classes of coping behavior. Most patients found in familial or social support and in positive actions and thoughts good strategies to cope with the illness. Further, women were observed to use spiritual and positive thoughts more frequently. At the opposite extreme, men preferred more concrete strategies drawn to improve their functional role in family and society. Males also reported negative thoughts more frequently than women. The psychological adjustment to the illness by males seemed to be more difficult. Interaction between measures showed that global Fact-Br score was better predicted by Kps and depression, while the SeiQol general score was mainly predicted by depression scores. Depression did not appear to change in correlation with age, sex, and time from first diagnosis. Our data confirm that patients with high-grade gliomas may cope with the illness without developing severe depression and anxiety. Though QoL is strongly affected by physical symptoms, patients are able to use different strategies to fit the situation, during aggressive therapy, radiotherapy, and chemotherapy. We think that the coping strategies used by patients help them to adjust to the illness and continue their treatment in a hopeful and protective context. This indicates the necessity to sustain patient's positive context with an adequate assessment and comprehension of the potential coping strategy. This could be done by adopting specific instruments that help to develop an understanding of the patient's subjective experience.

214. PRE-, POST-OPERATIVE AND FOLLOW-UP FMRI EVALUATION OF CONDITIONAL MOTOR ASSOCIATIVE FUNCTION IN PATIENTS WITH LOW-GRADE GLIOMAS

C. Amiez,[1] P. Kostopoulos,[1] A. Champod,[1] M. Petrides,[1] R. Del Maestro,[2] J. Doyon,[3] and L. Collins[4]; [1]Neuropsychology Unit, Montreal Neurological Institute [2]Brain Tumour Research Centre, Montreal Neurological Institute and Hospital; [3]Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal; [4]McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal; Canada

The goal of this study is to develop a functional Magnetic Resonance Imaging (fMRI) protocol that assesses higher cognitive functions, other than language and somatosensory functions, in patients with low-grade gliomas. These patients have a life expectancy that ranges between 2 and 10 years, and therefore it is crucial that great care be taken to preserve higher cognitive functions in order to preserve their autonomy and quality of life post-operatively. In this study, we developed an fMRI protocol for the pre-operative, post-operative (2 months after the surgery), and follow-up (8 months after the surgery) evaluation of higher cognitive functions in patients exhibiting gliomas near the premotor region. In order to assess the function of the premotor region in these patients, we developed a conditional visuo-motor associative protocol that was tested in 8 healthy control subjects. In this protocol, the subjects had to associate each one of four different colors with a specific key press on a mouse with four buttons. The fMRI results revealed that the premotor region and the supplementary motor area are specifically involved in conditional visuo-motor associative function. These data demonstrate that the protocol we developed provides robust and reliable measures of conditional visuo-motor associative function within the premotor region. We then conducted a pre-operative fMRI study using this protocol in three patients with tumors near the premotor

region. As in healthy control subjects, pre-operative fMRI data obtained in these patients showed activity increases within the premotor region. Coupled with the structural MRI, the clinical pre-operative fMRI data were transferred to an integrated image guided system in the operating room. The neurosurgeon was able to use the data in the planning of the surgery in order to spare functional tissue in the premotor region. As a result, the neurosurgeon was able to optimize the extension of the tumor resection in the three patients without any deficits in the conditional visuo-motor associative function. In order to study the long-term outcome in terms of higher cognitive function and the nature of any functional reorganization that might occur after brain tumor removals, we conducted a post-operative and a follow-up fMRI scan in these patients. In contrast to the pre-operative fMRI results, post-operative results showed decreased activity in the precentral gyrus. The follow-up fMRI revealed activity differences similar to the ones observed in the pre-operative fMRI scan. These results suggested that functional reorganizations occurred following the tumor resection. The results provided by such preoperative, postoperative and follow-up fMRI studies are a useful clinical tool for the patient's long-term prognosis.

215. QUALITY OF LIFE IN PEDIATRIC BRAIN TUMOR SURVIVORS

M.J. Bonner, K.K. Hardy, A.B. Guill, V.W. Willard, K.E. Carter, and S. Gururangan; Brain Tumor Center, Duke University Medical Center, Durham, North Carolina, USA

Studies suggest that survivors of pediatric brain tumors may be at risk for developing symptoms consistent with a nonverbal learning disability (NLD) as a consequence of their disease and its treatment. Rourke (1988) described NLD as affecting children's functioning across multiple domains including cognitive, academic, and social areas. As a result, individuals with NLD may experience learning difficulties in reading and math comprehension, math calculation, and written language. In addition, those with NLD may exhibit deficits in social perception, judgment, and interaction skills that put them at higher risk for social withdrawal or isolation. In contrast to these weaknesses, individuals with NLD often manifest relative strengths in rote skills and memorization, verbal processing, reading decoding and dictation skills. Preliminary data are now available that provide evidence of the cognitive and academic deficits associated with NLD; however, the social functioning of survivors is not well investigated. The primary objectives of the current study are to demonstrate that a sample of survivors of pediatric brain tumors have greater impairments in social functioning than do healthy controls, using both questionnaire and skill assessment data, and to determine what risk factors are associated with social impairment. To date, 24 patients (14 females and 10 males) ranging in age from 7 to 16 (M = 13.1, SD = 2.77) have been enrolled in the study. Diagnoses were varied, with 8 patients (33.3%) with medulloblastoma, 5 (20.8%) with ependymoma, 5 (20.8%) with pilocytic astrocytoma, and 6 (25%) with other tumor types. The majority of patients had been treated with surgery (n = 20, 83.3%), 17 (70.9%) had received hemotherapy, and 14 (58.3%) had been treated with radiation. Age at diagnosis ranged from 2 to14 (M = 6.58, SD = 3.20). Participants had been off treatment an average of 5.25 years (SD = 2.56; range = 1–11 years) at the time of study. Cognitive functioning of the sample was variable, with estimated IQs averaging 87.61 (SD = 16.18). Broadly speaking, parents rated their children's social skills as somewhat lower than would be expected by age-based norms. Subjects were also rated as having somewhat more social problems, including difficulties with nonverbal communication, than would be expected. Contrary to expectations, parent-reported social problems were not significantly associated with age at diagnosis, treatment type, functional or cosmetic impairment, or estimated IQ scores. However, social problems correlated significantly and negatively with scores of performance on a measure of ability to decode facial emotions. Findings from this study contribute to a more complete phenotype of the neuropsychological late effects experienced by survivors and to the development of interventions aimed at ameliorating disease and treatment-related deficits in the growing population of brain tumor survivors.

216. COGNITIVE FUNCTIONS AND APOE GENOTYPE IN LOW-GRADE GLIOMA PATIENTS

D. Correa,[1] L.M. DeAngelis,[1] W. Shi,[2] H.T. Thaler,[2] and L.E. Abrey[1]; [1]Neurology; [2]Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York, USA

The purpose of this study was to assess cognitive functions in patients with low-grade gliomas (LGG) who received radiation, chemotherapy, or no treatment. We also compared the cognitive performance of patients who carried the Epsilon-4 allele of the Apolipoprotein E (APOE) gene to those who carried other APOE alleles. Forty adult patients with LGG and no evidence of disease progression participated in the study; 16 patients had received conformal radiotherapy ± chemotherapy, and 24 patients had no adjuvant treatment. All patients underwent a neuropsychological evaluation, and test scores were compared to normative reference groups; 7 composite cognitive domain scores were calculated. APOE genotype was obtained in 33 patients who were classified in two groups based on the presence or absence of at least one APOE Epsilon-4 allele. Patients who received radiation ± chemotherapy had significantly lower scores than untreated patients on the Psychomotor ($P = 0.03$) domain. Analysis of covariance, adjusting for anticonvulsant regimen (i.e., monotherapy vs. polytherapy), suggested no significant adjuvant treatment effect on Psychomotor performance; the results showed that patients on anticonvulsant polytherapy had lower scores on the Psychomotor domain, regardless of tumor treatment status. Treated patients obtained significantly lower scores than untreated patients on the Non-Verbal Memory ($P = 0.02$) domain. Analysis of covariance, adjusting for age, showed that patients who completed treatment at intervals longer than 36 months had significantly lower scores on the Non-Verbal Memory domain than untreated patients. Over 60% of treated patients showed mild to moderate white matter confluence on MRI, whereas 90% of the untreated patients had either no or only minimal white matter changes ($P = 0.002$). Comparisons between APOE Epsilon-4 carriers (n = 8) and noncarriers (n = 25) on cognitive domain scores revealed no significant differences, but Epsilon-4 carriers had lower scores on the Verbal Memory domain than did noncarriers. The findings suggest that LGG patients treated with radiation ± chemotherapy had more difficulties in nonverbal memory and were more likely to show white matter abnormalities on MRI than untreated patients. Psychomotor slowing was most prominent among patients who were on anticonvulsant polytherapy, regardless of adjuvant treatment status.

217. THE COURSE OF NEUROCOGNITIVE STATUS IN HIGH-GRADE GLIOMA PATIENTS

I. Bosma,[1] M. Vos,[1] J. Heimans,[1] M. Taphoorn,[5] N. Aaronson,[6] T. Postma,[1] H. van der Ploeg,[2] W. Vandertop,[3] B. Slotman,[4] and M. Klein[2]; [1]Neurology, [2]Medical Psychology, [3]Neurosurgery, and [4]Radiation Oncology, Academic Hospital Vrije Universiteit, Amsterdam; [5]Neurology, Medical Center Haaglanden, The Hague; [6]Division of Psychosocial Research and Epidemiology, The Netherlands Cancer Institute, Amsterdam; The Netherlands

This work was conducted to study the course of neurocognitive functioning in newly diagnosed high-grade glioma patients and to determine the tumor, treatment, and patient-related factors affecting neurocognitive functioning in the course of the disease. Following baseline assessment (i.e., after surgery, prior to the start of radiotherapy), follow-up evaluations on neurocognitive functioning were performed at 8 and 16 months in newly diagnosed high-grade glioma (HGG) patients and in patients with non-small-cell lung cancer (NSCLC). HGG patients' level of functioning was compared to the level of functioning in NSCLC patients and to that of age- and sex-matched healthy controls. A battery of standardized tests was used to assess neurocognitive functioning. In order to accomplish data reduction, summary measures were calculated to detect possible deficits in the neurocognitive domains of (1) information processing speed, (2) psychomotor function, (3) attentional functioning, (4) verbal memory, (5) working memory, and (6) executive functioning. Follow-up data could be obtained in 35 of the 68 HGG patients initially included in the study. Of these, 20 patients had only one follow-up at 8 months, whereas 15 patients also had a 16-month follow-up. The patients who also had a follow-up at 16 months had a better neurocognitive status, were younger, had a significantly lower tumor grade, and received lower fraction doses than patients with only an 8-month follow-up. HGG patients with a shorter follow-up performed worse when compared to NSCLC patients with the same follow-up. Compared to the performance of NSCLC patients during the follow-up, no statistically significant deterioration was found in neurocognitive function in the course of the disease in the HGG patients. However, evaluation of HGG patients with versus HGG patients without tumor recurrence indicated that neurocognitive decline in these patients often precedes clinical signs of tumor progression. Neurocognitive functioning in HGG patients is mostly affected by tumor effects and not unequivocally by treatment effects. In the course of their disease and in the absence of tumor progression, no clear trend toward further worsening in neurocognitive functioning is to be expected in these patients. Additionally, neurocognitive function appears to be a prognostic factor in the course of the disease in these patients.

218. THE "END OF LIFE" HOSPITAL SETTING FOR PATIENTS WITH MALIGNANT GLIOMAS

W. Walter, S. Oberndorfer, E. Lindeck-Pozza, H. Lahrmann, *W. Struhal*, P. Hitzenberger, and W. Grisold; LBI for Neurooncology, Department for Neurology, KFJ-Hospital, Vienna, Austria

Despite aggressive treatment, outcome of patients with malignant gliomas is poor. In the terminal course of the disease, due to social, economical, individual, and cultural reasons, some patients are admitted to hospital care. For the terminal phase, there is a lack of investigations in the neurological literature. The purpose of this study was to evaluate the end of life phase in a hospital setting for patients with malignant glioma. Twenty consecutive patients with malignant gliomas were included in this analysis regarding symptoms, medication, diagnostic, and interventional procedures. The last ten weeks before death were divided into three periods: Period I; from ten weeks to six weeks before death, Period II; six weeks to two weeks before death; and period III; last two weeks before death. The patients were comparable regarding age, sex, and overall survival. The Karnofsky performance scale decreased continuously from period I (average absolute 70%) to period III (average absolute 10%). Relevant clinical complications, medications, and diagnostics as well as interventional procedures increased from period I to period III. For the period I symptoms of headache and seizures, treatment with antiepileptic drugs as well as steroids and analgesics was prescribed. Diagnostic procedures such as MRI, blood tests, or X ray of the chest, as well as interventional procedures (e.g., urinary catheter, intravenous drug administration), were less frequent compared to period II or III. In the last period, brain edema, fever, somnolence, and pneumonia were the most prominent clinical features. Almost all patients in period III received transdermal or subcutaneous opioids, fluid replacement, anticoagulation, intermittent oxygen, anticonvulsants, gastric protection, and urinary catheters, as well as a pressure relief mattress. The majority of patients died due to infection or respiratory distress. Providing adequate palliative care to dying patients with malignant gliomas is an important aspect of treatment. Our study demonstrates that the end-of-life phase of brain tumor patients has several periods. Contrary to the limited therapeutic implications, the number of therapeutic and diagnostic interventions increases toward the terminal phase. Future patient management needs to be more symptom-oriented and palliative.

219. PATIENT-HELD RECORDS: A PROSPECTIVE STUDY TO EVALUATE THE USE OF A STEROID BOOKLET BY PATIENTS WITH MALIGNANT INTRACRANIAL GLIOMA (MIG)

J. Bentley,[1] G. Hendry,[2] and P. Kane[2]; [1]Palliative Medicine; [2]Neurosurgery, James Cook University Hospital, Middlesbrough, UK

High-dose steroids are commonly used in the management of MIG and can be associated with significant side effects. This study investigated the use of a patient-held record as a possible approach to improving patient understanding of their medication and monitoring usage. The booklet was developed by consulting best current evidence, relevant health professionals, patients, and their carers. It provided information on steroids, side effects, and a chart to monitor treatment. Patients were recruited within one week of the diagnosis of MIG. All had been given verbal information about steroids by their clinician. Together with their main carer they completed a questionnaire to determine steroid knowledge. Patients were then randomized to receive the steroid booklet or not. Four months later they completed the same questionnaire again and also another to determine patient/carer satisfaction with steroid use. Twenty-one patients have been recruited to the study (M = 14, F = 7, age range, 38–75 yrs), and 13 have completed. Four patients did not complete (2 deaths, 2 disease progression). Fifty-four percent of the patients managed their own medication, and in the remainder it was handled by the carer. The mean steroid knowledge score at recruitment was 50%, and at follow up the mean score was 64%. There was no significant difference in the score between the groups. Improved score was due mainly to increased knowledge of the common side effects (somatic changes, sleep disturbance). Twenty-three percent of the patients were dissatisfied with the amount of written information given about steroids. None of these patients received the steroid booklet. Forty-three percent of the patients with the steroid booklet did not take it to consultations. All patients with the booklet reported that they had read it and found it useful. The study points to the important role of carers in patients with MIG. Preliminary results suggest that a steroid booklet does not improve patient/carer knowledge about steroids. However, the steroid booklet was welcomed by patients and their carers as a source of written information.

220. NEUROCOGNITIVE IMPROVEMENT FOLLOWING SURGICAL RESECTION IN ADULT PATIENTS WITH PRIMARY BRAIN TUMORS

E. Farace, M. Mut, and M.E. Shaffrey; Neurosurgery, University of Virginia, Charlottesville, Virginia, USA

The potential for surgical resection to increase or induce neuropsychological impairment in patients with primary brain tumors is clear. Analyses of the neurocognitive effects of treatment (radiation or chemotherapy) are complicated by the unknown neurocognitive effects of surgery. However, surgery may be the first and best treatment option, particularly in patients with meningiomas and other accessible tumors. Therefore, this pilot analysis of patients who underwent neuropsychological evaluation pre-surgery, and 6 weeks post-surgery, was undertaken to examine the effects of surgery in this population. Twenty-five patients with primary brain tumors who were clinically referred for baseline and post-operative neuropsychological evaluation were given a standard neuropsychological assessment battery measuring a broad range of domains (attention, memory, executive function, visual and verbal memory, psychomotor speed, depression). Scores were adjusted for patient age and education as norms were available. Scores were transformed into Z-scores to allow for ease of comparison across tests. Fifteen patients had a meningioma, and 10 patients had some form of glioma (grades 1–IV). Eighty-four percent of the patients showed improvement of at least one standard deviation, in at least one neurocognitive domain, at 6 weeks post-surgery. Thirty-six percent worsened by at least one standard deviation in at least one neurocognitive domain (percentages overlap because of some patients who improved in one domain and worsened in another). Language ability, when impaired at baseline, was most likely to improve post-surgery. Memory was the least likely neurocognitive domain to improve. Surgery was found to be, in certain conditions, beneficial for patients' neurocognitive function. Although surgery has the potential to induce deficits through focal damage to surrounding tissue, increased risk of hemorrhage, etc., it may also improve function in some patients, perhaps through resolution of mass effect, relief of intracranial pressure, or even a benefit of debulking. A better understanding of the neurocognitive effects of surgical resection is important as patients face decisions regarding their resection, and to aid researchers in delineating neurocognitive effects of treatments.

221. INTRACRANIAL MENINGIOMAS IN THE 8TH AND 9TH DECADES OF LIFE: RETROSPECTIVE ANALYSIS OF SURGICAL OUTCOME IN A CONSECUTIVE SERIES OF 70 PATIENTS

G. Zona, D. Criminelli Rossi, G. Spena, and R. Spaziante; San Martino Hospital, Neurosurgery, Genova, Italy

Long-lasting life expectancy, improved quality of life, and extensive availability of neuroradiological facilities (mainly CT scan and MRI) have dramatically increased the diagnosis of intracranial meningiomas in elderly population in the last years. As a consequence, more and more neurosurgeons have to face up to these slow-growing benign lesions whose surgical management in elderly patients remains quite controversial as the risk/benefit ratio is not always evident. In a consecutive series of 257 intracranial meningiomas operated on at the Department of Neurosurgery of the University of Genoa (Italy) between December 1995 and January 2004, we identified a subgroup of seventy patients older than 70 years. Age ranged from 70 to 86 years (mean, 75.6 $\pm$ 4.2), with an evident female preponderance (51 cases). Preoperative clinical status, expressed respectively as Karnofsky performing scale (KPS) and ASA risk, tumor size and location, extent of removal, age, and pathology were matched with the early outcome and, by means of Kaplan-Meier curves, with disease-free survival (DFS) at long-term follow-up. Mortality was 5.7% (4 cases). Neurological status improved in 25 patients and remained unchanged in 33 patients (35.7% and 47.1%, respectively). Eight patients worsened (11.4%); among them, three patients completely recovered after two weeks and two patients at long-term follow-up. Eleven patients presented medical complications, and four patients need re-operation: All of them completely recovered. Long-term follow-up ranging from 6 to 86 months was obtained in 53 patients (80.3%) out to 66 surviving patients. Four more patients died, raising the overall mortality to 11.4%. Neurological outcome, compared with the early postoperative period, improved in 9 patients (16.7%), remained stable in 38 patients (71.7%), and worsened in two cases (3.7%). None of the prognostic factors considered reached statistical significance. Nevertheless, subtotal resection showed to be slightly better than gross total removal in the Kaplan-Meier survival function. In conclusion, attentive selection criteria make surgery feasible with acceptable risks and good overall outcome in elderly patients. In our opinion, for dealing with slow-growing lesions, a mandatory aggressive attitude is not always justified, since subtotal resection, especially for a difficult location, to relieve mass effect can produce better results.

222. DETECTION OF SPECIFIC EXECUTIVE DEFICITS USING AN "ECOLOGICAL" BATTERY IN FRONTAL LOW-GRADE GLIOMAS

M. Roca,[1] T. Torralva,[1] L. Vita,[1] A. Lacroze,[1] M. Calcagno,[1] F. Manes,[1] and *B. Diez*[2]; [1]Cognitive and Behavioural Neurology Section and [2]Neuro Oncology Section, Raul Carrea Institute for Neurological Research (FLENI), Buenos Aires, Argentina

The most common cognitive symptoms of frontal lesions are impaired executive functions, social interaction, and personal regulation. However, this form of mental impairment is sometimes so subtle in daily activities and unsusceptible to testing that these patients seem intellectually intact. Deficits are not found in standard tests because they do not address the component of real life decision-making and social cognition. Therefore, the objective of this investigation was to detect specific executive deficits in patients with frontal low-grade gliomas (FG), using an "ecological" executive battery, that consists in real-life tests shown to be sensitive to damage to the prefrontal cortex (PFC). Patients with FG (n = 5) were compared with a group of normal controls (n = 5). All patients underwent a standard neuropsychological examination and the "ecological" executive battery. The "ecological" executive battery used in this study consists of five tests. (1) Frontal Assessment Battery (FAB): This consists in 6 subscales exploring frontal lobe functions. (2) IOWA Gambling Task (IGT): This test simulates personal real life decision-making activities. (3) Faux Pas Test: The subject is shown ten faux pas stories and ten stories without a faux pas; the subject is asked if something was said inappropriately and why. (4) The Hotel Task: It comprised six distinct activities that would plausibly need to be completed in the course of running a hotel. (5) MET–Hv: The purpose of this test, undertaken in the hospital and its surroundings, is to carry out 12 subtasks in a "real life" situation. Significant differences were found between FG and normal controls in both Trail Making Tests ($P = 0.14$ and $P = 0.27$), Faux Pas ($P = 0.007$), and total scores of the IGT ($P = 0.034$). In addition, tendencies were noted in the Met –hv total error score, which were not statistically significant, probably because of the limited size of the sample. These patients with low-grade gliomas in the frontal cortex did not exhibit the most typical disinhibited and socially inappropriate behavior, usually seen after frontal injury. However, the deficits in social interactions and decision making were captured by this new ecological executive battery. The present study has implications for the clinical assessment of the real-life problems faced by these patients, both within the family and whenever they might return to work.

223. ENHANCING COPING THROUGH NEURO-ONCOLOGY PATIENT EDUCATION MATERIALS

R. Calhoun-Eagan, *D. Allen*, C. Lentz, S. Jackson, J. Blivin, B. Guill, H. Quinn, S. Boulton, J. Cahill, and P. Lyons; Brain Tumor Center, Duke University Medical Center, Durham, North Carolina, USA

The diagnosis of a high-grade glioma forces the patient and family to make abrupt treatment decisions based on complex choices. At a time of crisis, the steep learning curve overwhelms many. While learning is enhanced by easily understandable information, not all patients or caregivers process information in the same manner. As more oncology offices prefer that specialized care be coordinated by neuro-oncology referral centers, the need for proactive communication and effective teaching strategies has increased. The Brain Tumor Center at Duke's response to this dilemma has been the development of a multi-modal approach to patient education. Designed to maximize comprehension and facilitate coping, it is theorized that individualized chemotherapy teaching sessions with the patient and caregivers are reinforced by the provision of a comprehensive patient education notebook. Since these sessions occur infrequently due to great travel distances, the notebook has been designed to orient patients to services and resources and to promote communications with the tertiary center. This educational tool has been utilized for the past two years with modifications. It is necessary to evaluate the tool to gain insight into its usefulness. A multidisciplinary education committee designed the notebook to address specific treatment information, clinical trials, early detection and management of complications, keeping track of symptoms, when to call, self-care, center-specific resources and national glioma resources, and advance directives. A survey was designed to measure self-confidence in coping with therapy and overall satisfaction with the notebook. The 5-min survey was given to the patient/caregiver at the clinic encounter after completion of their first cycle of chemotherapy. It is expected that 60 patients will be surveyed through a 6-month interval. To date, survey results (n = 15) suggest that patients and caregivers find the notebook's contents to be comprehensive, useful, appropriately tailored to their needs, and helpful in promoting a sense of control and confidence. Items cited as particularly helpful include fact sheets on treatment side effects, possible complications and seizure management. Reinforcing individualized educational sessions with customized information in a formulated notebook for ease of information processing promotes communication, confidence, and patient empowerment.

224. NEUROPSYCHOLOGICAL IMPACT OF BONE MARROW OR HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PROSPECTIVE STUDY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

H. Harder, M.J. van den Bent, A.R. Van Gool, J.J. Cornelissen, W. Eijkenboom, R. Barge, and H.J. Duivenvoorden; Neuro-oncology, Psychiatry, Hematology, and Radiotherapy, Daniel Den Hoed Cancer Center, Rotterdam; Hematology, Leiden University Medical Center, Leiden; Psychology and Psychotherapy, Erasmus University, Rotterdam; The Netherlands

Bone marrow or hematopoietic stem cell transplantation (SCT) is a current cancer treatment for patients with malignant hematological disorders. Better patient selection and development of reduced-intensity preparative regimens and new transplant techniques have expanded the use of SCT. SCT is preceded by the use of high-dose chemotherapy with or without total body irradiation (TBI) to eradicate the malignant disease and suppress the immune system to allow engraftment of the donor (or autologous) stem cells or bone marrow. The complications associated with SCT treatment are significant due to severe toxicity associated with myeloablative therapy (including central nervous system toxicity), the period of profound immunodeficiency, and the risk at graft failure or graft-versus-host reaction. The neurotoxic side-effects on cognitive functioning and the consequences on patients' quality of life are major concerns. Cognitive deficits following SCT have been documented in subgroups of patients. Unfortunately, no attempt has been made to evaluate a progressive decline or stability in cognitive functioning prospectively. The purpose of this study was to address the extent of cognitive changes associated with SCT in adult patients with hematological malignancies. A standardized neuropsychological test-battery assessing multiple cognitive domains was administered to a longitudinal cohort of 101 SCT patients before undergoing SCT (T1) and at 8 (T2) and 20 months (T3) after baseline. To control for SCT treatment, a reference group of 82 hematological patients treated with conventional systemic chemotherapy and/or involved-field radiotherapy was included. Effects of subjective cognitive functioning, quality of life (QOL), fatigue, and psychological functioning were measured with five self-report questionnaires. Results were compared to normative data. Analysis employed random regression modelling (RRM). No between-group differences were found in cognitive functioning at baseline. Changes over time were observed in attention ($P = .01$) and psychomotor functions ($P = .03$) with poorer functioning in SCT patients. Performance on verbal memory, visual memory, and visuospatial functions remained stable at follow-up. Negative effects of gender and age were found, suggesting poorer performance in, respectively, females and older patients. Positive effects of education were observed in all cognitive domains, reflecting that patients with higher educational had better test results. Impaired cognitive functioning in SCT patients was weakly correlated to mental fatigue, reduced motivation, and anxiety at follow-up. More cognitive deficits were observed in patients treated with TBI, in patients who received prednisone, and in patients who experienced long-term infections. Intensive myeloablative cancer therapy had an adverse impact on cognitive functioning over time, in particular, on psychomotor functions and attention.

225. LATE EFFECTS IN YOUNG ADULTS SURVIVING A CHILDHOOD PRIMARY TUMOR OF THE CNS

M.J. Verstegen,[1] R. Bem,[2] S. Leenstra,[1] C. Bos,[2] and D.A. Bosch[1]; [1]Neurosurgery, Academic Medical Centre; [2]Pediatric Oncology, Emma Childrenhospital, Amsterdam; The Netherlands

Central Nervous System (CNS) tumors account for about 20% of all childhood tumors. They are second in incidence only to leukemia. As treatment results have improved significantly, leading to an increased number of patients surviving over the last decades, attention is drawn toward the long-term effects and quality of life of these patients. In order to monitor the late effects of childhood cancer treatment, a long-term follow-up clinic was founded at the Academic Medical Centre in 1996. All patients had successfully completed their treatment 5 years before they were transferred to the long-term follow-up clinic. Participants in this study had to be aged 16 years or older and had to be treated for a primary malignancy of the CNS at childhood. The study included a questionnaire and interview for psychosocial and educational functioning, a physical examination, and a laboratory screening of endocrine-axis. Of 61 patients, the late effects were divided into 11 different groups: endocrinological, fertility, neurological, second malignancy, dermatological, psychological, orthopedic, hearing deficiency, urologic, visual deficiency and a rest group. The most serious late effects consisted of endocrine dysfunction comprising solitaire growth hormone (GH) deficiency (n = 13 [21%]) or a GH deficiency combined with a primary (n = 3 [5%]) or secondary hypothyroidism (n = 5 [8%]), late effects associated with fertility occurring in 15 (24%) patients. In four patients (15%), a secondary malignancy occurred. In total, 42 patients had a mild (70%), severe (22%), or total (8%) alopecia, of which 23 patients received a combination of radiation and chemotherapy. The psychological

problems consisted of a broad variety of social and cognitive disabilities. Twenty patients reported a learning disability, in fifteen cases leading to a decreased level of education compared with the pre-treatment level. Ten patients attended a special school for learning disabilities. Nine patients (15%) were treated by a psychiatrist or psychologist. Four out of seven patients (57%) who received carboplatine experienced hearing loss. The total number and severity of the late effects exceeded our expectations, and they should play a role in the assessment of future treatment strategies.

226. DEVELOPMENT OF NEW FMRI AND INTRA-OPERATIVE TOOLS FOR NEUROSURGERY GUIDANCE IN PATIENTS WITH BRAIN TUMORS INVADING THE PARIETAL LOBES

A.S. Champod,[1] C. Amiez,[1] P. Kostopoulos,[1] R. Del Maestro,[3] M. Petrides,[1] J. Doyon,[4] and D.L. Collins[2]; [1]Neurology and [2]McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal; [3]Brain Tumour Research Centre, Montreal Neurological Institute and Hospital, Montreal; [4]Centre de Recherche de l'Institut Universitaire de Gériatrie de Montréal, Montreal; Canada

The objective of this study is to develop new pre-operative neuroimaging and intra-operative behavioral tools for the assessment of high-order parietal functions in patients with brain tumors. Pre-operatively, we used an fMRI (Functional Magnetic Resonance Imaging) protocol to assess mental rotation function in three patients with brain tumors invading the parietal lobes. We aimed to localize functional regions surrounding the tumor that were involved in the task. The pre-operative fMRI showed that three regions were critically involved in this task, that is, the superior and inferior parietal lobules, as well as the cortex surrounding the intraparietal sulcus. These fMRI data were then transferred to a neuronavigation system to provide direct intra-operative guidance to the neurosurgeon. Using surgical navigation, the neurosurgeon precisely mapped out in the operating room not only the location of the tumor but also the critical areas involved in the mental rotation task. The neurosurgeon used the functional data to plan the neurosurgery and to determine the safest surgical route for the tumor resection of each patient. It was therefore possible, with the use of this technology, to remove as much of the tumor as possible while preserving critical areas in each patient. A standardized intra-operative procedure was also developed. During the operation, the patients were tested on the mental rotation protocol at regular intervals and at specific stages of the surgery. The online assessment of performance during surgery allowed the neurosurgeon to verify whether the cognitive and behavioral processes for mental rotation were preserved throughout the surgery and to adapt the surgical approach in order to minimize as much as possible the potential deficits post-operatively. Using this procedure, the neurosurgeon spared the areas that were critical for mental rotation in each patient. This was reflected in stable performance of the patients on the protocol during surgery. The results suggest that this procedure is of great clinical value. It provides the neurosurgeon with the possibility to balance the benefits of a complete tumor resection with the possible post-operative functional deficits. Ultimately, it allows an optimal tumor resection with the minimal post-operative neurological deficits. The use of this new procedure could therefore become crucial for the patients' post-operative quality of life and autonomy.

227. PALLIATIVE CARE IN BRAIN TUMOR PATIENTS: COMPLICATIONS AND SUPPORTIVE THERAPY IN 215 PATIENTS ASSISTED AT HOME

A. Pace, M. Giovannelli, P. Calabretta, M. Di Lelio, C. Parisi, O. Lembo, F. Guastella, S. Vita, *C. Carapella*, and A. Pompili; Regina Elena National Cancer Institute, Rome, Italy

Although the poor prognosis of malignant brain tumors has not been substantially modified in the last years by anticancer treatment, palliative care in neuro-oncology received very little attention. Since October 2000 at the Regina Elena National Cancer Institute of Rome, we started a palliative home-care program for patients affected by malignant brain tumor after hospital discharge, with financial support of Regional Health System. The aims of this model of assistance are to meet the patient's need of care during the evolution of the disease, to provide rehabilitation at home, to improve the patient's quality of life with palliative care, and to facilitate death at home. Neuro-oncologic home staff includes 1 neurologist, 5 nurses, 2 rehabilitation therapists, and 1 psychologist. In the first three years of our program 215 patients have been assisted at home and 131 died. The complications in the last phase of disease were pulmonary infections (10.6%), deep venous thrombosis (9.7%) with embolic complications in 6 cases, diabetes due to chronic steroid treatment (8.4%), and psychiatric syndromes (5.7%). Seventy-nine patients (37%) presented epilepsy despite anticonvulsant treatment; 24% presented adverse effects to medication (chemotherapy, antiepileptic drugs, and steroids). Sixty-nine percent (90/131) of the patients were able to die at home. Among the 131 patients who died, the most frequent symptoms in the terminal phase were lethargy (35.5 %), dysphagia (31.8 %), and headache (12.3 %). A cost/utility analysis showed that as a group, for the patients receiving continuing home care, the hospital readmission rate and the median time spent in hospital in the last four months of life are significantly lower than in a control group ($P < 0.01$). Future clinical research strategies should include a new model of care for brain tumor patients, with special attention to palliative home-care models.

228. EXTENDED ABSTRACT: PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: RECENT ADVANCES IN DIAGNOSIS AND TREATMENT

N. Arita; Department of Neurosurgery, Hyogo College of Medicine, Nishinomiya, Japan

Primary central nervous system lymphoma (PCNSL) has been considered to be a rare tumor, but most literature published since the 1980s has reported that PCNSL has been increasing in frequency. It now afflicts approximately 300 people in Japan and 1000 in the United States each year.

The pathogenesis of PCNSL remains obscure. It arises in the central nervous system where no lymphocytes are present in the normal state. Most PCNSL is B-cell derived non-Hodgkin's lymphoma (NHL). Patients frequently have multiple lesions in the brain. Compared to non–central nervous system (CNS) NHL, PCNSL is refractory to any treatment, and prognosis is dismal. Primary central nervous system lymphoma often relapses within the radiated field in which a dose sufficient to control non-CNS NHL is given. Primary central nervous system lymphoma arises in both immunocompetent and immunocompromised populations. These two groups present different clinical features. In this report, PCNSL in an immunocompetent population is described.

Primary central nervous system lymphoma affects any age, with a peak incidence in the fifth to seventh decade. Recently, PCNSL arising in the elderly seems to be increasing. Slight male preponderance has been noted at all ages. Primary central nervous system lymphoma has no specific diagnostic features. It may exhibit various clinical presentations, including increased intracranial pressure, focal neurological symptoms, and dementia. Uveitis is not rare as an initial symptom. Primary central nervous system lymphoma arises anywhere in the brain. Most common sites are the periventricular and cortical regions. The tumor is multifocal in 20% to 30% of cases. Unlike glioblastoma, which typically shows ringlike enhancement in CT and MRI, PCNSL exhibits circumscribed homogeneous enhancement. Peritumoral edema is common. Calcification, cyst formation, hemorrhage, and necrosis are not accompanied.

Histopathological confirmation is essential to establish diagnosis and determine treatment strategy. Stereotactic biopsy is currently the diagnostic procedure of choice. Total resection does not correlate with prolongation of survival in PCNSL. Therefore, extended resection of the tumor, enhancing the risk of new neurological deficits, is not warranted in most patients. Steroids should be withheld before biopsy because the tumor may disappear completely. T-cell–derived PCNSL has been rarely reported. But, the vast majority of PCNSLs correspond to B-cell NHL and are categorized into the subtype of diffuse large cell lymphomas. Immunostaining using various antibodies is required for the final diagnosis. Primary central nervous system lymphoma differs from systemic lymphomas in several aspects. However, until now, no evidence supporting that PCNSL is biologically or genetically different from systemic lymphomas has been proposed. From cytological and genetic observations, CNS B-cell-derived diffuse large cell lymphomas are related to lymphocytes in the germinal center in the differential stage.

Primary central nervous system lymphoma had been an incurable disease. If untreated, median survival time (MST) of patients was reported to be two to three months. Surgical resection alone did not provide any benefit to patients with MST of one to four months. The object of the surgery is to confirm histological diagnosis. Forty percent of PCNSL regresses with steroids, but duration of the tumor remission is several weeks. Primary central nervous system lymphoma is very sensitive to irradiation, and response rate is 60%. However, PCNSL soon recurs, and radiotherapy increased MST to only 10 to 18 months. There is no evidence that doses greater than 40 Gy to the tumor improve survival. The predominant cause of treatment failure is the recurrence of the tumor within the brain.

Chemotherapy has been applied to PCNSL since more than 20 years ago. Agents having the most effective antitumor activity against non-CNS NHL are anthracycline and cyclophosphamide. Combined regimens such as CHOP that include these two agents have been established as a standard treatment of choice in non-CNS NHL. These two agents cannot cross the blood-brain barrier. When patients with PCNSL were treated by CHOP or CHOD, the MST was 45 weeks to 16 months, not exceeding the result of radiotherapy alone. Since the early 1990s, preirradiation high-dose methotrexate (HD-MTX) was reported to produce a higher response rate as well as prolonged survival. Subsequently, several clinical trials were published to

suggest the superiority of preirradiation HD-MTX over radiotherapy alone. High-dose methotrexate followed by radiotherapy brings a response rate of 80% to 90% and MST of 30 to 40 months. Long-term survival or even cure can be expected in a limited number of patients. Clinical results supported that HD-MTX followed by irradiation produces better treatment results than irradiation followed by chemotherapy. Any combined chemotherapy without HD-MTX did not surpass HD-MTX. Now, systemic HD-MTX followed by whole cranial irradiation is recommended as a standard treatment. These reports were phase 2 trials, and efficacy of HD-MTX has not been validated by randomized prospective phase 2 studies. Because of the small number of patients and large difference of survival between these two treatments, phase 2 study has not been accepted to perform by most physicians.

The molecular weight of MTX is 454, and MTX is not lipid soluble. When given in a low dose, MTX cannot cross the blood-brain barrier. When a dose greater than 50mg/kg or 1g/m^2 is administered, the cerebrospinal fluid (CSF) level of MTX was demonstrated to elevate over 1 ÏM, which has an antitumor effect against lymphoma cells. In most clinical trials, 'high-dose' is defined as greater than 1g/m^2 of intravenous MTX. Optimal dose and number of cycles of HD-MTX has not been established. We reported a single institutional trial of HD-MTX followed by whole brain irradiation. Median survival time and median relapse-free survival were 39.3 and 35.2 months, respectively, for 28 assessable patients. Response rate was 93.8% in rapid (three-hour) infusion and 58.3% in regular (six-hour) infusion. Rapid infusion produced a higher level of MTX in the CSF and significant tumor volume reduction. Thus, HD-MTX improved the prognosis of the patients with PCNSL; however, the five-year survival rate still remains 20% to 25%. High-dose methotrexate is not effective in all cases. A few negative trials were also reported. Why some tumors are resistant to MTX has not been clarified. For relapsed or refractory tumors, there is no established standard treatment.

For further improvement, new trials are being tested. Combined chemotherapy trials based on HD-MTX have been reported with MST of up to 60 months. High-dose combined chemotherapy with bone marrow or stem cell transplantation was also reported. The role of intrathecal chemotherapy to PCNSL is not clear. New therapeutic modalities have been tested. In systemic NHL, response rate to the humanized anti-CD20 antibody rituximab is 50%. Most PCNSL expresses the cell surface molecule CD20. If rituximab(146kDa) is administered intravenously, its CSF levels are very low. Intrathecal rituximab may be of advantage. Currently, there is limited evidence for effectiveness of rituximab against PCNSL. Temozolomide, an alkylating agent recently used to treat gliomas, has a modest but true activity against PCNSL. These agents have been recently used in relapsed or refractory tumors after HD-MTX as a salvage therapy.

Delayed neurologic toxicity due to a combination of HD-MTX and whole brain irradiation has been recognized. As survival is prolonged by chemotherapy, this complication overshadows quality of life, especially in the elderly. Patients with this complication present cognitive dysfunction, dementia, and ataxia. In MRI, diffuse brain atrophy and leukoencephalopathy are observed. Delayed neurotoxicity is more frequent in patients older than 60 years. To reduce this complication, several trials have been reported. Radiotherapy is excluded in aged patients or deferred in patients with complete response with chemotherapy.

In immunocompetent persons, PCNSL will be expected to be more common in the country where the aged population further increases. Basic research to understand the pathogenesis of PCNSL is mandatory. Multi-institutional prospective trials will ensure the establishment of evidence-based standard treatment strategies.

229. EXTENDED ABSTRACT: NOVEL THERAPIES AGAINST PRIMARY CNS LYMPHOMAS

A.J.M. Ferreri and M. Reni; Medical Oncology Unit, Department of Oncology, San Raffaele H. Scientific Institute, Milan, Italy

Despite recent progress, primary central nervous system lymphomas (PCNSL) still exhibit one of the worst prognoses among non-Hodgkin lymphomas (NHL). Chemotherapy followed by radiotherapy is the most commonly used strategy. The most effective drug is high-dose methotrexate (HD-MTX) (response rate: 52%–100%; two-year overall survival: 58%–72%), while chemotherapy regimens without this drug comprehensively do not perform any better than radiotherapy alone (Ferreri et al., 2003a). Several attempts to improve outcome by adding other drugs, empirically chosen on the bases of extracerebral NHL experience or of the capability to permeate the blood-brain barrier (BBB), to HD-MTX have been performed. However, only a few drugs had been previously evaluated as single agents in phase 1/2 trials, in patients with relapsed or refractory PCNSL. New strategies aimed to intensify chemotherapy as well as to replace consolidation radiotherapy are now a matter of investigation. Moreover, investigators are focusing on improving drug bioavailability in different areas of the CNS, such as eyes and meninges. This paper summarizes new drugs and strategies against PCNSL and discusses their current role and future developments.

New Drugs: The reduced number of available active drugs limits further improvements in chemotherapy efficacy, which remains the most pressing issue in PCNSL. Preliminary results from small phase 2 studies in relapsed patients are now available with temozolomide, topotecan, and rituximab, and some retrospective evidence suggests that the addition of high-dose cytarabine to HD-MTX could be associated with survival improvement (Ferreri et al., 2002).

Temozolomide is an oral second-generation alkylating agent that spontaneously undergoes chemical conversion to MTIC (5–[3methyl-1–triazeno] imidazole-4–carboxamide), resulting in O-6 methylguanine-DNA methyltransferase depletion. This drug has been associated with excellent tolerance and a 26% overall response rate, mostly complete remissions, in a multicenter phase 2 trial on 23 patients with PCNSL relapsed or refractory to HD-MTX (Reni et al., 2004). Considering it permeates the BBB, is well tolerated even in elderly patients, and exhibits additive cytotoxic activity with radiotherapy, temozolomide could be used as induction, maintenance, or radiomimetic treatment against PCNSL.

Topotecan, a camptothecin derivative that inhibits enzyme topoisomerase I, has been tested in 16 patients with refractory or relapsed PCNSL, obtaining four complete remissions and two partial remissions (overall response rate: 38%) and a one-year progression-free survival rate of 13% (Fischer et al., 2004). Promising results but on small groups of patients with relapsed PCNSL have been reported by using ifosfamide and trofosfamide (Jahnke et al., 2005), while infusional 5 bromo-2'-deoxyuridine given as radiomimetic with whole brain radiotherapy has been associated with modest disease control and unacceptable neurotoxicity (Dabaja et al., 2003).

Rituximab, a human-mouse chimeric anti-CD20 antibody active against B-cell lymphomas, is an intriguing investigational drug. High doses of this drug can be safely infused to attain higher cerebrospinal fluid (CSF) concentrations ($\leq$ 1.7% of serum level) (Raizer et al., 2000). However, anecdotal experience with intravenous rituximab shows disappointing results (Harjunpaa et al., 2001). Conversely, promising results and excellent tolerance were reported in some cases of leptomeningeal lymphoma treated with intraventricular rituximab (Schulz et al., 2004). However, these patients died early because of progression of intraparenchymal lesions (Schulz et al., 2004), and duration of response of leptomeningeal disease remains to be defined. Recently, rituximab was used in association with temozolomide in seven patients with CNS lymphoma, obtaining five complete remissions and two partial responses, with a median response duration and survival of six and eight months, respectively (Wong et al., 2004). This combination was well tolerated and active in elderly and heavily pretreated patients (Wong et al., 2004). However, it is not possible to know if response was due to one or both drugs.

New Strategies: Chemoradiotherapy is associated with a higher risk of neurotoxicity in PCNSL patients. Thus, some authorities focused their efforts on new strategies, that is, BBBD and high-dose chemotherapy supported by autologous peripheral-blood stem-cell transplantation (APBSCT), to dose intensify chemotherapy and eliminate the need for consolidation radiotherapy. BBBD by intra-arterial infusion of hypertonic mannitol followed by intra-arterial cytostatics delivery is a strategy leading to increased drug concentrations in the lymphoma-infiltrated brain to enhance survival. BBBD plus HD-MTX has been associated with five-year survival of 42%, and a 14% cognitive loss rate at one year (Kraemer et al., 2002). In relapsed patients, carboplatin-based chemotherapy plus BBBD produced a 36% response rate, with a median duration of 6.8 months (Tyson et al., 2003). Given its good efficacy and acceptable complication rates, the role of BBBD deserves further investigation in PCNSL.

Preliminary results indicate that high-dose chemotherapy supported by APBSCT is feasible in PCNSL patients. In one study on 28 patients with newly diagnosed PCNSL (Abrey et al., 2001), HD-MTX and HD-cytarabine, followed by BEAM consolidation chemotherapy and APBSCT was well tolerated, but only five remained in remission at a median of 26 months after transplantation. In an ongoing study, 19 of 24 enrolled patients have achieved a complete remission, without relevant toxicity, after a combination of MTX, thiotepa, and cytarabine, followed by high-dose BCNU and thiotepa and hyperfractionated radiotherapy (Illerhaus et al., 2001). In a study on 22 patients with recurrent or refractory primary CNS or intraocular lymphoma, induction cytarabine and etoposide followed by high-dose thiotepa, busulfan, and cyclophosphamide produced a complete remission rate of 72%, with a three-year overall survival of 64%, but with a significant treatment-related morbidity/mortality in elderly individuals and risk of neurotoxicity in pre-irradiated patients (Soussain et al., 2001). The role of high-dose chemotherapy and APBSCT in PCNSL remains to be defined, considering that worldwide experience is still limited, and further studies will need to be done to identify the optimal induction and high-dose chemotherapy regimens.

Primary central nervous system lymphomas infiltrate the subarachnoid space and eyes in a variable proportion of cases (Ferreri et al., 2002). These areas are considered as "sanctuaries" for conventional chemotherapy. Even if MTX doses greater than or equal to 3 g/m^2 lead to therapeutic concentrations and eradication of neoplastic cells from CSF (Guha-Thakurta et al., 1999; Shapiro et al., 1975), some authorities suggest adding intrathecal chemotherapy for meningeal treatment, mostly in cases with positive CSF cytology. Drugs (MTX, cytarabine, and steroids) are delivered by intrathecal or intraventricular (Ommaya's reservoir) route, and, importantly,

a sustained release formulation of cytarabine (liposomal cytarabine) for intrathecal injection is available and allows dosing once every 14 days (Jaeckle et al., 2001). Indications and efficacy of intrathecal chemotherapy are, however, debatable. In fact, this strategy is associated with increased risks of neurotoxicity and chemical meningitis (Bessell et al., 2002; Ferreri et al., 2002), and its efficacy in PCNSL patients has not been prospectively assessed. Moreover, leptomeningeal relapse is almost always associated with brain recurrence (Ferreri et al., 2002), which constitutes the cardinal prognostic event in PCNSL, obscuring the effect of concurrent leptomeningeal relapse on survival and, consequently, the potential benefit of intrathecal chemotherapy.

Chemotherapy efficacy against intraocular lymphoma is dependent on intraocular pharmacokinetics, which is not well understood. One case series suggests that micromolar concentrations of MTX are achieved in the aqueous and vitreous humor when the drug is given at a dose of 8 g/m (Batchelor et al., 2003; Ferreri et al., 2002). However, intravitreal drug concentration is erratic, it is not predictive of response, and it is lower in the vitreous humor, where lymphomatous cells usually grow, with respect to the aqueous humor (Batchelor et al., 2003). These difficulties have induced investigators to establish protocols using repeated intravitreal injections of MTX, with or without thiotepa, which are associated with promising results and reduced morbidity (Smith et al., 2002).

Perspectives: The optimum treatment of PCNSL remains a relevant challenge for international cooperation (Ferreri et al., 2003b). Collaborative efforts should be focused on the identification of new active drugs and combinations and on the role of emerging strategies against NHL. Different combinations of strategies may be needed because of the capability of lymphomatous cells to infiltrate more than one compartment of the CNS. To improve our knowledge of the molecular mechanisms underlying genesis and dissemination of malignant lymphocytes constitutes an essential step to improve therapeutic efficacy, and the establishment of PCNSL animal models will allow us to investigate a variety of novel molecules to be included in the armamentarium against PCNSL.

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232. EXTENDED ABSTRACT: STROKES IN CANCER PATIENTS: CEREBRAL INFARCTION, CEREBRAL HEMORRHAGE, DISSEMINATED INTRAVASCULAR COAGULATION, NEOPLASMS

Lisa R. Rogers; Hermelin Brain Tumor Center, Henry Ford Hospital Detroit, MI, USA

Stroke in the cancer patient is rarely associated with the common causes of stroke in patients without cancer. The type of stroke is tumor-specific and is often associated with the stage of cancer and type of antineoplastic therapy (Cestari et al, 2004; Graus et al., 1985).

Nonmetastatic

1. Coagulopathy

Infarction/Thrombosis: A hypercoagulable state frequently accompanies carcinomas due to a complex interplay of immature cancer blood vessels, inflammation, and interaction of the host blood vessels with procoagulant substances secreted by the tumor. In nonbacterial thrombotic endocarditis (NBTE), sterile platelet-fibrin vegetations develop on heart valves. This material embolizes to the brain and is accompanied by cerebral intravascular thrombosis due to the underlying hypercoagulable state. Other cancer patients have disseminated intravascular coagulation (DIC) and diffuse small-vessel cerebral thrombosis without NBTE. Hypercoagulability, sometimes from chemotherapy administration, underlies venous sinus thrombosis, typically in patients with leukemia and lymphoma (Raizer and DeAngelis, 2000). Nonbacterial thrombotic endocarditis results in focal or multifocal cerebral signs from TIA (transient ischemic attack) or infarction (Rogers et al., 1987). Confusion alone or with focal signs or partial seizures may result from disseminated thrombosis in NBTE or DIC. Venous occlusion typically causes a headache that is accompanied by seizures or focal signs if infarction or hemorrhage develops. Signs of systemic thrombosis may be observed in NBTE and DIC, but laboratory tests of coagulation function are often not diagnostic. Cardiac vegetations visualized on transesophageal echocardiography and evidence of systemic arterial or venous thrombosis are clues to NBTE. MRI in NBTE will typically reveal varying sizes of infarctions in multiple territories (Singhal et al., 2002). MRI or magnetic resonance venography (MRV) is diagnostic of venous occlusion. Optimal therapy for cancer-related hypercoagulability is not known and should be individualized. Heparin should be considered for NBTE and DIC. Venous occlusion may require anticoagulation, thrombolysis, or thrombectomy but more often can be observed.

Hemorrhage: Acute DIC is most common in leukemia, especially myelogenous leukemias. In acute promyelocytic leukemia, procoagulants released from the tumor activate the clotting pathway and deplete clotting factors. In other cancer patients, cerebral hemorrhage results from thrombocytopenia that is due to marrow metastasis, marrow suppression from radiation or chemotherapy, or microangiopathic hemolytic anemia or liver dysfunction. Acute or subacute headache, focal signs, vomiting, and/or encephalopathy are signs of parenchymal or subdural hemorrhage from coagulopathy. There may also be systemic bleeding. In acute DIC there may also be systemic thrombosis. Microangiopathic hemolytic anemia also causes pulmonary edema, hypertension, and renal insufficiency. CT or MRI will show single or multiple parenchymal or subdural hemorrhages. Low platelets and fibrinogen, elevated prothrombin time, activated partial thromboplastin time, and D-dimer are signs of acute DIC. Treatment for DIC is directed to the tumor and replacement of clotting factors. Sometimes anticoagulation is indicated. Subdural hemorrhages from coagulopathy can usually be managed conservatively (Graus et al., 1996).

2. Treatment-Related

Infarction/ Thrombosis: Arterial or venous thrombosis is an uncommon complication of chemotherapy, possibly related to vasospasm, vasculitis, or effects on the coagulation system. It is most common in children with leukemia who develop venous sinus thrombosis after induction therapy with L-asparaginase. Arterial infarction is also reported with cisplatin administration and in breast cancer patients receiving tamoxifen and multiagent chemotherapy (Bushnell and Goldstein, 2004). Therapeutic radiation to treat head and neck cancer is associated with accelerated carotid atherosclerosis (Dorresteijn et al., 2002). Venous thrombosis typically causes headache. There are focal signs if infarction or hemorrhage ensues. Chemotherapy and radiation–related thrombosis may result in TIA or infarction. Long segments of carotid stenosis confined to the area of radiation are visualized on angiography in radiation-induced atherosclerosis. Radiation-induced carotid disease is effectively treated surgically.

Hemorrhage: Radiation or chemotherapy with marrow suppression may result in thrombocytopenia and brain, subdural, or subarachnoid hemorrhage. The hemolytic-uremic syndrome is a complication of some chemotherapies, especially mitomycin (Gordon and Kwaan, 1999).

3. Other

Infarction: Fungal septic embolus is a rare cause of stroke, resulting in symptomatic bland or hemorrhagic infarctions, most often in leukemia patients after bone marrow transplantation. Granulomatous angiitis is a rare complication of lymphoma or leukemia.

Metastatic

1. Vessel Compression/Infiltration

Infarction/Thrombosis: Metastatic tumor in the skull or meninges can produce thrombosis in an underlying venous sinus due to compression or infiltration. Parenchymal arterial compression or spasm is a rare complication of leptomeningeal metastasis. Intravascular lymphomatosis causes infarction from proliferation of lymphoma cells within cerebral vessels. Metastatic venous occlusion causes gradual signs of increased intracranial pressure. Papilloedema is often present. Subacute and progressive focal signs or encephalopathy result from lymphomatosis. MRI or MRV shows venous occlusion and skull or dural enhancement in metastatic venous occlusion. Infarction, enhancement, and nonspecific white matter changes are seen on MRI in lymphomatosis (Williams et al., 1998). Treatment for neoplastic vessel compression or infiltration includes brain radiation therapy and/or chemotherapy.

Hemorrhage: Brain or dural metastasis can result in acute or subacute hemorrhage. The most common parenchymal tumors are lung cancer, melanoma, germ cell tumors, and thyroid or hepatocellular carcinoma. Dural metastasis is most common in breast and prostate carcinoma, less frequent in leukemia and lymphoma. Hyperleukocytosis in acute leukemia with leukostasis and brain hemorrhage is now rare. Signs of parenchymal hemorrhage are typically a sudden headache and focal signs. Subdural hemorrhages present subacutely with headache and focal signs or encephalopathy.

A clue to neoplastic parenchymal hemorrhage on MRI is early edema and enhancement, heterogeneous signal, and other brain areas of enhancement. Metastatic subdural hemorrhages are accompanied by dural and/or skull enhancement. A dural biopsy may be required for diagnosis. Steroids are often indicated to treat tumor-associated edema. Removal of the parenchymal hematoma or drainage of subdural fluid, followed by brain radiation, is indicated if the patient is symptomatic.

2. Embolism

Infarction: Cerebral TIA or infarction results from mucin or large tumor emboli, typically from the lung (O'Neill et al., 1987), less often from cardiac or aortic arch tumors. Embolization may occur from surgical manipulation of the lung to remove cancer. Brain CT or MRI shows focal or multifocal infarctions and may show enhancement from growing tumor. Echocardiography is diagnostic of cardiac tumor. The treatment for tumor embolus is brain radiation and treating the systemic tumor in order to prevent further episodes.

Hemorrhage: Neoplastic aneurysms with brain hemorrhage develop from tumor embolic material that results in vessel invasion and rupture. This rare disorder is usually described in choriocarcinoma or lung carcinoma (Murata et al., 1993). Angiography can be diagnostic but is normal if the aneurysm is obliterated by the hemorrhage. Treatment is chemotherapy for the systemic tumor and brain radiation.

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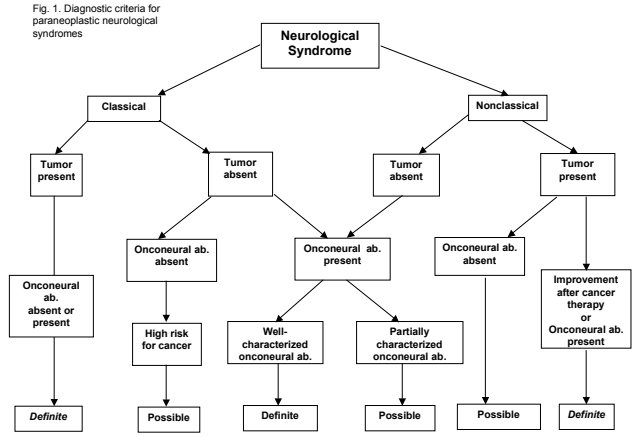
233. EXTENDED ABSTRACT: PARANEOPLASTIC NEUROLOGICAL SYNDROMES

F. Graus; Service of Neurology, Hospital Clinic, Barcelona, Spain

Paraneoplastic neurological syndromes (PNS) are important in clinical practice because they are associated with specific types of tumors and usually antedate the diagnosis of the cancer that usually is in a localized stage where the chances to cure the tumor are highest. The clinical evaluation of patients with suspected PNS is difficult because similar syndromes may occur in the absence of cancer and the tumor is not evident at the onset of the neurological disorder in the majority of patients. Some neurological syndromes defined as classical must suggest a paraneoplastic etiology as one of the leading diagnoses (Table 1). Most PNS have in common the subacute onset, severe neurological deterioration, and, in those involving the central nervous system, frequent evidence of mild cerebrospinal fluid pleocytosis or IgG oligoclonal bands. The most helpful test that suggests the paraneoplastic etiology of the syndrome is the detection of onconeural antibodies (Table 2). Recently, the term "well-characterized" onconeural antibody has been introduced to designate those onconeural antibodies (Hu, Yo, Ri, Ma2, CV2, and amphiphysin) for which (1) there are recognizable patterns on routine immunohistochemistry and for which immunoblotting on recombinant proteins is available to confirm their specificities, (2) the number of cases reported is associated with tumors, (3) the description of well-characterized neurological syndromes is associated with the antibodies, (4) the unambiguous identification of the antibodies occurs among different studies, and (5) the frequency of these antibodies is less in patients without cancer. Most onconeural antibodies are tightly associated with particular PNS and tumor types. However, the predictive value depends on the onconeural antibody and the PNS.



Recently, a panel of neurologists interested in PNS suggested that there should be two levels of diagnostic evidence to define a neurological syndrome as paraneoplastic: definite and possible. Each level can be reached combining a set of criteria (Fig. 1). The panel recognized that the term "possible" may include true PNS but also the coincidental association of two unrelated disorders (the neurological syndrome and cancer). However, this level of evidence may be useful to identify disorders that in the future may be upgraded to definite PNS and to recognize PNS based on the identification of specific trends such as a higher than expected association with a specific type of cancer. The panel emphasized that definite and possible PNS have in common the need to exclude other known causes that could explain the neurological syndrome under study even if onconeural antibodies are positive.

Early diagnosis of the underlying tumor affords the best chance to cure the neoplasm. In addition, effective treatment of the neoplasm contributes to improving or stabilizing the PNS. Early tumor diagnosis requires a high index of suspicion by the radiologist who performs the radiological examination. Recently, positron emission tomography showed a better sensitivity than thorax CT to demonstrate the underlying neoplasm. Sometimes the tumor discovered is not the one usually associated with the PNS or the onconeural antibody. In this situation, the tumor may be responsible for the PNS, or the patient may harbor another tumor that is responsible for the PNS. A way to solve this dilemma is to determine if the tumor expresses the antigen recognized by the onconeural antibody.

The clinical course of PNS is not always uniform. Spontaneous improvement is reported in a few patients with several PNS. Furthermore, some patients with PNS may present with a slowly indolent clinical course over years in absence of any treatment. Several immunosuppressor therapies including corticosteroids, plasmapheresis, and intravenous high-dose immunoglobulins have been used in the treatment of PNS. These therapies are useful in the opsoclonus-myoclonus syndrome associated with neuroblastoma, with LEMS, with multineuritis with vasculitis, with dermatomyositis, and in a few patients, with limbic encephalitis, particularly those with anti-Ma2 antibodies. In most of these disorders, the damage to the nervous system is functional more than structural, so a clinical improvement may be expected after treatment.

In PNS with neuronal degeneration such as paraneoplastic cerebellar degeneration, immunosuppressor therapies have been not successful. However, we favor a trial of immunosuppressor or immunomodulating drugs based on the evidence that these PNS probably are immune mediated and on occasional case reports that they improved with intravenous immunoglobulins or other immunotherapies. Although theoretically, immunosuppression could exacerbate tumor growth, we did not find that these treatments were an adverse prognostic factor for survival.

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234. TREATMENT OF HUMAN GLIOMA CELLS WITH HUMAN ANTI-HUMAN TNF-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL/APO2L) RECEPTOR MONOCLONAL ANTIBODIES

M. Nagane, K. Kobayashi, and Y. Shiokawa; Department of Neurosurgery, Kyorin University School of Medicine, Mitaka, Japan

TNF-related apoptosis-inducing ligand (TRAIL/Apo2L), a member of TNF family, induces apoptosis preferentially in human tumor cells but not in normal cells, suggesting TRAIL, through its cognate death receptors DR4 or DR5, may serve in a potential therapeutic role for intractable malignant gliomas. We applied complete human anti-human TRAIL receptor monoclonal antibodies (mAbs) to specifically target one of TRAIL's death receptors in human glioma cells, which could reduce potential TRAIL-induced toxicity in human. All mAbs were provided by the Kirin Brewing Co. Ltd, Tokyo. Fourteen human glioma cell lines were treated with either a human anti-DR4 mAb (clone B12), or anti-DR5 mAbs (clones E11 and H48), and their cytotoxic effects were determined by using MTT assays. Anti-TRAIL receptor mAb-induced cytotoxicity was compared with that induced by soluble human TRAIL. Caspase activation was evaluated by Western blot analyses using whole cell lysates prepared after mAb treatments. Anti-DR5 mAb treatments induced significant cytotoxicity in a majority of human glioma cell lines tested, which was blocked in the presence of DR5-Fc, a TRAIL-neutralizing fusion protein composed of the extracellular domain of DR5. Sensitivity to anti-DR5 mAb correlated with that to soluble TRAIL in these cells, suggesting that the apoptosis signals triggered by ligation of DR5 with human mAbs may be transduced similarly to that by soluble TRAIL. In contrast, anti-DR4 mAb treatment was ineffective in most human glioma cell lines except two, and only one cell line exhibited cross sensitivity to both mAbs. Established TRAIL-resistant sublines, T98G. TR and LNZ308.TR, showed lower response rates to the mAb treatments. Anti-DR5 mAb treatment resulted in cleavage and activation of initiator and executioner caspases, as well as cleavage of poly(ADP-ribose) polymerase, an intrinsic substrate of caspase 3. Furthermore, treatment with anti-DR5 mAbs suppressed growth of subcutaneous xenografts derived from LNZ308 cells in athymic mice. These results suggest that DR5 may represent the major functional TRAIL receptor mediating receptor-induced apoptosis in human glioma cells, and targeting DR5 with human mAb agonistic to DR5 could provide a potential therapeutic strategy against intractable malignant gliomas.

235. INHIBITION OF C-JUN N-TERMINAL KINASE ENHANCES TEMOZOLOMIDE-INDUCED CYTOTOXICITY IN HUMAN GLIOMA CELLS

Y. Hirose, S. Ohba, and T. Kawase; Neurosurgery, Keio University, Tokyo, Japan

Previous studies revealed that the p38, a member of stress-activated protein kinases (SAPKs), cooperates with the Chk1-pathway to bring about TMZ-induced G2 arrest, and the inhibition of either pathway alone is sufficient to sensitize U87MG glioma cells to TMZ-induced cytotoxicity. We hypothesized that other SAPKs might be involved in cellular responses to DNA damage and that blocking of such protein might sensitize glioma cells to chemotherapeutic agents. In the present study, we analyzed alteration of c-Jun N-terminal kinase (JNK), another SAPK, in U87MG cells treated with DNA-methylating agent temozolomide (TMZ). Immunoblot analysis showed that JNK was phosphorylated 1 to 2 days after TMZ treatment. Since a previous study suggested that TMZ induces severe DNA damage 1 to 2 days after the drug exposure through activation of DNA mismatch repair system, we speculate that activation of JNK is triggered in response to the creation of severe DNA damage, probably DNA double strand breaks which are potentially lethal to the cells. To analyze the role of JNK phosphorylation in survival of glioma cells with DNA damage, we pre- (for 24 h) and post-(for 72 h) treated U87MG cells with JNK inhibitor (Calbiochem, USA) in combination with TMZ treatment. Colony formation efficiency assay revealed that the clonogenicity of TMZ-treated U87MG cells was remarkably reduced by JNK inhibitor at 200 nM or higher concentration. Immunoblot for phosphorylated cdc2 revealed that this potentiation of TMZ-induced cytotoxicity was not associated with abrogation of G2 checkpoint pathway. Phosphorylation of JNK target protein c-Jun was inhibited with 200 nM JNK inhibitor. However, phosphorylation of ATF-2, another JNK target, was not affected by this concentration of JNK inhibitor, and it was suggested that c-Jun-related responses were more important in JNK-mediated survival of glioma cells with DNA damage. Finally, we performed similar experiments using another human glioma cell line, U251, and confirmed that the events mentioned above were not cell line-specific. The mechanism of the JNK inhibitor-induced enhancement of the cytotoxicity of TMZ is still unclear, and JNK inhibitors are not available for clinical use. Nonetheless, further investigation based on the present data may provide a viable approach for the sensitization of human gliomas to TMZ-induced cytotoxicity.

236. BLOCKADE OF THE PI3-KINASE P110A CATALYTIC SUBUNIT INDUCES G2M ARREST AND APOPTOSIS IN HUMAN GLIOMA CELL LINES

W. Weiss,[1] Q.-W. Fan,[1] Z. Knight,[2] and K. Shokat[2]; [1]Neurology, Pediatrics, and Neurological Surgery, [2]Cellular and Molecular Pharmacology, University of California, San Francisco, California, USA

Amplification of *EGFR* occurs in 40% of astrocytomas and correlates with advanced disease. Activation of phosphatidylinositol-3-kinase (PI3-kinase) also occurs commonly in glioma and occurs in part through loss of the tumor suppressor *PTEN*. Because the EGFR and PI3-kinases are activated in glioma and in other human cancers, combination therapies directed against these kinases offer a mechanistic rationale to improve therapy. In published work, we showed that inhibition of EGFR cooperated with inhibition of PI3-kinase in the preclinical therapy of glioma. The PI3-kinases constitute a complex protein family classified according to structure and substrate specificities. Despite known differences in upstream activation, the physiological roles of individual PI3-kinase isoforms and the contributions of individual isoforms to specific malignancies remains poorly understood. The small-molecule PI3-kinase inhibitors LY294002 and wortmannin have been instrumental tools to dissect basic elements of PI3-kinase signaling. As a consequence of indiscriminately inhibiting all PI3-kinases and a large number of related proteins, LY294002 and wortmannin are too toxic to be used in patients. Thus, the utility of small-molecule PI3-kinase inhibitors in clinical practice requires development of new, more selective inhibitors that can be safely and effectively used in patients. To address the role of particular PI3-kinase isoforms in glioma, we have synthesized 12 isoform-selective inhibitors of particular PI3-kinase subunits likely to contribute to glioma and have characterized the IC50 values against 20 recombinant kinase targets. These agents represent the first new tools available in a decade for analysis of PI3-kinase signaling. We have screened all of these compounds against a panel of astrocytoma cell lines. Although most of these inhibitors were quite potent in blocking the PI3-kinase downstream target Akt, only one inhibitor (selectivity: p110a = DNA-Protein Kinase > p110g) induced significant growth arrest and apoptosis in the entire panel of human glioma cell lines and was most effective against cells wild-type for *PTEN*. This study demonstrates that the PI3-kinase catalytic subunit p110a plays an important role in proliferation and survival of glioma.

237. LOW-MOLECULAR-WEIGHT EGFR/KDR TYROSINE KINASE INHIBITOR OFFERS COMBINATORIAL BENEFIT WITH A RAPAMYCIN DERIVATIVE BASED ON PTEN STATUS

J. Rich,[1] Q. Shi,[2] M. Hjelmeland,[2] R. Goudar,[1] S. Keir,[2] R. McLendon,[3] E. Reese,[2] P. Traxler,[4] H. Lane,[4] D. Bigner,[3] and H. Friedman[2]; Departments of [1]Medicine, [2]Surgery, and [3]Pathology, Duke University Medical Center, Durham, North Carolina, USA; [4]Novartis Institutes for BioMedical Research Basel, Oncology, Basel, Switzerland

Malignant gliomas are highly lethal tumors that display striking genetic heterogeneity. Novel therapies that inhibit a single molecular target may slow tumor progression, but tumors are likely not dependent on a signal transduction pathway. We recently reported the first completed trial of an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, gefitinib, in recurrent glioblastomas. Although a subset of patients experienced long-term control of tumor growth, the majority of patients suffered progression of their tumors. The molecular mechanisms by which cancers develop resistance to EGFR inhibitors remains poorly understood. As EGFR may have significant impact on tumor growth through its pro-angiogenic effects, independent vascular endothelial growth factor receptor 2 (kinase domain region, KDR) activity may provide an important survival advantage with the withdrawal of EGFR effects. Additionally, PTEN-deficient glioma cell lines display increased sensitivity to mammalian target of rapamycin (mTOR) inhibition as compared with those with wild-type PTEN. AEE788 is a novel orally active tyrosine kinase inhibitor that decreases the kinase activity associated with EGFR and KDR. RAD001 [everolimus] is an orally available mTOR inhibitor structurally related to rapamycin. We hypothesized that combined inhibition of upstream EGFR and KDR receptors with AEE788 and inhibition of the downstream mTOR pathway with RAD001 would result in increased efficacy against gliomas compared to single-agent therapy. In vitro experiments showed that the combination of AEE788 and RAD001 resulted in increased rates of cell cycle arrest and

apoptosis, and reduced proliferation more than either agent alone. Combined AEE788 and RAD001 administered orally to athymic mice bearing established human malignant glioma tumor xenografts expressing mutant Pten resulted in greater tumor growth inhibition and greater increases in median survival than monotherapy. In contrast, a malignant glioma xenograft expressing a wild-type Pten was more significantly growth inhibited upon AEE788 treatment as monotherapy but the addition of RAD001 had only a marginal impact. These studies suggest that simultaneous inhibition of growth factor receptor and mTOR pathways offer increased benefit in glioma therapy with preferential benefit in tumors with disruption of Pten function. This work was also supported by grants from Accelerate Brain Cancer Cure, Pediatric Brain Tumor Foundation of the United States, and NIH grant NS047409. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

238. HUMAN, TUMOR-FOUNDING NEURAL STEM CELLS AND TUMOR STEM CELL LINES FOR THE DIAGNOSIS AND THERAPY OF GLIOBLASTOMAS

A. Vescovi,[3] R. Galli,[2] E. Binda,[2] S. Piccirillo,[2] G. Broggi,[1] and *F. DiMeco*[1]; [1]Neurosurgery, Istituto Nazionale Neurologico, C. Besta, Milan; [2]Stem Cell Research Institute, Fondazione San Raffaele, Milan; [3]University of Milan-Bicocca, Milan; Italy

We have recently provided the initial evidence that, unlike other brain malignancies, the lethal glioblastoma multiforme (GBM) contains neural precursors endowed with all of the critical features expected from NSCs. Similar, yet not identical, to their normal NSC counterpart, these precursors emerge as unipotent (astroglial) in vivo and multipotent (neuronal-astroglial-oligodendroglial) in culture. More importantly, these cells can act as tumor-founding cells down to the clonal level and can establish tumors which closely resemble the main histological, cytological, and architectural features of the human disease, even when challenged through serial transplantation. Thus, cells possessing all the characteristics expected from tumor neural stem cells from GBMs, including the typical infiltrating and migratory capacity expected from malignant glioma cells, appear to be involved in the growth and recurrence of adult human GBMs. Such features have never been observed previously in the use of common xenograft or allograft-based brain tumor models. Our report also describes tumor neural stem cells (TNSCs) that can be used to routinely establish single patient-derived GBM cell lines in a quick and reproducible fashion. Importantly, TNSCs from different patients retain their distinctive, line-specific proliferation and differentiation attributes which appear to be genetically determined, as shown by the establishment of clonal TNSCs, which possess stable properties identical to those of their parental bulk cultures. TNSCs remain unaltered after multiple in vitro passages and even serial in vivo orthotopic transplantation. Using these lines we are now exploring the possibility that the same key genetic, epigenetic, and extracellular cues that are involved in the maintenance of stem cells and their fate regulation may also be at work in TNSCs so as to prove that the body of knowledge that has emerged from studying basic brain stem cell physiology may be harnessed to identify new therapeutic targets and approaches in neuro-oncology. We shall present recent findings which show that some cues act upon neural stem cells. In our hands, TNSCs also provide an invaluable tool for the in vivo modeling and studying of GBMs, particularly in view of their patient-specific features. As a result, these cells may provide the means to improve diagnosis and develop patient-tailored therapies.

239. A BISPECIFIC IMMUNOTOXIN (DTAT13) TARGETING HUMAN INTERLEUKIN-13 AND UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTORS IN A MOUSE XENOGRAFT MODEL

W.A. Hall,[1,3] E. Rustamzadeh,[1] D.A. Vallera,[2] D.A. Todhunter,[3] and D.A. Vallera[3]; [1]Neurosurgery; [2]Therapeutic Radiology; [3]Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

A bispecific immunotoxin (IT) DTAT13 was synthesized in order to target simultaneously the urokinase-type plasminogen activator receptor (uPAR)-expressing tumor neovasculature and IL-13 receptor expressing glioblastoma cells with the goal of intratumoral administration for brain tumors. The recombinant hybrid was created by using the non-internalizing N-terminal fragment of uPA (ATF) and the IL-13 molecule for binding plus the catalytic and translocation portion of diphtheria toxin (DT) for killing. The 71 kDa protein was highly selective for human glioblastoma in vitro showing no loss on binding compared with DTAT and DTIL13 controls. In vivo, DTAT13 caused the regression of small tumors when administered at 10 μg/day given on a five-dose schedule every other day. DTAT13 was able to target both overexpressed uPAR and the vasculature, as demonstrated by its ability to kill HUVEC cells. Also, mortality studies indicated that DTAT13 was less toxic than DTAT or DTAT13. These findings indicate that bispecific IT may allow treatment of a broader subset of antigenically diverse patients while simultaneously reducing the exposure to toxin that is required if two separate agents were employed.

240. GENOME-WIDE ALLELIC IMBALANCE ANALYSIS OF PEDIATRIC GLIOMAS BY HIGH-DENSITY SINGLE NUCLEOTIDE POLYMORPHIC ALLELE (SNP) ARRAY

K. Wong, Y.T.M. Tsang, Y. Chang, and C.C. Lau; Pediatric/Hematology-Oncology, Texas Children's Cancer Center, Baylor College of Medicine, Houston, Texas, USA

In the Children's Cancer Group high-grade glioma study CCG-945, out of 250 high-grade glioma cases diagnosed by local pathologists, 70 cases were reclassified as low-grade glioma after central consensus pathology review. This indicates a need for additional criteria other than morphology, such as genome-wide genotyping (allelic imbalance analysis), to improve the accuracy of histopathologic classification for these tumors. More importantly, the etiology and molecular pathogenesis of pediatric gliomas remain unclear. Brain tumor tissues were obtained under an IRB-approved protocol after informed consents were obtained from patients undergoing tumor resection at Texas Children's Hospital, Baylor College of Medicine. Portions of the tumors were fixed in 10% formaldehyde and embedded in paraffin for sectioning and pathological diagnosis, and the residual tissues were snap-frozen in liquid nitrogen and stored at −80°C for DNA extraction. All tumor tissues were obtained at initial diagnosis with no prior exposure to chemotherapy or radiation. Totally, 7 low-grade gliomas (5 JPA, 1 ganglioglioma, 1 astrocytoma) and 9 high-grade gliomas (GBM) were analyzed by SNP array that contains 11,560 SNP alleles spanning the human genome with a median intermarker distance of 105 kb. Sixteen pediatric gliomas were analyzed by SNP arrays. No loss of heterozygosity (LOH) was detected in any of the of the 11,562 SNP loci for the five JPA tissues studied. The ganglioglioma has LOH in 7 SNP loci on chromosome 9p while the astrocytoma has LOH in 28 SNP loci on chromosome 6q. On the other hand, high-grade gliomas are very heterogeneous in that the number of SNP loci with LOH varied from 52 to 2125. Significant LOH cytoband regions in GBM include 4q, 6q, 9p, 12, 13q, 14q, 17, 18p, and 19q. We also detected amplification of SNP loci near the genes *EGFR* and *PDGFRA* in two different cases of GBM. No observable allelic imbalance was detected in JPA, and allelic imbalance in other low-grade gliomas only involves a single chromosome. On the other hand, allelic imbalance in high-grade gliomas is quite variable and involves multiple chromosomes. The simultaneous measurement of DNA copy number changes and LOH by SNP arrays should enhance our ability to discover cancer-causing genes and to refine the diagnosis of pediatric gliomas.

241. DISSECTING INTRATUMORAL HETEROGENEITY IN UNTREATED GLIOBLASTOMA: TALES FROM THE EDGE

T. Van Meter,[1] C. Dumur,[2] N. Hafez,[2] C. Garrett,[2] H. Fillmore,[1] and W. Broaddus[1]; [1]Neurosurgery; [2]Pathology, Virginia Commonwealth University, Richmond, Virginia, USA

Glioblastoma multiforme is the most aggressive and treatment-resistant adult primary brain tumor. The molecular changes that underlie tumor heterogeneity in glioblastoma have not been studied in detail. We have undertaken a detailed analysis of tissue prospectively collected intra-operatively using Stealth imaging-assisted tissue extraction. This method was used to isolate tissue samples from multiple intra-tumoral regions in untreated glioblastoma, corresponding to the enhancing tumor rim and areas of hypoxic tumor core. Affymetrix HG-U133A high-density oligonucleotide arrays were used to assess differences in gene expression profiles in the different regions. Our approach used an RNA extraction protocol paired with in-process histological scoring of tissue samples using H&E staining of frozen sections. Tumor gene expression profiles from different tumor regions were compared and correlated with percent tumor, percent necrosis, and other histological features. In this report, we focused on genes upregulated in the enhancing periphery of regions bearing >90% tumor versus normal brain, compared to the core regions. We have analyzed the resulting normalized data sets using a series of 3 algorithms (MBEI, MAS5 and RMA) to provide a consensus profile of transcriptomic differences between periphery and core samples. Previously, we have reported that EGFR and AKT signaling are upregulated in the tumor periphery. Here we report that tumor cells in the periphery of untreated GBMs overexpress additional survival factors in the TNF superfamily, putative regulators of MAP kinase signaling, and several previously unreported transcriptional regulators including LHX2, FoxF1, Sox11, and Shox. Upregulation of EGFR and AKT signaling seen in the tumor periphery, and the putative survival pathways reported here, are consistent with the notion of apoptotic suppression in cells of the invasive rim in GBM. Novel mechanisms of upregulated survival signaling may be important to the infiltrative phenotype and treatment resistance in GBM.

242. CHROMOSOMAL TILE PATH ARRAY-CGH ANALYSIS IN ASTROCYTIC TUMORS LEADS TO IDENTIFICATION OF A NOVEL CANDIDATE TUMOR SUPPRESSOR GENE ON CHROMOSOME 22

K. Ichimura, T.J. Seng, D.M. Pearson, L. Liu, and V.P. Collins; Molecular Histopathology, University of Cambridge, Cambridge, UK

Adult astrocytic tumors have complex and diverse genotypes. A number of oncogenes and tumor suppressor genes (TSGs) have been implicated in the development and/or progression of these tumors, including *CDKN2A/CDKN2B/p14ARF*, *RB1*, *CDK4/CDK6*, *MDM2*, *TP53*, *EGFR*, and *PTEN*. In addition, numerous studies of microsatellite analysis and metaphase CGH have revealed frequent losses, gains, and amplifications in many chromosomal regions in astrocytic tumors, suggesting the presence of as yet identified novel TSGs and oncogenes. One such chromosome is chromosome 22. However, those conventional techniques have failed to narrow down the critical regions and to pin-point the target genes because of low resolution and/or lack of correlation to sequence. We have constructed a chromosome 22 tile path microarray for CGH (array-CGH) in order to precisely investigate the chromosomal 22 abnormalities of astrocytic tumors. The technique has a number of advantages in that (1) it does not rely on naturally occurring polymorphisms, (2) the findings can be directly linked to published human genome sequences, and (3) it allows quantitative assessment of copy number at each clone. A tile path clone set for chromosome 22, which covers 82% of 22q with 443 BAC/PAC/cosmid/fosmid clones, has been obtained from the Wellcome Trust Sanger Institute. The array was constructed according to the published protocol using modified DOP-PCR method. A total of 126 astrocytic tumors consisting of 92 glioblastomas (GB), 29 anaplastic astrocytomas (AA), and 5 diffuse astrocytomas (A) were subjected to the study. These tumors have also been examined for allelic status using 28 microsatellite markers distributed along the entire 22q. Approval for the study has been obtained from the local ethical committee. The results showed good concordance between microsatellite and array-CGH data. As a result of combined analysis using chromosome 22 array and microsatellite analysis, we identified 22q abnormalities in 38% of GB, 33% of AA and 5% of A. Among several candidate regions identified, we further investigated two overlapping homozygous deletions on 22q12.3 that spanned three clones. The region harbored three genes, *YWHAH*, *C22ORF24*, and *DEPDC5*. Homozygous deletions were confirmed by Southern hybridization and multiplex PCR. Gene-by-gene mutation analysis revealed 3 different protein truncating mutations in 3 glioblastoma cell lines and another somatic nonsense mutation in a primary glioblastoma and its xenograft in *DEPDC5*. We have thus demonstrated that chromosomal 22 tile path array can accurately discriminate single copy number change at each clone and that it is a powerful tool to identify critical regions. Tile path arrays for chromosome 6, 7, and 10, as well as an array that covers the whole genome with less than 1Mb interval, have also successfully been constructed and are being used for further investigation of astrocytic gliomas.

243. MOLECULAR SIGNATURES REFLECTING DIFFERENTIATION STATUS OF HIGH-GRADE ASTROCYTOMA IDENTIFY PROGNOSTIC SUBCLASSES OF TUMOR AND DELINEATE A PATTERN OF DISEASE PROGRESSION

H.S. Phillips,[1] S. Kharbanda,[1] R.H. Soriano,[1] W.F. Forrest,[1] T.D. Wu,[1] J. Nigro,[1] P.M. Willams,[1] L. Soroceanu,[1] A. Misra,[3] B.G. Feuerstein,[3] and K.D. Aldape[2]; [1]Genentech, Inc., San Francisco, California; [2]M.D. Anderson Cancer Center, Houston, Texas; [3]University of California San Francisco, San Francisco, California; USA

Gene expression profiling of a set of 76 newly diagnosed high-grade astrocytomas reveals molecular subtypes of tumor that differ in both survival times and in the subsets of genes whose expression is most strongly associated with survival. Based on relative expression of 35 marker genes, tumors are segregated into 3 groups, each of which has an expression signature characteristic of a distinct set of tissues. One tumor subclass displays a median survival time (175 weeks) that is substantially longer than that of the other subtypes (61 weeks and 71 weeks) and is distinguished by a marker signature that resembles that of fetal brain or undifferentiated neural stem cell lines. Markers of this tumor subtype include genes implicated in neurogenesis and, among this tumor subset, one of the genes most strongly correlated with survival is Numb, a Notch pathway antagonist critical for regulation of forebrain neurogenesis. Tumors of the subclasses with poorer prognoses display signatures dominated by features characteristic of either proliferating tissues or tissues of mesenchymal origin. A Cox proportional hazards model suggests that many genes strongly associated with survival within one tumor subgroup do not exhibit equivalent effects across all tumor groups. Utilizing matched primary-recurrent pairs of tumor samples to evaluate changes in molecular signatures that accompany recurrence following surgery and radiation therapy, we find that some tumors show subtype switching, most notably into the mesenchymal phenotype. Comparative genomic hybridization analysis on a subset of samples reveals a strong association between loss of chromosome 10, the most commonly occurring aberration in GBM, and the mesenchymal signature. These findings suggest that aggressiveness of high-grade astrocytomas is governed in large part by genetic and epigenetic changes that determine whether the tumor displays neural lineage markers or adopts a phenotype indicative of an alternate differentiation state. This work was supported in part by the National Brain Tumor Foundation and NIH grants CA85799 and NS42927.

244. PIK3CA IS MUTATED IN OLIGODENDROGLIOMAS, ASTROCYTOMAS, AND MEDULLOBLASTOMAS AND ASSOCIATED WITH CHROMOSOME 1p, 19q LOH IN OLIGODENDROGLIOMAS

D.C. Adamson,[1] D.K. Broderick,[1] C. Di,[1] Y.R. Samuels,[2] R.E. McLendon,[1] D.W. Fults,[3] V.E. Velculescu,[2] A. Rasheed,[1] D.D. Bigner,[1] and H. Yan[1]; [1]Pediatric Brain Tumor Foundation Institute, Duke University Medical Center, Durham, North Carolina; [2]Johns Hopkins University Medical Institutions, Baltimore, Maryland ; [3]University of Utah, Salt Lake City, Utah; USA

The phosphatidylinositol 3'-kinase (PI3K) pathway helps to maintain a delicate balance between cell survival and death and is interrupted by inactivating mutations of *PTEN*, a PI3K phosphatase, in some cancers, including glioblastomas. Mutations in *PIK3CA*, a member of the PI3K family, have also been identified in glioblastomas, colorectal cancers, and gastric cancers, and in a smaller fraction of breast and lung cancers. The 1p and 19q LOH in oligodendrogliomas is a well-established genetic phenomenon which has important prognostic and therapeutic implications for patients with these tumors. Evidence from several retrospective studies suggests that allelic losses of 1p and 19q serve as molecular markers for response to chemotherapy and radiation therapy and are an indicator of prolonged survival in patients with oligodendroglioma. We explored the role of *PIK3CA* and *PTEN* mutations in brain tumors by sequencing it in a panel of 332 tumors in 7 different histological categories, as described by two board-certified neuropathologists. We also performed a microsatellite analysis on 44 tumors with a panel of 6 markers for 1p and 19q LOH. A total of 19 *PIK3CA* mutations were identified in 17% of the anaplastic oligodendrogliomas (6/36), 6% of the oligodendrogliomas (2/32), 5% of the glioblastoma multiforme (5/105), 7% of the anaplastic astrocytomas (1/31), and 6% of the medulloblastomas (5/78). No mutations were seen in low-grade astrocytomas or ependymomas. Furthermore, no *PTEN* mutations were identified in tumors with *PIK3CA* mutations. We also found 1p and 19q LOH in 60% (13/22) of the well-differentiated oligodendrogliomas and 81% (18/22) of the anaplastic oligodendrogliomas. The eight discovered unique missense mutations in oligodendrogliomas clustered in the helical and catalytic domains. Of note, all of the tumors with *PIK3CA* mutations possessed the LOH of 1p and 19q. These observations demonstrate that *PIK3CA* and *PTEN* mutations occur in a mutually exclusive manner in brain tumors. This novel discovery of relatively common *PIK3CA* mutations in anaplastic oligodendroglioma and the clear association with 1q, 19p LOH gives new insight into the molecular pathogenesis of these tumors and suggests the need to evaluate outcomes of patients with *PIK3CA* mutations. *PIK3CA* mutations may serve as important prognostic markers for brain tumors with anaplastic behavior.

245. MOLECULAR CLASSIFICATION OF OLIGODENDROGLIOMA IDENTIFIES TRANSCRIPTS ASSOCIATED WITH RESPONSE TO CHEMOTHERAPY

P. French,[1] M. Kouwenhoven,[2] S. Swagemakers,[3] P. van der Spek,[3] and T. Luider,[1] J. Kros,[2] M. van den Bent,[1] and P. Sillevis Smitt[1]; [1]Departments of Neurology, [2]Pathology, and [3]Bioinformatics, Erasmus MC, Rotterdam, The Netherlands

Malignant gliomas are the most common primary central nervous system tumor in adults. One glioma subtype, the oligodendrogliomas, have a markedly better prognosis than most others gliomas (median survival is 5–10 years). A common genomic aberration in oligodendrogliomas is a combined loss of the 1p and 19q chromosomal arms. Loss of these arms is significantly correlated with response to treatment (combined radiation therapy and chemotherapy). In order to better understand the molecular mechanisms that underlie chemosensitivity and potentially identify molecular pathways affected in oligodendrogliomas, we compared mRNA expression profiles of 28 oligodendrogliomas and 6 control brains using Affymetrix HU133-plus 2 microarrays. We first performed unsupervised clustering to group samples on their similarities in mRNA expression profile. Three subgroups can clearly be distinguished. Subgroup 1 consists of low-grade tumors and control brains; subgroup 2 consists mainly of chemosensitive tumors with loss of 1p, while subgroup 3 consists of chemoresistant tumors that have retained both copies of 1p. Unsupervised clustering therefore readily identifies subgroups that are associated with tumor grade and prognosis.

We next performed supervised clustering to identify genes associated with loss of the short arm of chromosome 1. Both FISH and LOH-PCR were used to determine loss of 1p and 19q in our samples. Supervised clustering identified 95 probesets as being differentially expressed between tumors that have lost one copy of 1p compared to those that have retained both copies. Interestingly, 81/95 (85%) of these probesets are located either on chromosome 1p or on 19q, and the average difference in expression level is 0.55 ± 0.14. As allelic loss of these genes virtually halves their expression level, it can be hypothesized that these genes have an allele-number-dependent expression level. These genes can therefore serve as markers to identify chromosomal aberrations. Finally, supervised clustering was performed to identify genes associated with chemotherapeutic response. We identified 16 probesets that are significantly differentially expressed between chemosensitive and chemoresistant tumors. These genes can provide a prognostic value as to whether the tumor will respond favorably to chemotherapy and may identify molecular mechanisms that underlie chemosensitivity.

246. MEASURING GROWTH RATES OF LOW-GRADE GLIOMAS MAY PREDICT EARLY MALIGNANT TRANSFORMATION

J. Rees, H. Watt, C. Benton, N. Fox, P. Tofts, H. Jager, and A. Waldman; Molecular Neuroscience, Institute of Neurology, UCL, London, UK

A majority of adult low-grade gliomas (LGGs) grow slowly for many years and then, unpredictably, undergo malignant transformation. The clinical management of LGGs is controversial as there is no proven benefit to intervention prior to transformation. We have developed a reproducible and sensitive method of measuring tumor volumes on MRI scans in order to model growth rates of untreated adult LGGs and to determine whether acceleration of tumor growth occurs prior to transformation. Coronal-Oblique FLAIR and thin section Gd T1w sequences were obtained six monthly from 33 patients with untreated LGGs who were recruited into a multimodality imaging study. Tumor volumes were measured on 5-mm-thick sections with an interslice thickness of 1.5 mm from FLAIR images with a semi-automatic intensity gradient-based thresholding program with manual editing used when required. The tumor was contoured on all covering slices, the contoured areas were saved as a region file, and their combined volume was calculated. Percentage tumor growth rates per year were derived from hierarchical regression modeling and compared between different tumor histologies and between non-transformers (NT) and transformers (T). Transformation was defined as clinical deterioration, or the appearance of new or increased enhancement on the Gd studies, and confirmed by biopsy of the enhancing region or resection of the tumor. Seventeen patients transformed and 16 remained clinically and radiologically stable (NT). The average annual growth rate in the NT group was 13% (95% CI, 9%–18%) and in the T group was 26% (95% CI, 21%–32%) up to six months prior to the transformation scan. This equates to a 12% higher growth rate in the T group (95% CI, 5%–18%; $P < 0.0001$). Within the T group, the average growth rate increased in the final six-month transforming period to 57% per annum (95% CI, 19%–100%; $P = 0.08$ compared with earlier growth rates). Non-transforming astrocytomas grew at the same rate as oligodendrogliomas (14%–15% per year). We conclude that LGG which subsequently transform grow significantly faster prior to malignant transformation than non-transforming tumors. There is no difference between growth rates of astrocytomas compared with oligodendrogliomas. Accurate measurement of tumor growth may offer an early marker of malignant transformation and enable intervention before symptomatic or radiological progression.

247. THE USE OF DIFFUSION TENSOR IMAGING IN SURGICAL PLANNING FOR RESECTION OF LEFT FRONTAL LOBE TUMORS

A. Beaumont,[1] H. Krouwer,[2] J. Ulmer,[3] and W. Mueller[1]; Departments of [1]Neurosurgery, [2]Neurology, and [3]Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

The goal of surgical intervention for primary and metastatic intracerebral tumors is to maximize resection and minimize post-operative deficit. Techniques including awake surgery, electrocorticography, and functional mapping help delineate functionally important cortical tissue prior to resection. Diffusion Tensor Imaging (DTI) is an MR technique that can demonstrate white matter pathways. This study explores the use of DTI and white matter tractography in surgical planning for resection of posterior left frontal lobe tumors. Thirty-two cases of posterior left frontal lobe tumor resection performed by one surgeon (WM) were retrospectively analyzed. Fourteen patients had pre-operative DTI in addition to standard surgical planning and resection techniques. DTI was performed by using established protocols with post-processing to determine white matter pathways. Short- and long-term post-operative neurological deficits were recorded. Statistical significance was assessed by using the Fisher exact test. The median age of patients with and without DTI was 43.7 years (range, 28.8–56.8) and 42.0 years (range, 19.3–90.4), respectively. There were no significant differences in sex distribution (18 males, 14 females) or median time of follow-up (5.4 months; range, 0.4–27.7) in the two groups. Histopathological diagnosis was similar in the two groups (40.6% grade II, 15.6% grade III, 31.2% grade IV, and 12.5% metastatic/other). In the DTI group, 7 patients (50.0%) had no new neurological deficits, and 7 (50.0%) developed post-operative speech or motor deficit. In the non-DTI group, 10 patients (55.6%) had no new post-operative deficits, and 8 (44.4%) had speech or motor deficits. New deficits were transient and completely resolved in 5 (83.3%) patients with pre-operative DTI and in 1 (12.5%) patient without DTI ($P = 0.025$). One DTI patient had persistent speech and motor deficit, and one patient had rapid tumor recurrence with clinical decline during rehabilitation for mild weakness and aphasia. In this study DTI patients had a trend for higher rate of post-operative deficit but with a high rate of recovery. Deficits in the non-DTI group were more permanent ($P = 0.025$). This is consistent with DTI permitting more aggressive resection without causing prolonged deficit. White matter tractography may be a useful adjunct in planning the extent of surgical resection for tumors in functionally significant areas.

248. MR IMAGING BASED BIOLOGICAL CHARACTERIZATION OF NEWLY DIAGNOSED GBM

A. Pirzkall,[1] E. Ozturk,[2] R. Choy,[2] J. Lupo,[2] M. Lee,[2] S. Chang,[3] M. Berger,[3] and S. Nelson[2]; [1]Radiation Oncology, [2]Center of Molecular and Functional Imaging, and [3]Neurological Surgery, University of California San Francisco, San Francisco, California, USA

The objective of this study was to characterize metabolic and physiologic properties of newly diagnosed GBM with respect to its morphologic properties derived from MRI. Fourteen patients with newly diagnosed GBM, prior to any intervention, were studied with MRI and multivoxel 3D Proton magnetic resonance spectroscopy imaging (MRSI), perfusion- (PWI) and diffusion-weighted imaging (DWI) to assess morphologic, metabolic, and physiologic properties of the tumors studied. The MRI protocol included axial T2-weighted (FLAIR) and post-contrast T1-weighted (SPGR) sequences. Three-dimensional MRSI was acquired with lactate editing and a 1-cc nominal spatial resolution. Diffusion and perfusion data were resampled to match the resolution of the MRSI allowing for a voxel-by-voxel analysis. Evaluated parameters included choline (Cho), creatine (Cr), *N*-acetyl aspartate (NAA), lactate (Lac) and lipid (Lip), and Cho- to-NAA index (CNI) for MRSI; normalized (to normal appearing white matter) Apparent Diffusion Coefficient (nADC) for DWI; and Peak Height (PH) for PWI. Manually defined, mutually exclusive regions of interest (ROI) included macroscopic necrosis (Necr), contrast enhancement (CEL) and T2 hyperintensity (T2L) lesion, and normal appearing white matter (NAWM). CNI contours of ⩾2 (CNI2), previously confirmed to correspond with tumor infiltration, were generated. A total of 3453 data voxels within the MRSI selected volume were studied. All GBM exhibited CEL and T2L, and macroscopic necrosis was present in 12/14 patients. A number of significant differences in metabolic and physiologic imaging characteristics were found with respect to the defined anatomic and anatomic/metabolic ROIs. The CEL in newly diagnosed GBM was found particularly heterogeneous and, apart from Necr, exhibited the highest levels of Lac and Lip (within the CNI4-inside-CEL) reflecting micronecrosis and hypoxia, while simultaneously exhibiting significantly increased perfusion PH which was highest in CNI2,3-inside-CEL indicating increased cell division and neovasculature. Most importantly, the CNI regions extending beyond the CEL, on the other hand, exhibited clearly tumor suggestive metabolism with highest Cho, increased PH, increased Lac, and to a lesser extent Lip, demarcating the "leading edge" of the tumor. Regions of T2 hyperintensity had similar Cho, decreased Cr and NAA, slightly increased Lac/Lip, much increased nADC, and similar PH values compared to NAWM indicating a mixture of edema and tumor cell infiltration. Metabolic and physiologic imaging provides additional insight into biological characteristics, extent, and heterogeneity of brain gliomas likely enabling us to optimize our treatment planning both for surgery and for radiation therapy and allowing us to improve patient selection for respective treatment protocols.

249. HIGH SPECIFICITY AND SENSITIVITY OF HIGH-RESOLUTION, BIPLANE DIGITAL SUBTRACTION ANGIOGRAPHY IN THE DIAGNOSIS OF PATIENTS WITH SUSPECTED GLIOBLASTOMA MULTIFORME: IMPACT ON THE MANAGEMENT OF NONSURGICAL CASES

L. Mariani,[1] L. Schaller,[1] A. Fathi,[1] L. Remonda,[2] G. Schroth,[2] and R. Seiler[1]; [1]Universitätsklinik für Neurochirurgie, Inselspital; [2]Institut für Pathologie, Universität Bern, Switzerland

In a significant subset of patients suspected to have supratentorial glioblastoma multiforme (GBM) based on MRI, biopsy does not have therapeutic consequences, is performed to rule out a hypothetic curable lesion, is not always conclusive, and carries a significant risk of bleeding and clinical deterioration. Less invasive, but equally reliable diagnostic techniques are warranted for these patients with a limited life expectancy. We routinely perform preoperative digital subtraction angiography (DSA) in patients suspected to have a GBM. We investigated the diagnostic specificity and sensitivity of DSA in this patient population. Patients diagnosed between 1993 and 2002, with a supratentorial lesion compatible with a GBM based on MRI, with a preoperative high-resolution, biplane DSA (1024 × 1024 matrix), and with a histological diagnosis were included. Neuroradiologists blinded for the histological diagnosis analyzed the angiograms and assessed the image quality and the presence of tumor-blush, pathological vessels (PV), and an early venous drainage (EVD). They were asked to answer following questions about the lesion: Is it a tumor? Is it a malignant tumor? Is it a GBM? One hundred eighty-six patients were eligible for the study (32 stereotaxic biopsies, 154 surgical resections). No major complications resulted from angiography. Preliminary data showed following results: (1) The presence of PV associated with EVD at DSA was 100% specific for the presence of a malignant tumor (77% a malignant glioma, 20% a metastasis, 3% a PNET). (2) PV associated with EVD at DSA was present in 82% of GBMs and 75% of all malignant tumors including GBM (sensitivity). We conclude that in patients with supratentorial, intracerebral lesions compatible with a GBM based on MRI, the presence of pathological vessels and of an early venous drainage in high-resolution biplane DSA is both extremely specific and highly sensitive for the diagnosis of a malignant tumor, mostly a malignant glioma. In these patients, especially those in whom no therapy is planned, the risk of biopsy can be avoided.

250. NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME: PROTON MR SPECTROSCOPIC CHARACTERISTICS AND CORRELATION WITH CLINICAL OUTCOME

J. Chang,[1] X. Li,[2] N. Butowski,[3] S. Chang,[3] M. Prados,[3] K. Lamborn,[3] M. Berger,[3] S. Nelson,[2] and S. Cha[1]; Departments of [1]Neuroradiology, [2]Radiology, and [3]Neurosurgery, University of California at San Francisco, San Francisco, California, USA

Proton magnetic resonance spectroscopic imaging (1H MRSI) is a noninvasive means of analyzing metabolic integrity within both normal and diseased areas of the brain. The purpose of this study was to use 1H MRSI to separately quantify levels of lactate (Lac), lipid (Lip) and choline to *N*-acetyl aspartate ratio index (CNI) in two groups of glioblastoma multiforme (GBM) patients differentiated by time to progression (TTP) to assess for relationships between clinical outcome and metabolic markers. Twelve newly diagnosed GBM patients were recruited for this study. These patients were divided into two groups based on clinical status: rapid progression (RP, progression before 6 months; n = 8) and moderate progression (MP, progression after 6 months; n = 4). In addition to pre-operative anatomic MR imaging, all patients underwent 3D J-difference lactate-edited MRSI using a PRESS volume selection technique. The lactate-edited method required two acquisitions in each phase encoding step and provided reliable separated quantifications of Lip and Lac. Spectroscopic data was analyzed for levels of Lip, Lac, and CNI. The metabolite levels were quantified by using a software program developed in-house. The quantification was based on a z-score, defined as the number of standard deviations (SD) a voxel presented away from the control voxels, given a normal distribution with a mean of 0 and a SD of 1. A z-score of 4+ for Lac/Lip and 2+ for CNI was considered abnormal. The following measurement variables were derived from each patient's 1H MRSI exam: number of voxels with significant z-scores (sigZ), average z-score (avgZ), and Max z-score (maxZ). The average volume of the T2L hyperintensite captured by the PRESS box was similar among both groups. MRSI voxels contaminated with lipid artifact were excluded from analysis. Results were as follows.

Group score derived from an average of each patient's preoperative exam	# + voxels			avg Z			max Z		
	lac	lip	cni	lac	lip	cni	lac	lip	cni
Rapid Progression	26.3	21.8	28.9	6.8	10.6	3.8	11.8	20.1	6.3
Moderate Progression	19.0	8.0	21.0	6.4	7.8	4.4	10.3	13.1	8.4

Overall, the presence of Lac and that of CNI were comparable in the RP and the MP groups. Lip, however, was found in higher quantities in the RP group than in MP. This trend was observed under all measurement variables, and was particularly notable under sigZ. Our preliminary data suggests that lipid may predict clinical outcome in GBM patients and that lipid is also a better predictor of outcome than Lac or CNI. The results of this study suggest that metabolic information derived from 1H MRSI may be a predictive marker of clinical outcome in patients with GBM. Further study with larger sample size and longer follow-up will be conducted to further evaluate 1H MRS variables in predicting clinical outcome.

253. ADVANCES IN MANAGEMENT OF EPENDYMOMA

R. Grundy; Childhood Brain Tumor Research Centre, University of Nottingham, UK

Pediatric ependymomas are enigmatic tumors, and their clinical management remains one of the more difficult in pediatric oncology. There are a number of controversies in ependymoma, and these re highlighted in my address. Ependymomas are thought to arise from the ependymal lining of the ventricles and lining of the central canal, though the cell of origin is unknown. Indeed our understanding of the biology of this disease is still limited. While a number of prognostic factors have been identified, these are based on predominantly single-institution studies over long time periods covering many different attitudes to both the diagnosis and treatment of ependymoma. The most widely accepted prognostic factor is the degree of surgical resection. However, 1/3 of the children with complete resection relapse. The relationship between histological grading and tumor outcome is unclear. Indeed, consistent histological grading of ependymomas has proven difficult because a spectrum of pathological features exists, and the distinction between classic and anaplastic is difficult. A clear international consensus on this difficult issue is now required. Postoperative radiation therapy to the tumor bed is an important component in the treatment of localized ependymoma, and its role in the treatment of very young children is changing. Although there is increasing evidence that a proportion of ependymomas are chemoresponsive, a convincing role for chemotherapy is still to be demonstrated. We still need to define which drugs are active in ependymoma. This in part reflects the difficulties in interpretation of post chemotherapy imaging, and more sophisticated imaging studies are now needed. Defining which patients are likely to benefit from chemotherapy is clearly an important albeit significant challenge. Current recommendations for chemotherapy in ependymoma include (i) infants when the aim is to delay or avoid radiotherapy and (ii) patients with residual tumor after initial surgery. The systematic genetic analysis of tumor specimens obtained following chemotherapy may provide useful information for future development. A majority of tumor recurrences occur as a result of failure of local tumor control and are identified between 9 and 24 months after therapy. The prognosis for relapse is relatively poor, and overall only 25% of children survive first relapse. Better strategies for the management of relapse are needed A number of important issues remain to be addressed. We need to arrive at an international consensus on tumor grade, identify biological correlates of outcome, devise imaging methods for assessing chemosensitivity, investigate which agents are active, and define the role of newer radiotherapy treatments. Significant challenges for the years ahead.

254. ADVANCES IN MANAGEMENT OF MEDULLOBLASTOMA

M. Grotzer; Division of Oncology, University Children's Hospital of Zurich, Zurich, Switzerland

Medulloblastoma is the most common malignant brain tumor of childhood, and yet it makes up only 1% of adult brain tumors. Medulloblastoma is sensitive to chemotherapy and radiation, but successful surgical resection continues to be an important component of therapeutic success. Advances in the management of medulloblastoma have occurred in multiple areas from improved neurosurgical techniques, refined dosing and delivery of radiation, and optimized chemotherapy. Medulloblastomas are currently risk-stratified as average risk or high risk depending on clinical factors such as age, extent of resection, and presence of metastases. Molecular biology and histological subtyping are beginning to improve prognostication and may soon provide the means to accurately predict response to therapy. Treatment for average-risk medulloblastoma has achieved a level of success that allows efforts to be focused on the limitation of long-term sequelae of therapy. Therapy for high-risk and relapsed medulloblastoma has been positively affected by the advent of high-dose chemotherapy with peripheral stem cell rescue. In addition, molecular targets are being elucidated and new therapeutic agents are being tested for safety and efficacy.

255. ADVANCES IN THE MANAGEMENT OF CHILDHOOD GLIOMAS: MOLECULAR MARKERS OF PROGNOSIS AND NOVEL TREATMENT APPROACHES

I. Pollack; University of Pittsburgh, Pittsburgh, Pennsylvania, USA

With the exception of certain rare childhood glioma variants, pediatric gliomas are histologically similar to lesions that arise in adults. However, they have distinct molecular features, suggesting age-related pathways of tumorigenesis. Whereas high-grade gliomas in adults, particularly grade IV lesions, typically have amplification of EGFR and mutations of PTEN, such changes are rare in pediatric high-grade gliomas. In contrast, approximately 40% of pediatric malignant gliomas have p53 mutations, which appear to constitute an adverse prognostic factor. P16 or Rb deletions are observed in approximately half of tumors, and correlate with p53 mutations. Unlike the situation in adults, chromosome 1p and 19q deletions, although present in a subset of gliomas, are not associated with a favorable prognosis. Studies are in progress to determine whether prospective categorization of tumors by these molecular features and markers of drug resistance identifies prognostically distinct tumor subgroups. In parallel with these analyses, studies are in progress to test new therapeutic approaches for these tumors. One approach that is being examined in several studies involves the use of concurrent chemotherapy with irradiation, in addition to standard post-radiation chemotherapy. Studies are also in progress that aim to counteract drug resistance as a way of improving response to conventional chemotherapeutic agents. A second general strategy involves targeting of the growth signaling mediators that may contribute to tumor growth, such as PDGFR, EGFR, and Ras, using small molecule inhibitors. A third approach incorporates targeted inhibition of pathways that contribute to tumor angiogenesis and/or invasion. A fourth strategy applies convection-enhanced delivery of high-molecular-weight macromolecules targeted to receptors that are overexpressed on tumor cells compared to normal brain. Finally, it is likely that increased understanding of the molecular features of childhood gliomas will suggest additional relevant therapeutic targets.

256. RESPONSE OF ASYMPTOMATIC BRAIN METASTASES OF SMALL CELL LUNG CANCER TO SYSTEMIC (FIRST-LINE) CHEMOTHERAPY

T. Seute,[2] P. Leffers,[3] G.P.M. Ten Velde,[1] J.T. Wilmink,[4] and A. Twijnstra[2]; Departments of [1]Pulmonology, [2]Neurology, [3]Epidemiology, and [4]Radiology, University Hospital Maastricht, Maastricht, The Netherlands

The objective of our study was to investigate the radiological response of asymptomatic brain metastases (BM) from small cell lung cancer (SCLC) to first-line chemotherapy. BMs are a frequent and devastating complication in SCLC patients. The standard treatment of symptomatic BM is whole-brain radiotherapy (WBRT) in combination with corticosteroid medication. However, earlier studies found that BMs respond well to systemic chemotherapy. It is postulated that the response rate (RR) of BM to systemic chemotherapy reflects the response of the primary tumor. Therefore, the current prevailing opinion is that BM from SCLC should be treated initially with systemic chemotherapy. Whereas previous reports studied the RR of symptomatic BM to chemotherapy, the present study investigated the RR of asymptomatic BM to first-line chemotherapy. From 1980 to 2003, 462 consecutive patients with SCLC were enrolled in this study. Patients were examined by a neurologist on a regular basis. Routine imaging of the brain (CT or MRI) was performed before and after systemic chemotherapy. All patients were initially treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, and etoposide. Clinically manifest BMs were treated with WBRT. Patients with asymptomatic BM did not receive cranial irradiation. The RR of asymptomatic BM to chemotherapy was assessed by changes in the size or the number of enhanced lesions on MRI, using standard criteria. The MRI scans were blindly reviewed by an experienced neuro-radiologist. We found 70 patients (15%) with BM at diagnosis of SCLC. In 24 (5%) of these patients, BMs were asymptomatic. The asymptomatic BMs were all diagnosed by MRI. Twenty-two patients with asymptomatic BM completed five cycles of chemotherapy and could be evaluated for response. In six patients (27%) the BM responded (two complete remissions, four partial remissions) to chemotherapy. In 16 patients (73%) there was a systemic response. In ten patients the systemic response was consonant with the cranial response. In 19 patients, BM became symptomatic during follow-up, after completing chemotherapy, with a median duration of 2.3 months. The progression-free survival did not differ among cranial responders and non-responders. The median survival for patients with asymptomatic BM was 8.3 months, whereas that for patients with symptomatic BM was 8.0 months. The prevalence of asymptomatic BM in SCLC is 5%. The RR of asymptomatic BM of SCLC to first-line systemic chemotherapy is 27%. We could not confirm the high RR found by former studies. Furthermore, the response of the BM was considerably worse than the systemic response to chemotherapy. We therefore propose that the treatment of BM from SCLC should consist of chemotherapy and cranial irradiation.

257. BRAIN METASTASES FROM DIFFERENT TUMOR TYPES: A SURVEY ANALYSIS FROM A MULTIDISCIPLINARY EXPERIENCE

A. Fabi, A. Felici, A. Mirri, I. Sperduti, E. Bria, G. Lanzetta, G. Mansueto, L. Moscetti, A. Pace, S. Telera, A. Vidiri, and *C. Carapella*; Latium Neuro-Oncology Group, AINO, Regina Elena National Cancer Institute, Rome, Italy

In order to evaluate trend and clinical behaviour of patients (pts) affected by brain metastases (BM), the Latium Neuro-Oncology Group members (Neurology, Neurosurgery, Oncology, and Radiotherapy Units) applied a multi-institutional survey to clarify the commonly employed therapeutic strategies and to indicate the most effective approach arising from a multidisciplinary experience. Primary outcomes have been identified the median overall survival (OS) and median overall survival after BM appearance (BM-OS). The outcome monitoring was accomplished through a questionnaire with several items regarding patients' characteristics and therapeutic approach to BM from different primary tumors. From March 2003 to November 2004, 203 patients were registered for the study. The median age was 60 (range, 20–88), male/female distribution 107/96 (53%/47%). The primary tumor sites were as follows: NSCLC 98 (48%), breast 44 (22%), colorectal 23 (11%), melanoma 18 (9%), SCLC 8 (4%), unknown primary tumor 7 (3.5%), kidney 2 (1%), others 3 (1.5%). Sites of BM were as follows: supratentorial in 118 pts (58%), infratentorial in 54 (27%), and infra-supratentorial in 31 (15%). Neurological symptoms were present in 118 pts (58%), and the RPA-RTOG scale was I for 70 pts (34%), II for 115 pts (57%), III for 12 pts (6%), and unknown for 6 pts (3%). One lesion was observed in 90 pts (44%), 2 to 3 lesions in 35 pts (17%), and more than 3 lesions in 78 pts (39%). First- and second-line treatment for BM was given at 197 and 87 pts, respectively. Surgery was the first- and second-line treatment in 46 (23%) and 8 (9%) pts, chemotherapy in 45 (23%) and 40 (46%) pts, whole brain radiation (WBRT) in 91 (46%) and 35 (40%) pts, and radiosurgery in 14 (7%) and 4 (5%). At a median follow-up of 18.5 months, the median OS was 11 (8–15) months; with respect to different tumor type the 1-year survival rate was lung 52.5%, breast 47.7%, melanoma 30%, and colorectal 12.7%. Pts with 1 and >2 lesions present a median OS of 16 (9–24) months and 7 (5–9) months, respectively ($P = 0.0005$). Local aggressive treatment (surgery and radiosurgery), mainly considered in pts with 1 to 3 lesions and limited systemic disease, was demonstrated to be superior in terms of median OS compared with CT and WBRT (17 and 9 months, $P = 0.004$). In our survey, no significant differences were seen in OS from BM diagnosis between patients affected by either lung or breast cancer, but a significant OS difference was observed for colorectal cancer pts in which BM presents the worst prognosis. Numbers of lesions, limited systemic disease, and local aggressive treatment are predictive factors for OS.

258. LEPTOMENINGEAL METASTASIS: A DESCRIPTIVE META-ANALYSIS OF 2765 PATIENTS

L. Junck; Neurology, University of Michigan Hospital, Ann Arbor, Michigan, USA

The purpose of this study was to characterize leptomeningeal metastasis by meta-analysis of published series. The literature was searched for articles describing series of 18 or more patients with multiple primary malignancies, mainly or entirely adults, published between 1974 and 2004 in English. Each analyzed feature, e.g., proportion of cases with each type of primary malignancy, was normalized to the appropriate subset of patients. Results were compared to U.S. statistics for all cancer deaths from the SEER registry. Forty-nine series were found. Among common malignancies, the primary malignancy was as follows: breast 24%, leukemia 20%, lymphoma 17%, lung 14%, CNS 11%, melanoma 5%, colorectal 0.7%, prostate 0.4%. An index of relative incidence of leptomeningeal metastasis was calculated in relation to all cancer deaths, adjusted so that the incidence averages 1.0 for all cancers. This relative incidence is as follows: leukemia 5.1, CNS 4.9, lymphoma 3.9, melanoma 3.6, breast 3.1, lung 0.51, prostate 0.08, colorectal 0.06. Among patients with lung cancer, adenocarcinoma and small-cell carcinoma are overrepresented, accounting for 45% and 35% of cases of leptomeningeal metastasis, respectively, though these subtypes each account for a small minority of lung cancer cases. Average survival, including both treated and untreated cases, was 2.6 months. Among patients who underwent lumbar puncture (LP), the initial positive cytology was obtained with the first LP in 73%, the second LP in 22%, and the third LP in only 3%. CSF analysis revealed increased WBC in 68%, decreased glucose in 47%, increased protein in 82%, and normal analysis in 6%. The risk of leptomeningeal metastasis varies widely by cancer type. Although lung cancer is considered a common source, the risk of leptomeningeal metastasis with lung cancer in relation to cancer death is less than 1/6 that of several other types of malignancy. Survival is poor. Performing CSF cytology on two LPs adds to diagnostic yield, but a third LP rarely helps. While CSF WBC, glucose, and protein are non-specific, some of the findings are nearly always abnormal and can aid in diagnosis.

259. A PHASE 2 TRIAL OF INTRA-CSF ETOPOSIDE IN THE TREATMENT OF NEOPLASTIC MENINGITIS

M. Chamberlain; Interdisciplinary Oncology, H. Lee Moffitt Cancer Centre, Tampa, Florida, USA

The objective of this study was to determine the toxicity and response rate of intra-CSF etoposide [VP-16] in the treatment of patients with neoplastic meningitis [NM]. NM, a metastatic complication of both primary CNS and systemic cancer, occurs in 1% to 5% of patients with known cancer. Currently available treatment options are limited and provide only modest benefit. Twenty-two patients (median age 55 years) with clinical and cytologically documented NM received intra-CSF VP-16. Tumor histologies included the following: lung (6 patients), breast (4), brain (4), non-Hodgkin's lymphoma (3), melanoma (3), colon (1), and prostate (1). Concurrent involved-field radiotherapy (16/22 patients) or systemic chemotherapy (15/22) was administered based on clinical indications. VP-16 was administered at a fixed dose (0.5 mg every day given 5 times per week every other week for 8 weeks [induction]). Patients were evaluated by CSF cytology and neurological examination at the conclusion of induction therapy. Responding patients continued to receive VP-16 (5 consecutive days every 4 weeks) with monthly evaluations. Seven of 22 patients (32%) treated with VP-16 had a cytological response and either stable or improved neurological status at the conclusion of induction. Six of 22 patients (27%) progressed during induction therapy and did not receive the 8-week induction course of therapy. In responding patients, duration of response ranged from 8 to 40 weeks (median 24 weeks). Toxicity was manifested as transient chemical arachnoiditis (4/22 patients; 10% of all treatment cycles). There was no evidence of myelosuppression nor were treatment-related hospitalizations or deaths seen. It is concluded that VP-16 has modest activity against NM and easily managed toxicity.

260. PHASE 3 STUDY COMPARING RADIOTHERAPY WITH SUPPORTIVE CARE IN OLDER PATIENTS WITH NEWLY DIAGNOSED ANAPLASTIC ASTROCYTOMAS (AA) OR GLIOBLASTOMA MULTIFORME (GBM): AN ANOCEF GROUP TRIAL

F. Keime-Guibert, O. Chinot, L. Taillandier, S. Cartalat-Carel, M. Frenay, G. Kantor, J. Guillamo, E. Jadaud, P. Colin, P. Bondiau, P. Meneï, H. Loiseau, V. Bernier, J. Mazeron, J. Honnorat, M. Barrié, A. Bissery, and J. Delattre; Association des Neuro-Oncologues d'Expression Française

Despite evidence of increased incidence, the optimal treatment of malignant astrocytomas in elderly patients is unsettled, ranging from palliative care to a vigorous approach with radiotherapy and chemotherapy. To start evaluating this issue, patients with AA or GBM, age 70 years or older, and a Karnofsky performance status of at least 70 were randomly assigned after biopsy or surgical excision to receive either supportive care (corticosteroids, anticonvulsants, physical therapy, and palliative support) or supportive care and focal RT (50 Gy/28 fractions/38 days). Randomization was stratified by center. The primary end point was overall survival. The secondary end points were tolerance and quality of life. Between February 2001 and December 2004, 84 patients with a median age of 73 years (range, 70–85) were randomized in 11 medical centers. A sequential triangular design was used. and the trial reached a stopping boundary at the first interim analysis ($P = 0.004$). Median survival of the 40 patients who received RT was 28 weeks compared with 17 weeks for the 44 patients who had only supportive care. There were no clinically severe adverse reactions related to RT. Quality of life evaluation is ongoing. This trial, which is the first phase 3 study specifically devoted to the management of malignant astrocytomas in the elderly (age 70 years or more), demonstrates that focal RT increases survival in this population.

261. THE ROLE OF FRACTIONATED STEREOTACTIC RE-IRRADIATION IN RECURRENT GLIOMAS

S. Combs, S. Gutwein, D. Schulz-Ertner, C. Thilmann, P. Huber, and J. Debus; Radiation Oncology, University of Heidelberg, Heidelberg, Germany

The present analysis evaluates the effectiveness of fractionated stereotactic re-irradiation (FSRT) in recurrent gliomas. From January 1995 to July 2003, 156 patients with recurrent gliomas were treated with FSRT. At primary diagnosis, 63 patients had WHO grade II tumors, WHO grade III astrocytomas were diagnosed in 40 patients, and 53 patients suffered from glioblastoma multiforme (GBM). All patients underwent neurosurgical interventions at primary diagnosis. Median time between primary diagnosis and re-irradiation was 50, 31.5, and 10 months for grade II, III, and GBM, respectively. Using 3-4 non-coplanar fields formed with a multi-leaf-collimator, a median dose of 36 Gy was applied in a median fractionation of 5x2 Gy/week depending on the size and the localization of the lesion. No concomitant chemotherapy was applied. Radiation was well tolerated by all patients. No severe side effects occurred. Median overall survival was 111 months for patients with grade II gliomas, 48 months for grade III, and 21 months for patients with GBM. Calculated from the initiation of FSRT, median survival was 23, 16, and 8 months for grade II, III, and IV gliomas. Main prognosticators for overall survival were histology, and extent of neurosurgical resection. We conclude that stereotactically guided fractionated re-irradiation is a safe and effective treatment modality in selected cases with recurrent gliomas. Further investigations to continuously improve overall survival in patients with astrocytomas are warranted, especially with regard to newer radio-chemotherapeutic strategies.

262. LONG-TERM FOLLOW-UP OF PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA TREATED WITH A HIGH-DOSE METHOTREXATE-BASED REGIMEN WITH OR WITHOUT WHOLE BRAIN RADIOTHERAPY

I. Gavrilovic, A. Hormigo, A. Kim, J. Yahalom, L. DeAngelis, and L. Abrey; Neurology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

The primary objective of this study is to report the long-term outcome of newly diagnosed primary central nervous system lymphoma (PCNSL) patients treated with high-dose methotrexate (MTX)-based chemotherapy with or without whole brain radiotherapy (WBRT). Our initial report of this regimen (J. Clin. Oncol. 18, 3144, 2000) projected an overall median survival of 60 months and an excellent outcome for patients younger than age 60. We have now followed all surviving patients for nearly 10 years and have detailed information regarding relapse and delayed treatment toxicity. There were 57 patients with a median age of 65 years (range, 22–89 years) and median Karnofsky performance status of 70 (range, 30–100). Patients received five cycles of MTX 3.5 g/m^2 and vincristine 1.4 mg/m^2. Procarbazine 100 mg/m^2/d for 7 days was given with the first, third, and fifth cycle of MTX. Three weeks after WBRT or cycle 5 of MTX, cytosine arabinoside 3 g/m^2 was given for two cycles for a total of 4 doses. Thirty-one patients received WBRT (45 Gy) and 26 older patients deferred WBRT in an effort to reduce treatment-related neurotoxicity. Thirty-seven patients have died, and the overall median survival was 51 months with a median follow-up of the 20 surviving patients 115 months (range, 12–144 months). Recurrent disease developed in 22/57 patients (39%) typically within 3 years of diagnosis; one patient developed an isolated systemic non-Hodgkin lymphoma 7.4 years after PCNSL diagnosis. Thirty percent (17/57) of all patients have developed treatment-related dementia; the risk was higher in patients age 60 and older who received WBRT (75%), but 26% of those patients <60 who received WBRT also developed cognitive deficits or dementia. Late neurotoxicity has not developed in any surviving patient who deferred WBRT. At last follow-up, a total of 20/57 patients (35%) are alive, including 74% of the patients under the age of 60 at diagnosis plus 19% of older patients who received chemotherapy alone. Cause of death was tumor in 23 patients (40%), acute toxicity during salvage treatment in 2 (3.5%), late toxicity in 9 (16%), other cancers in 2 (3.5%), and unknown in one (1.8%). Long-term follow-up confirms our initial observation of excellent overall survival with this regimen. Younger patients do particularly well, but have a significant risk of developing late neurotoxicity. A subset of older patients who deferred WBRT enjoyed prolonged survival and an excellent quality of life without evidence of treatment-induced neurotoxicity.

263. MODIFIED BORON NEUTRON CAPTURE THERAPY (BNCT) FOR MALIGNANT GLIOMAS USING EPITHERMAL NEUTRON AND TWO BORON COMPOUNDS WITH DIFFERENT ACCUMULATION MECHANISMS: EFFECTIVENESS OF BNCT ON RADIOGRAPHIC IMAGES

S. Miyatake[1], Y. Kajimoto[1], S. Kawabata[1], K. Yokoyama[1], K. Kuroiwa[1], and O. Ono[2]; [1]Neurosurgery, Osaka Medical College, Takatsuki; [2]Radiation Oncology, Kyoto University, Research Reactor Institute, Kumatori; Japan

Boron neutron capture therapy (BNCT) is tumor-specific radiotherapy. To improve the effectiveness of BNCT for malignant gliomas, we utilized epithermal neutron and two different boron compounds, sodium borocaptate (BSH) and boronophenylalanine (BPA) with different accumulation mechanisms to increase the boron level in tumors. Thirteen patients—ten glioblastoma, one gliosarcoma, one anaplastic astrocytoma, and one anaplastic oligoastrocytoma (6 were new and 7 were recurrent)—were treated with this modified BNCT from January 2002 to December 2003. Only one glioblastoma patient had no postoperative enhanced lesion. The patients received ^{18}F-labeled BPA PET to assess the accumulation and distribution of BPA before neutron irradiation. Irradiation time was determined not to

exceed 13 Gy-Eq to the normal brain and as much as we could to contrast enhanced tumor for the new cases. For the recurrent cases, irradiation time was determined in each case according to the previous irradiation dose and field. Five grams of BSH and 250 mg/kg of BPA were administrated 12 h and 1 h before neutron irradiation, respectively. The patients received volumetric assessments, by MRI or CT scanning. Improvements in the images were assessed at 2 to 7 days after irradiation as initial effects, and their maximum effects on serial radiographic images were also analyzed. Survival period was also estimated after diagnosis and BNCT. Six cases out of 13 were analyzed the peak activity of chorine and *N*-acetyl aspartate (NAA) by MR spectroscopy (MRS) to analyze tumor-specific cytotoxicity and neuronal preservation The lesion/normal brain (L/N) ratio of BPA before BNCT on PET varied from 2.65 to 7.8. There was a tendency of low L/N ratio in recurrent cases and high in primary cases. The neutron irradiation time varied from 60 to 120 min. The mean of initial tumor volumes prior to BNCT was 42.3 (2.2–107.6) cm^3. Irrespective of initial tumor volume, in all patients who had assessable lesions, the improvements on MRI/CT images were recognized both on initial assessments (volume reduction rate: 17.4%–71.0%, mean 46.4%) and on follow-up assessments (30.3%–87.6%, mean 58.5%). More than 50% of the contrast-enhanced lesions disappeared in 8 out of the 12 patients during the follow-up period. Mean survival after the diagnosis was over 19 months. Three patients are still alive. Four cases were lost by CSF dissemination, and 2 cases were lost by other cause of death. MRS showed preserved NAA activity while Chorine activity decreased after BNCT, which showed tumor-specific destruction and neuronal preservation by this treatment. By this BNCT method, good improvements of malignant glioma patients on radiographic images and improved survival were obtained, and tumor-specific destruction was proven.

264. PRELIMINARY RESULTS OF A PHASE 2 STUDY OF TP-38 IMMUNOTOXIN DELIVERED VIA CONVECTION-ENHANCED DELIVERY TO PATIENTS WITH RECURRENT GLIOBLASTOMA MULTIFORME: REPORT OF THE TP-38 STUDY GROUP

F. Weber; Neurosurgery, Klinikum Saarbrücken, Saarbrücken, Germany

TP-38 is a recombinant chimeric protein composed of the epidermal growth factor receptor (EGFR) binding ligand (TGF-a) and a genetically engineered form of the *Pseudomonas* exotoxin PE-38. We report preliminary results of a randomized, phase 2 study conducted at multiple European centers. Either of two dose levels of TP-38 (50 ng/ml or 100 ng/ml) was administered to patients with recurrent glioblastoma in a single treatment consisting of a continuous, intratumoral infusion. This was a nonresection study; patients did not undergo tumor resection immediately prior to treatment. Three catheters were stereotactically placed in investigator-determined locations within the enhancing tumor area. The infusion rate was 0.2 ml/min per catheter. Each catheter delivered 13.4 ml over 67 h. The total volume infused was approximately 40 ml, and the total dose of TP-38 infused was approximately 2 μg or 4 μg. Patients were followed until death. Tumor responses were assessed by MRI at every 8 weeks, beginning 4 weeks after treatment. Safety was closely evaluated. Time to progression, progression-free survival, and overall survival were measured end points. Thirty-four of the planned 38 patients have been treated thus far. Safety and tolerability has been excellent. The 4-week and 12-week post-infusion MRI scans often showed treatment-related changes that make response assessment difficult. These changes usually resolved by 20 weeks post treatment MRI. Preliminary data show that one patient has a complete response 48 weeks after infusion, and another patient showed a partial response over 60 weeks, 24 patients remained stable. These results in a not highly selected group of patients suffering from recurrent glioblastoma are promising, and we are proceeding with a second trial where the tumor gets first resected and the tumor adjacent area will be treated by CED of TP38.

265. IMMUNOLOGIC TARGETING OF THE TUMOR-SPECIFIC ANTIGEN CONTAINED IN THE DELETION MUTATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR (EGFRVIII) WITH PEPTIDE LOADED DENDRITIC CELLS IN PATIENTS WITH MALIGNANT GLIOMAS

G. Archer,[1] D. Bigner,[1] A. Friedman,[1] H. Friedman,[1] K. Penne,[1] A. Paolino,[2] D. Smith,[1] D. Reardon,[1] and J. Sampson[1]; [1]Duke University Medical Center, Durham, North Carolina; [2]Emory University, Atlanta, Georgia; USA

The use of immunotherapy for the treatment of malignant gliomas requires the generation of a strong immune response in what has traditionally been considered an immunologically privileged site. Most importantly, the immune response must be specific for the tumor so as not to cross-react with normal brain, which could result in a devastating autoimmune encephalomyelitis. The deletion mutation of the epidermal growth factor receptor (EGFRvIII) is a tumor specific antigen, which is expressed on approximately 47% of all malignant gliomas (MGs). The in-frame EGFRvIII deletion combines distant parts of the molecule producing a novel glycine at the fusion junction. We have initiated a phase 1 clinical trial for patients with newly diagnosed MGs to determine the safety of vaccinating with mature DCs loaded with a peptide spanning the fusion junction of the EGFRvIII conjugated to keyhole limpet hemocyanin (PEPvIII-KLH). The vaccination protocol consist of 3 vaccines 2 weeks apart of PEPvIII-KLH loaded, mature DCs beginning 2 weeks following completion of post-resection radiotherapy. To date, 19 patients have been enrolled with 16 completing vaccination with no adverse events. No patient showed a positive delayed-type hypersensitivity reaction to KLH or PEPvIII before vaccination and of the patients who could be evaluated after vaccination, 14/15 patients (93.3%) reacted to KLH and 11/15 (73.3%) reacted to PEPvIII. In vitro proliferation in response to PEPvIII was seen in 11/12 (92%) and to KLH in 9/12 (75%) of patients tested. At two weeks following the completion of the vaccination protocol, no humoral response to PEPvIII was found. Two patients, one with anaplastic astrocytoma and one with glioblastoma multiforme (GBM) with residual radiographic disease after resection, and radiation, have had a nearly complete response following completion of vaccination. These patients have remained stable for 921 and 1216 days. Of the 14 patients without radiographically evident disease, 4/14 (28.6%) have not progressed at 418, 914, 964, 2738 days with a median overall time to progression of 315 days. For patients with GBM, the median survival time was 609 days, which compares favorably with recently published trials evaluating newly diagnosed patients with GBM treated with Gliadel (417 days [Westphal et al., Neuro-Oncology, 79, 2003]), radiation and concurrent Temodar (409 days [Stupp et al., ASCO June 2004]), or radiolabeled anti-tenascin monoclonal antibodies (556 days [Reardon et al., J. Clin. Oncol. 1389, 2002]). These findings suggest that autologous mature DCs loaded with the tumor-specific antigen PEPvIII are safe and may induce a beneficial immunologic response in patients with MGs.

266. PHASE 1/2 CLINICAL TRIAL OF CTL PRECURSOR-ORIENTED PERSONALIZED PEPTIDE VACCINATION THERAPY FOR PATIENTS WITH RECURRENT GLIOMA

R. Yamanaka[1], N. Yajima[1], N. Tsuchiya[1], J. Honma[1], M. Sano[1], Y. Obata[3], M. Shigemori[2], T. Mine[3], K. Itoh[3], and R. Tanaka[1]; [1]Neurosurgery, Brain Research Institute, Niigata University, Niigata; [2]Neurosurgery and [3]Immunology, Kurume University School of Medicine, Kurume; Japan

The purpose of this study was to investigate the safety and immunological responses of personalized peptide vaccination in patients with malignant glioma. Twenty-six patients with recurrent malignant glioma entered in the phase 1 clinical study of personalized peptide vaccination. Peripheral blood mononuclear cells (PBMCs) and plasma prior to vaccination were provided for cellular and humoral responses in vitro to each of 31 or 36 peptides in cases of HLA-A24+ or HLA-A2+ patients, respectively, and then only the reactive peptides (maximum: 4) were allowed to in vivo administration. Post-vaccination PBMCs and plasma, and also pre-and post-vaccination cerebrospinal fluid, were provided for their reactivity to the vaccinated peptides. The protocol was generally well tolerated, although the majority of patients developed grade 1 or 2 local redness and swelling at the injection site. Increase in cellular and humoral responses specific to at least one of the vaccinated peptides was observed in the post-vaccination (6th) PBMCs and plasma from 62% and 73%, respectively. More importantly, peptide-specific IgG were found in the post-vaccination cerebrospinal fluid of tumor sites. Clinical responses were 4 cases with partial response, 9 with stable disease, and 10 with progressive disease. Mean survival time has not been reached at the mean observation time 217 days. Personalized peptide vaccination is well tolerated and has the ability to induce immune responses in the majority of malignant glioma patients, along with several cases of major tumor regression. These results would encourage the phase 2 clinical study of personalized peptide vaccination for patients with recurrent malignant glioma.

267. THE TGF-BETA2 SPECIFIC ANTISENSE OLIGONUCLEOTIDE AP 12009 AS IMMUNOTHERAPY IN HIGH-GRADE GLIOMA: A CLINICAL PHASE IIB STUDY

P. Hau,[1] W. Grisold,[2] M. Mahapatra,[3] M. Mehdorn,[4] V.E. Parfenov,[5] J. Pichler,[6] M. Zaaroor,[7] U. Bogdahn,[1] K.-H. Schlingensiepen,[8] and the AP 12009 glioma study group; [1]Clinic and Policlinic for Neurology, University Regensburg, Regensburg, Germany; [2]Kaiser Franz Josef Spital, Neurologische Ambulanz, Wien, Austria; [3]All India Institute of Medical Sciences, Neurosciences Centre, New Delhi, India; [4]Klinik für Neurochirurgie, Universitätsklinikum Kiel, Kiel, Germany; [5]Department of Neurosurgery, Military Medical Academy, St. Petersburg, Russia; [6]Department of Neurosurgery, Landesnervenklinik Wagner-Jauregg, Linz, Austria; [7]Department of Neurosurgery, Rambam Medical Center, Haifa, Israel; [8]Antisense Pharma GmbH, Regensburg, Germany

Transforming growth factor-beta2 (TGF-beta2) is an important factor in malignant progression by inducing proliferation, invasion, metastasis, and angiogenesis. Furthermore, TGF-beta is the most potent immunosuppressor known. High-grade (malignant) gliomas are highly aggressive tumors characterized by distinct overexpression of TGF-beta2, responsible for the immunodeficient state of glioma patients, especially in advanced stage. AP 12009, a phosphorothioate antisense oligodeoxynucleotide specific for the human TGF-beta2 mRNA, has been developed as a targeted anti-tumor therapy and already has proven safety and shown anti-tumor activity in phase 1/2 clinical studies in recurrent high-grade glioma. Based on these results, an open-label active-controlled phase 2b multi-national study in adult patients with recurrent high-grade glioma has been initiated and is currently ongoing. Patients are randomized into 3 treatment groups to receive one of two doses of AP 12009 or standard chemotherapy, i.e., temozolomide (TMZ), or if patients had already received TMZ before the combination regimen procarbazine/CCNU/vincristine (PCV). Primary objectives are the response rate (RR), progression-free survival (PFS), and overall survival at different time points. AP 12009 is administered intratumorally as continuous high-flow microperfusion over a 7-day period every other week for up to six months. Both efficacy and safety will be used as criteria for evaluation. In the phase 1/2 studies with 24 patients, the median overall survival time was longer as compared to recent literature (Yung et al., 2000; Theodosopoulus et al., 2001; Chang et al., 2004) on standard chemotherapy. Response data after AP 12009 in phase 1/2 include several patients with stabilizations and two patients with long-lasting complete tumor remissions. As of November 2004 more than 120 patients have been enrolled in the current phase 2b study.

268. PRE-RADIATION CHEMOTHERAPY MAY IMPROVE SURVIVAL RATE OF DIFFUSE BRAINSTEM GLIOMAS: FINAL RESULTS OF BSG98 TRIAL

D. Frappaz,[1] M. Schell,[1] P. Marec-Berard,[1] C. Mottolose,[2] D. Perol,[1] C. Bergeron,[1] T. Philip,[1] A. Ricci,[2] S. Galand-Desme,[1] A. Szathmari,[2] and C. Carrie[1]; [1]Neuro-oncology, Centre Léon Bérard, Lyon; [2]Hôpital Wertheimer, Lyon; France

Despite numerous attempts (standard RT, hyperfractionated RT, standard or high-dose chemotherapy), the usual median survival of patients with diffuse brain stem tumor does not exceed 9 months. We prospectively proposed to delay radiotherapy as long as no progression was observed under multidrug chemotherapy. As soon as MRI showed a diffuse BSG, cycles of chemotherapy were initiated. Each cycle included three monthly courses, alternating BCNU and CDDP (40 mg/m²/d × 4 days in continuous infusion), course and high-dose MTX (12 g/m²). Cycles were delivered until progression, or to a maximum of 4 (1 year of treatment). Standard radiation therapy was then delivered to the tumor with maintenance hydroxyurea. Twenty-three patients were prospectively included. They were compared with a historical control group of 14 patients treated in the same institution, by front-line radiotherapy only and/or procarbazine or carboplatin. The median number of cycles was 3 (1 to 4). Iatrogenic severe infections occurred in 4 patients, and 11 patients required platelet transfusions. The median survival is **significantly increased** as compared to historical controls (17 months [95% CI, 10–23 months] vs. 9 months [95% CI, 8–10 months]; $P = 0.015$), and survival from time of radiotherapy is similar in both groups, though the number of days in hospital was prolonged (57 vs. 25 days; $P = 0.001$). Steroids could be at least transiently withdrawn in half of the patients of both groups ($P = 0.79$). We conclude that front-line chemotherapy alternating hematotoxic and nonhematotoxic schedules significantly increases overall median survival. However, the price to be paid (infections and hospitalization) deserves honest discussion with the children and their parents.

269. OUTCOME OF INTENSIVE INDUCTION CHEMOTHERAPY FOLLOWED BY CONSOLIDATIVE MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE (AUSCR) IN YOUNG CHILDREN WITH NEWLY DIAGNOSED NON-CEREBELLAR PRIMITIVE NEUROECTODERMAL TUMORS (PNET): THE "HEAD START" I AND II PROTOCOLS

J. Finlay,[1] J. Allen,[2] B. Diez,[3] S. Kellie,[4] S. Chi,[5] S. Asgharzadeh,[1] S. Zacharoulis,[1] and S. Gardner[2]; [1]Neural Tumors Program, Childrens Hospital Los Angeles, Los Angeles, California, USA; [2]Pediatric Oncology, New York University, New York, New York, USA; [3]Neuro-oncology, Institute FLENI, Buenos Aires, Argentina; [4]Pediatric Oncology, The Children's Hospital at Westmead, Westmead, Australia; [5]Pediatric Neuro-Oncology, Dana Farber Cancer Institute, Boston, Massachusetts, USA

The outcome for young children with non-cerebellar PNET has been dismal with conventional chemotherapy attempting to either delay or avoid irradiation. Between 1991 and 2002, 42 children (median age 2.9 years; range, 0.2–7 years) were treated with two serial studies: "Head Start" I (1991–1997) and "Head Start" II (1997–2002) in an effort to avoid or reduce irradiation. Head Start I induction chemotherapy included 5 cycles of vincristine, cisplatin, cyclophosphamide, and etoposide at 3- to 4-week intervals, followed by consolidation with thiotepa, carboplatin, and etoposide with AuSCR. Head Start II therapy was identical, except for the addition of high-dose (400 mg/kg) methotrexate during each induction cycle for patients with metastatic (M1+) disease. No irradiation was administered to patients. The 1-, 2-, and 5-year Kaplan Meier analyses of event-free survival (EFS) are 66.2 ± 4.4, 42.8 ± 7.9, and 40%, and of overall survival (OS) are 71 ± 7, 49.6 ± 8.6, and 43.4%. A significant difference in outcome was noted for children 36 months; 1- and 5-year OS for children 36 months are 74% and 57%. A trend for improved survival with radical resection was noted: 5 of 13 patients with less than a radical resection survive without disease compared with 14 of 24 with radical resection. All 4 children with brainstem PNET died of tumor progression. Patients with M1+ disease at diagnosis fared poorly: Of 5 pineoblastoma, 1 brainstem PNET, and 1 other supratentorial PNET, two survive without disease. The outcome for children with non-cerebellar PNET with this intensive chemotherapy strategy still remains poor for children.

270. SALVAGE TREATMENT FOR EPENDYMOMA (EPD) AFTER SURGERY ONLY: PITFALLS OF OMITTING "AT ONCE" ADJUVANT TREATMENT

M. Massimino,[1] L. Gandola,[1] F. Giangaspero,[2] A. Sandri,[2] M. Garrè,[2] L. Genitori,[2] G. Perilongo,[2] G. Scarzello,[2] F. Spreafico,[1] M. Mascarin,[2] P. Modena,[1] and E. Madon[2]; [1]Istituto Nazionale Tumori, Milano, Italy; [2]AIEOP Neuro-Oncology group

From 1993 to 2002, while 63 children were electively treated with the Italian post-surgical protocol for EPD (hyperfractionated radiotherapy [HFRT] ± VCR/CTX/VP16), 14 other patients were referred for adjuvant treatment after 2 (n = 12) or 3 (n = 2) excisions of the tumor whose treatment had been considered exclusively surgery by the referral neurosurgeon. Mean time to local progression had been 14 months, mean age at diagnosis, 5 years. Tumor originated in posterior fossa (PF) in 10 children and was supratentorial (ST) in the other 4; 11 tumors had been completely excised at both surgical report and radiological evaluation, 3 had macroscopic residues. Histological diagnosis was classic EPD in 9 pts and anaplastic in 5. Eight children were referred NED and 6 ED after second or further surgery, 5 had low cranial nerves palsy (1 requiring tracheostomy), 1 had recurrent surgery-related meningitis, and 2 had persistent hydrocephalus. All children were treated with RT to tumor bed (6 HFRT: 70.4 Gy; 8 standard RT: 54–59.4 Gy) and 5 also with pre-RT CT. Five of 14 pts (5/10 with PF tumors) had a further relapse at a mean of 6 months after last surgery; all have died, thus obtaining a PFS of 66% and an OS of 74% at a mean follow-up of 3 years after referral. Considering only pts with PF tumors, PFS and OS were 53% and 61%, respectively. Patients with relapsing EPD after surgery only, especially if originating in PF, have a more severe prognosis despite surgery completeness and non-anaplastic subtype than pts receiving adjuvant treatment after first diagnosis (PF tumors 3 years, PFS: 53% vs. 63%, $P = 0.05$); moreover, subsequent surgical acts for tumor re-growth are followed by severe neurological sequelae.

271. OUTCOME OF INTENSIVE INDUCTION CHEMOTHERAPY FOLLOWED BY CONSOLIDATIVE MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL RESCUE (AUSCR) IN YOUNG CHILDREN WITH NEWLY DIAGNOSED EPENDYMOMA

S. Zacharoulis,[1] A. Levy,[4] S. Gardner,[2] I. Dunkel,[3] J. Allen,[2] S. Asgharzadeh,[1] and J. Finlay[1]; [1]Pediatric Neuro-Oncology, Childrens Hospital Los Angeles, Los Angeles, California; [2]Pediatric Oncology, New York University, New York, New York; [3]Pediatric Oncology, Memorial Sloan- Kettering Cancer Center, New York, New York; [4]Pediatric Oncology, Montefiore Medical Center, New York, New York, USA

The outcome of young children with ependymoma has been dismal with conventional chemotherapy attempting to either delay or avoid irradiation. This study investigates the efficacy of high-dose chemotherapy followed by stem cell rescue in pediatric patients with ependymoma. The patients were 28 children from "Head Start I" (1991–1997) and "Head Start II" (1997–2002). Overall, the mean age at diagnosis was 2.4 years. Twenty-four patients with local disease received an induction regimen of 5 cycles of chemotherapy (cisplatin, vincristine, etoposide, cyclophosphamide at 3–4 week intervals). All four patients with leptomeningeal dissemination received the same drugs with the addition of high-dose methotrexate and leucovorin rescue. After induction, individuals in both groups without evidence of disease proceeded to marrow ablative chemotherapy (thiotepa, carboplatin and etoposide) with autologous stem cell rescue. Twenty patients (71%) had posterior fossa tumors and eight supratentorial tumors. Seventeen patients (61%) had a gross total resection (GTR) at diagnosis. The 1-, 2-, and 5-year Kaplan Meier analyses of progression-free survival (PFS) of these patients are 70 ± 9%, 43 ± 10%, and 14 ± 7%. The 1-, 2-, and 5-year overall survival (OS) are 75 ± 8%, 63 ± 9%, and 38 ± 10%. Survival data did not differ significantly for patients 3 years old at diagnosis. Thirty-seven percent of the patients with supratentorial tumors are long-term survivors compared to 55% of the patients with posterior fossa tumors. Half of the patients who underwent a GTR survived compared to 36% of the ones for whom GTR was not achieved. Radiation was administered to 15/28 patients overall: 75% of patients with prolonged survival received RT. Two of the four patients with disseminated disease are long-term survivors. The toxic mortality within this group of 28 patients was 14%. Outcome with this treatment strategy does not appear superior to other strategies seeking to avoid or delay RT.

272. GENE THERAPY

J. Yoshida; Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

Gene therapy is a new medicine based on a technique for correcting defective genes responsible for disease development. It holds potential for treating or even curing formidable diseases such as monogenic metabolic disorders, cancers, infectious diseases, and vascular diseases. More than 1000 clinical trials involving 6000 patients were identified worldwide in 2004, but almost all trials are aimed at establishing the safety of gene therapy rather than the effectiveness. In fact, gene therapy still faces many scientific or ethical obstacles before it can become a good practical medicine for man. A major blow to disturb the success of gene therapy came in 1999, which is the death of a patient with ornithine transcarbamylase deficiency (OTCD). Another major blow came in 2003, when the Food and Drug Administration (FDA) placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells, because children treated with French gene therapy for X-linked severe combined immunodeficiency disease (X-SCID) had developed a leukemia-like condition. In malignant glioma, on the other hand, a suicide gene therapy using herpes simplex virus-thymidine kinase gene/ganciclovir is the first clinical trial in the world, which started in 1992. Thereafter, immuno-gene therapies using cytokine genes such as interleukin or interferon (IFN) were opened one after another. For the past five years, 7 of 11 clinical trials in USA have included immuno-gene therapies. We have been also developing a new immuno-gene therapy in Japan. This therapy using interferon (IFN)- and cationic liposomes is a first original protocol developed in Japan. A pilot clinical trial of the therapy began in 2000, and thereafter the safety and effectiveness were confirmed. In addition, researchers belonging to Experts on the Gene Therapy Advisory Committee in the UK have been given approval to carry out a large clinical trial in 2004, which involves injecting oncolytic herpes simplex virus into the glioma, because the first patient to receive the treatment had a long survival more than 7 years. From this evidence, it is considered that gene therapy for malignant brain tumors is one of the most promising strategies in all gene therapies. In this lecture I introduce the current status of gene therapy research and clinical trials for malignant brain tumors, including ours. In addition, I comment on the status of gene therapy regulation in Japan, a new molecular targeting therapy based on the results of genetic analysis in patients treated with our IFN-gene therapy and the combination of gene therapy and cell therapy. In the near future I believe that gene therapy can offer hope to patients with malignant brain tumors, including glioma.

273. THE P75 NEUROTROPHIN RECEPTOR MEDIATES GLIOMA INVASION

A. Johnston,[1] D. Senger,[2] X. Lun,[2] H. Muzik,[2] Z. Shi,[2] M. Slovack,[2] S. Robbins,[1] and P. Forsyth[2]; [1]Biochemistry and Molecular Biology and [2]Clinical Neuroscience, University of Calgary, Calgary, Canada

Malignant gliomas are highly invasive brain tumors. This invasive tendency renders these tumors surgically incurable and associates them with a high mortality rate. Glioma cell invasion is poorly understood, and we believe that increased understanding of this process will lead to the development of novel therapeutics. Through the use of a serial in vivo selection procedure, we have isolated highly invasive and highly noninvasive cell subpopulations from the human glioma cell line U87. Microarray comparison of the two subpopulations was then used to identify novel genes not previously implicated in glioma invasion. One of the differentially expressed genes was the p75 neurotrophin receptor. This receptor is present at both the RNA and protein level in the invasive cell population, but is undetectable in the noninvasive population. Importantly, we have also observed that treatment of these cells with p75 ligands increases the migration and invasion of the invasive, p75-positive cells, but has no effect on the noninvasive, p75-negative population. In addition, we found that p75 is present in other glioma cell lines and that a strong positive correlation exists between levels of p75 expression and neurotrophin-induced migration in these cells. Subsequently, we upregulated p75 levels in the original U87 cell line and demonstrated that this upregulation conferred an increased migratory and invasive ability in vitro. Conversely, downregulation of p75 in a human glioma cell line expressing high levels of p75 decreased the migration and invasion of these cells in vitro. Finally, we demonstrated that p75 is overexpressed in human glioma patient specimens. These data suggest that p75 is present, functional, and involved in glioma migration and invasion. Future experiments are aimed at identifying the downstream signaling molecules that mediate these effects.

274. HUMAN SKIN–DERIVED STEM CELLS INHIBIT BRAIN TUMOR GROWTH

F. Acerbi,[1] C. Giussani,[1] F. Pisati,[2] G. Carrabba,[1] M. Belicchi,[2] V. Lucini,[1] S.M. Gaini,[1] A. Bikfalvi,[3] Y. Torrente,[2] and *L. Bello*[1]; [1]Neurosurgery and [2]Neurology, University of Milano, Milano, Italy; [3]INSERM Unit EPI 0113, University of Bordeaux 1, Talence, France

Neural stem cells (NSC) implanted into rodent intracranial gliomas distribute themselves and surround infiltrating tumor cells. A number of questions are left unanswered (the difficulty of isolating NSC from adult tissues, an ethical issue linked to the use of fetal derivatives, and the advantages of NSC in allogenic transplantation). We isolated human skin-derived stem cells (SDSC) from samples of skin obtained from glioma patients and studied their behavior in the presence of brain tumors. We initially studied SDSC targeting ability by implanting SDSC in the contralateral and homolateral hemisphere of well-established U87 nude mice glioma. SDSC demonstrated a high tumor target ability. Eight days after injection, SDSC migrated extensively within the tumor and surrounded the invading tumor border. In short- and long-term studies, mice injected with SDSC 11 days after tumor cell implantation showed a decrease in tumor volume and a longer survival than the controls (50% survival longer of 11 days). Treated tumors were associated with a decrease in tumor vascularization and invasion in the area of injected SDSC. When SDSC were implanted 5 days after glioma cell injection, they did not form tumors and resulted in a more significant decrease in tumor volume. The ability of SDSC to decrease tumor vascularization and invasion was confirmed in CAM assay implanted with U87 tumors. The antitumor effect of SDSC was further studied in tyrp1-Tag mice that develop retinal pigmented epithelium tumors, rapidly migrating along the optic nerve and invading the brain. In these mice, intracerebral SDSC injection decreased the intracranial and extracranial portions of the tumors. Tumor growth inhibition was associated with a marked decrease in tumor vascularization and invasion. These data suggest a potential treatment of intracranial tumors by the use of SDSC from the same patients.

275. INFLUENCE OF ANTI-ANGIOGENIC SUBSTANCES ON NEURAL PROGENITOR CELL MIGRATION, DIFFERENTIATION, AND TUMOR HOMING

M. Kirsch,[1] O. Heese,[2] G. Schackert,[1] and H. Schackert[1]; [1]Neurosurgery, Carl Gustav Carus University Hospital Dresden, Dresden; [2]Neurosurgery, Hamburg-Eppendorf University Hospital, Hamburg; Germany

Neural progenitor cells (NPC) have been used to track solid brain tumors in vivo as well as to express a therapeutic molecule intratumorally. In addition to their antitumoral properties, a restorative potential of these NPC is hoped for. We have investigated the use of local expression of the angiogenic inhibitor endostatin in a model of hematogenous cerebral melanoma metastases using neural progenitor cells as therapeutic vehicles. Concomitantly, we have investigated the effects of endostatin and of additional angiogenesis inhibitors (angiostatin, Su5416, Su5614, and Cox2-inhibitor) on in vivo migration and differentiation. In a second step, the therapeutic potential of endostatin-transfected NPCs was investigated. Migration of NPCs was tested with a modified Boyden-chamber assay and a modified wounding assay. Endostatin levels were measured by ELISA. Proliferation and differentiation patterns were analyzed by immunohistochemistry using antibodies against GFAP, NeuN, Huc, MBP, GalC, nestin, vimentin, and Ki67. The primary NPC from GFP-transgene mice as well as the myc-immortalized C17.2 NPC line were exposed to murine recombinant endostatin and angiostatin and the tyrosine kinase inhibitors Su5416 and Su5614. The cerebral metastasis model used murine melanoma cells either injected into the internal carotid artery of mice or placed stereotactically into the frontal lobe and concomitant injection of NPCs with/without transgenic endostatin secretion. Survival was recorded, and the brains were subjected to immunohistochemical analysis. Endostatin and Su5614 led to a significant reduction in migration, whereas angiostatin and Su5416 did not alter migration. Phenotypic and proliferative changes compared to control cells were not observed. Endostatin-transfection of NPCs did not result in prolonged survival although endogenous endostatin expression by tumor cells demonstrated survival advantage. Upon inspection of tumors, a reduced migration of NPCs into metastases was noted if compared to non-endostatin transfected NPCs. Endostatin inhibited NPC migration. This observation concurs with the in vivo findings of reduced NPC homing toward the tumor and therefore abolishing the therapeutic properties of NPCs using endostatins anti-angiogenic therapeutic paradigm.

276. ANTIANGIOGENIC AGENT THALIDOMIDE INCREASES THE ANTITUMOR EFFECT OF SINGLE HIGH-DOSE IRRADIATION (GAMMA-KNIFE RADIOSURGERY) ON THE RAT ORTHOTOPIC GLIOMA MODEL

D. Nam and J. Lee; Department of Neurosurgery, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea

We investigated the effect of gamma-knife radiosurgery (GKR) on a rat model of glioma with or without simultaneous administration of temozolomide or thalidomide. Combined GKR (20 Gy single maximal dose) and/or pharmacotherapy (for 3 days including the radiosurgery day) was delivered on the 18th day after stereotactic implantation of C6 glioma cells. The animals were sacrificed 24 h after GKR for evaluation of apoptosis, PCNA index, microvessel density, and expression of basic fibroblastic growth factor (bFGF) and of vascular endothelial cell derived growth factor (VEGF). To determine the tumor size reduction, other groups of animals were sacrificed at 5 days after GKR (with or without pharmacotherapy), which was delivered 14 days after the tumor inoculation. Compared with the rat which received GKR alone, there was significant increase of tumor cell apoptosis in GKR + TMZ as well as GKR + thalidomide, significant decrease of MVD in GKR + thalidomide, and significant decrease of proliferative index in GKR + TMZ. Five days after the GKR, shrinkage of tumor was most prominent in the GKR and thalidomide combination group. In addition to the reduction of bFGF and VEGF expression, reduction of MVD and induction of apoptosis by thalidomide may be associated with the prominent shrinkage of tumor among the GKR treatment groups. Our data suggest that radiosurgery combined with antiangiogenic therapy may be the most promising combination to control malignant glioma.

277. THE ROLE OF AUTOTAXIN IN GLIOMA INVASION

B. Hoelzinger,[1,2] C. Beaudry,[1] T. Demuth,[1] L. Reavie,[1] L. Mariani,[3] M. Nakada,[1] and M. Berens[1]; [1]Neuro-genomics, Translational Genomics Research Institute, Phoenix, Arizona, USA; [2]Arizona State University, Tempe, Arizona, USA; [3]Department of Neurosurgery, Inselspital, Bern, Switzerland

One of the salient features of malignant astrocytomas is the propensity to diffusely invade brain parenchyma; it is from these invading cells that the tumor invariably recurs. A better understanding of the molecular mechanisms of this locally invasive behavior could yield potential therapeutic targets aimed exclusively at this tumor cell subpopulation. To this end, a gene expression profile of GBM invasion was created by comparing mRNA from invasive cells to that of matched noninvasive cells from the tumor core. Differences in expression patterns between the two laser capture microdissected cell populations revealed multiple genes related to invasion. Among these was the autocrine motility factor Autotaxin (ATX), which is secreted by invasive melanoma and as well as by metastatic breast carcinoma and other malignant tumors. Recently, its role as a lysophospholipase D was identified, catalyzing lysophosphatidyl choline (LPC) into lysophosphatidic acid (LPA). LPA stimulates motility in various cell types, including glioma cells, which express high levels of LPA1 receptor. Evaluation of ATX expression in a glioma revealed a high expression in the invading cells of astrocytic tumors of all grades. To investigate the functional role of ATX in astrocytoma invasion, stable transfectants carrying wild-type ATX and a catalytically inactive form (H316Q-U251) were created by using the U251 glioma cell line. ATX-U251 migrated significantly faster than H316Q-U251 in a radial migration assay, an effect that was not reversed by LPA dependent motility stimulation. Cells expressing high levels of ATX also proved more adherent to laminin, specifically in the presence of its substrate LPC. To test the property of invasion we used an orthotopic rat brain slice assay, which showed ATX-U251 to be more highly invasive than H316Q-U251 or control cells. ATX knockdown with siRNA resulted in lowered invasion of spheroids in a collagen I matrix, an effect that was reversed with the addition of LPA. Changes in motility that are induced by LPA receptors result in cytoskeletal rearrangements brought about by the rho family of GTPases. We examined the actin cytoskeleton in the poorly motile H316Q-U251 cells and found normal stress fibers, enhanced filopodia formation and reduced membrane ruffles, suggesting modulation of rac signaling. Further studies consist of elucidating whether the motility inhibition of the secreted protein H316QATX functions in a paracrine or autocrine manner.

278. INTERPLAY BETWEEN EPHB2 AND EPHRIN-B ON GLIOMA CELLS PROMOTES GLIOMA MIGRATION AND INVASION

M. Nakada,[1] J. Niska,[2] W. McDonough,[2] N. Tran,[2] A. Joy,[2] and M. Berens[2]; [1]Neurology Research, Barrow Neurological Institute, Phoenix, Arizona; [2]The Translational Genomics Research Institute, Phoenix, Arizona; USA

The receptor tyrosine kinases of the EphB family and their ephrin-B ligands play a key role in neurodevelopmental processes such as boundary formation, vasculogenesis, and cell migration. The Eph receptors transmit forward signals via their kinase domain and reverse signals via their transmembrane ephrin-B ligands. EphB2 are upregulated in glioblastoma, especially in invading glioma cells (Nakada et al., Cancer Res. 64, 2004). Here we use a soluble EphB2 ectodomain fusion protein (EphB2/Fc) to demonstrate that ephrin-B transduces signals that regulate cell migration and invasion. EphB2/Fc induced ephrin-B tyrosine phosphorylation, migration, and invasion in in vitro and in ex vivo rat brain slice model using U87 and U251 glioma cells. Confocal imaging showed ephrin-B localized in lamellopodia of motile U87 and U251 cells. Activation of ephrin-B by EphB2/Fc induced phosphorylation of Akt. The phosphatidylinositol 3-kinase (PI3-K) inhibitor LY294002 reduced EphB2/Fc stimulated migration and invasion concomitant with phosphorylation of Akt. Human brain tumor specimens portrayed higher expression of ephrin-B in glioblastoma than in low-grade astrocytomas or normal brain. Immunohistochemistry showed phosphorylated form of ephrin-B localization primarily in glioblastoma cells. Taken together, ephrin-B stimulated by EphB2 transduces "outside-in" signals that affect migration and invasion in glioma through the PI3-K/Akt pathway. This study was supported by the American Brain Tumor Association (MN), NS042262 and NS043446.

279. HIF-1A EXPRESSION IS ASSOCIATED WITH GLIOMA CELL INVASION

T. Demuth,[1] D. Hoelzinger,[1] J. Rennert,[1] E. Newcomb,[2] M. Berens,[1] and D. Zagzag[2]; [1]Neurogenomics Department, Brain Tumor Unit, TGen, Phoenix, Arizona; [2]Department of Pathology, NYU Medical School, New York, New York; [3]Arizona State University, Phoenix, Arizona; USA

Hypoxia is associated with adverse outcome for a number of solid tumors, including gliomas. We reported expression of hypoxia inducible factor-1a (HIF-1a) in two different microenvironmental areas of gliomas: (1) pseudopalisading cells at the tumor core, and (2) invasive tumor cells located in brain adjacent to tumor (BAT). Using CA9, a carbonic anhydrase, as a surrogate marker for tumor hypoxia, we showed by immunohistochemistry (IHC) that HIF-1a expression within these two brain tumor areas may be driven by different mechanisms (possibly leading to distinct cellular responses): Hypoxic cells within the pseudopalisades exhibited staining for HIF-1a and CA9, whereas all the invading HIF-1a positive glioma cells in the BAT were negative for CA9. These results suggest that upregulation of

HIF-1a in invading glioma cells assessed by IHC might be controlled by molecular mechanism(s) other than those induced by hypoxia. The association of HIF-1a expression with glioma invasion was further studied by comparing the gene expression profile of invasive and stationary glioma cells isolated from human biopsies by laser capture microdissection. In two of three tumor samples HIF-1a was found to be upregulated in invasive cells while VEGF, a gene known to be induced by HIF-1a under hypoxic conditions, was downregulated. Transcriptional regulation of HIF-1a was also examined by microarray analysis of in vitro migration assays. Three of ten GBM cell lines induced to migrate on glioma-derived extracellular matrix showed HIF-1a upregulation. Gene expression changes coincident with upregulation of HIF-1a and glioma invasion will be reported by comparing in vivo transcriptomes from human glioma biopsy specimens with those obtained from in vitro invasion assays. These data and the results from IHC for CA9 and HIF-1a suggest that HIF-1a may be activated and signal through a distinct and separate pathway unique for glioma cell invasion. We propose dual modes of activated expression leading to detection of HIF1a-protein in invasive and stationary glioma cells by IHC: (1) In the hypoxic environment of tumor core HIF-1a is stabilized and activates transcription of hypoxia induced genes; (2) in glioma cells at the invasive edge, increased transcription of HIF-1a may compensate for its ubiquitination/degradation under normoxic conditions. Disparate drivers of HIF-1a expression in glioma cells in response to microenvironental cues may point to exploitable targets in the invasive cells.

280. THE SOLUBLE DECOY RECEPTOR FOR VEGF, VEFG TRAP, INDUCES ANTIGLIOMA EFFECT IN VIVO

C. Gomez-Manzano,[1] J. Holash,[4] J. Xu,[1] O. Lee,[1] J. Fueyo,[1] J. Rudge,[4] X. Zhou,[2] B.N. Bekele,[2] K. Aldape,[3] and W.K.A. Yung[1]; [1]Neuro-Oncology, [2]Biostatistics, and [3]Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [4]Regeneron Pharmaceuticals, Inc., Tarrytown, New York; USA

Pathological angiogenesis is a hallmark of cancer, and specifically of glioblastomas, the most malignant and common form of primary brain tumors. VEGF is the key molecular player involved in the mechanism of vascular growth. We undertook this project to test VEGF Trap, a new antiangiogenic agent that acts as a soluble decoy receptor for VEGF. This molecule incorporates domains of both VEGFR-1 and VEGFR-2 and binds VEGF with high affinity. U-87 MG human glioma cells were implanted in the brain of nude mice, and VEGF Trap was administered (25 mg/kg sc, twice a week for a total of 3 weeks) at days 0, 4, and 10 after cell implantation. hFc and PBS were used as control treatments. Serum was collected three days after the initial dose to assess circulating levels of VEGF Trap. Serial temporal examination of the brains of untreated mice showed that tumors grew to a volume of 0.3 mm^3 and exhibited a very low microvascular density (MVD = 6 vessels/0.5 mm^2), with central necrosis within four days of implantation, and peripheral reactive vasculature. At that time point, treatment of glioma-bearing animals with VEGF Trap increases significantly the survival time of animals compared to that of control-treated animals ($P < 0.007$, log-rank test). Another group of animals was treated starting day 10 of the experiment, at the time that tumors were masses of cells with volumes of 30–45 mm^3 and had fully developed vasculature (MVD = 30–35 vessels/0.5 mm^2). At this stage of the disease, VEGF Trap treatment induced a significant prolongation of animal survival ($P < 0.0001$, log-rank test). VEGF Trap was detected in the serum of the animals at levels of about 50 μg/ml or greater. Taken collectively, our data indicate that treatment with VEGF Trap results in prolongation of survival in this intracranial human glioma xenograft model.

281. DOMINANT-NEGATIVE INHIBITION OF THE RECEPTOR TYROSINE KINASE RECEPTOR AXL SUPPRESSES GLIOMA CELL MIGRATION AND INVASION AND PROLONGS SURVIVAL

P. Vajkoczy,[1] A. Kunkel,[1] R. Erber,[1] H. Capelle,[1] U. Eichelsbacher,[2] and A. Ullrich[2]; [1]Department of Neurosurgery, Klinikum Mannheim der Universitat Heidelberg, Mannheim; [2]Department of Molecular Biology, Max-Planck-Institute for Biochemistry, Martinsried; Germany

Receptor tyrosine kinases (RTKs) play an important role in growth and progression of brain tumors. Our previous work has shown that the RTK AXL, whose biological function has remained obscure so far, is overexpressed by glioma cell lines and human astroglial tumors. The aim of the present study was to analyze the role of AXL in glioma biology. Two glioma cell lines, one expressing high levels of AXL (i.e., SF126) and the other lacking intrinsic AXL (i.e., SF767) were transfected to overexpress either the human wild-type form (AXL-WT) or a truncated, dominant-negative mutant form (AXL-DN). Glioma cell morphology and cell behavior with respect to proliferation, aggregability, migration, and invasion were assessed in vitro. To study the relevance of AXL for tumor growth, the glioma cell lines were implanted subcutaneously and into the brains of nude mice. Finally, glioma cells were implanted into the dorsal skinfold chamber model to assess tumor cell behavior, tumor angiogenesis, and tumor perfusion in vivo by intravital multi-fluorescence microscopy. SF126-AXL-DN cells were characterized by reduced cell-to-cell contacts, a moderately reduced proliferative activity, and most importantly by a severe impairment of tumor cell migration and tumor cell invasion. In vivo, subcutaneous SF126-AXL-DN tumor growth was reduced by 97%, and tumor cell invasion into the adjacent tissue was suppressed. Finally, survival following intracerebral implantation of SF126-AXL-DN cells was significantly prolonged compared to SF126-AXL-WT cells. In contrast, inhibition of AXL signaling in SF767 cells had no significant effects. Our study provides the first evidence that the tyrosine kinase receptor AXL modulates migration and invasion of human glioma cells and that inhibition of AXL signaling suppresses tumor expansion and prolongs survival by blocking tumor cell invasion. Thus, AXL may represent a novel molecular target for the treatment of malignant glioma.

282. INHIBITION OF GLIOBLASTOMA ANGIOGENESIS AND INVASION BY COMBINED TREATMENTS DIRECTED AGAINST VEGFR-2, EGFR AND VE-CADHERIN

K. Lamszus,[1] M.A. Brockmann,[1] C. Eckerich,[1] P. Bohlen,[2] C. May,[2] R. Fillbrandt,[1] and M. Westphal[1]; [1]Department of Neurosurgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]ImClone Systems, New York, New York, USA

Increasing evidence suggests that inhibition of tumor angiogenesis can influence tumor cell invasion and metastasis. We previously showed that systemic antagonization of vascular endothelial growth factor receptor-2 (VEGFR-2) with the monoclonal antibody (mAb) DC101 inhibited glioblastoma growth in an orthotopic model, but caused increased tumor cell invasion along the preexistent vasculature. In human glioblastoma cells, signaling through the epidermal growth factor receptor (EGFR) predominantly stimulates tumor cell invasion. Therefore, we attempted to inhibit tumor cell invasion caused by DC101 therapy by combined systemic treatment with a mAb against EGFR (C225). In addition, we analyzed whether antagonization of vascular endothelial (VE)-cadherin as a different anti-angiogenic target can also inhibit glioblastoma growth and whether this also stimulates invasion. Treatments were either initiated on day 1 after intracerebral tumor cell injection or on day 6 when tumors were already established. Increased tumor cell invasion caused by DC101 monotherapy was inhibited by 50%–66% through combined treatment with C225 and DC101. C225 inhibited glioblastoma cell migration in vitro, but had no effect on the volume of the main tumor mass or on tumor cell proliferation or apoptosis in vivo, neither alone nor in combination with DC101. The anti-VE-cadherin mAb E4G10 also inhibited tumor angiogenesis and growth, although with weaker effects than DC101, and the effects of E4G10 were dependent on early initiation of treatment. E4G10 treatment caused increased tumor cell invasion along the host vasculature, although also with weaker effects than DC101. Our findings show that anti-angiogenic glioblastoma therapy targeting either VEGFR-2 or VE-cadherin can inhibit tumor growth, but can increase tumor cell invasion in an orthotopic model. The increased tumor cell invasion caused by DC101 treatment can be inhibited by simultaneous antagonization of EGFR, which in the context of human glioblastomas has been implicated in tumor cell invasion.

283. CONTACTIN IS EXPRESSED IN HUMAN ASTROCYTIC GLIOMAS AND MEDIATES REPULSIVE EFFECTS

C. Eckerich,[1] S. Zapf,[1] U. Ulbricht,[1] S. Müller,[2] R. Fillbrandt,[1] M. Westphal,[1] and *K. Lamszus*[1]; [1]Department of Neurosurgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]AGY Therapeutics, Inc., San Francisco, California, USA

Using subtractive cloning, we identified contactin as overexpressed in glioblastomas compared with normal brain. Contactin is a cell surface adhesion molecule that is normally not expressed by astrocytes. It is expressed by neurons and oligodendrocytes at particularly high levels during development. Contactin can mediate adhesive or repulsive intercellular interactions depending on the molecular context. We analyzed the expression of contactin in human astrocytomas and determined its functional relevance for glioma cells. Western blotting, immunohistochemistry and confocal immunocytochemistry were used to analyze contactin expression in astrocytomas and astrocytes. Adhesion and migration assays were performed to study effects of contactin on glioma cells. Contactin cDNA was transfected into glioma cells to determine the effect of contactin overexpression on attachment to extracellular matrix (ECM) molecules. Expression of the receptor-type protein tyrosine phosphatase , (RPTP,), a specific contactin ligand that is also overexpressed in glioblastomas, was downregulated by stable siRNA

transfection to study interactions with contactin overexpressing cells. Contactin expression was detected in GFAP positive tumor cells but was absent in normal astrocytes. Levels of contactin in gliomas were associated with increasing malignancy grade. None of 10 glioblastoma cell lines adhered to contactin or was stimulated in its motility by contactin. In contrast, increasing coating concentrations of contactin caused progressive cell repellence. Co-presentation with contactin reduced the haptotactic migration induced by fibronectin in several cell lines. Overexpression of contactin in human glioblastoma cells by cDNA transfection had no effect on cell proliferation or adhesion to various ECM molecules. Contactin expression also did not alter the adhesion to cells expressing normal or downregulated levels of RPTP,. The expression of astrocytic as well as neuronal markers within glioma cells may reflect an ability of these cells for multilineage differentiation, a phenomenon that has recently been described also for the so-called glioma stem cells. While adhesive and proliferative properties of glioma cells are unaltered by contactin overexpression, confrontation of glioma cells with contactin has repulsive effects, which may contribute to the diffuse infiltration pattern characteristic of these cells in human brain.

284. INHIBITION OF GLIOBLASTOMA GROWTH BY SIRNA-MEDIATED ANTAGONIZATION OF RPTPβ

U. Ulbricht, C. Eckerich, R. Fillbrandt, M. Westphal, and *K. Lamszus*; Department of Neurosurgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany

We previously identified the receptor-type protein tyrosine phosphataseβ (RPTPβ) and its ligand pleiotrophin (PTN) as overexpressed in glioblastomas using cDNA arrays (Müller et al., Oncogene, 2003). Both molecules have been implicated in neuronal migration during central nervous system development. We confirmed RPTPβ and PTN overexpression in glioblastomas at the protein level and showed that matrix-immobilized PTN stimulates glioma cell migration (Ulbricht et al., J. Neuropathol. Exp. Neurol., 2003). In the present study, we analyzed the effect of RPTPβ expression on glioma growth in vitro and in vivo using siRNA technology. RPTPβ expression was downregulated in U251-MG glioblastoma cells by stable siRNA transfection. Downregulation was detected by Northern and Western blotting. Colorimetric proliferation assays and modified Boyden chamber haptotactic migration assays were performed in vitro. Tumor growth in vivo was studied using a subcutaneous nude mouse model. Tumors were analyzed immunohistochemically to assess cell proliferation, apoptosis and vascularization. Two siRNA transfected clones with strong downregulation of RPTPβ expression (RPTPβ–) were obtained, and these clones were subsequently compared with 2 mock transfected control clones (RPTPβ+). Proliferation of RPTPβ– clones was inhibited by 56% to 90% compared with RPTPβ+ clones. Haptotaxis (migration toward substrate-bound molecules) induced by PTN was inhibited by 52% to 76% in RPTPβ– clones compared with controls. Collagen I and fibronectin were also haptotactic. However, no differences were observed between RPTPβ– and RPTPβ+ clones, suggesting that the inhibition of haptotaxis toward PTN is specifically mediated through RPTPβ. In vivo, growth of RPTPβ– clones was inhibited by 93% to 98% compared with controls. The fraction of proliferating tumor cells was reduced by 49% to 74% in tumors derived from RPTPβ– clones, whereas no differences were observed for apoptosis or microvessel density. Glioma growth in vivo can be inhibited by downregulation of RPTPβ expression. In vitro, RPTPβ contributes to glioma cell proliferation and mediates PTN-induced haptotactic migration. PTN was demonstrated earlier to be secreted by U251-MG cells. Therefore, our findings suggest that upregulated expression of PTN and RPTPβ in human astrocytoma cells can create an autocrine loop that contributes to tumor growth in vivo.

285. REGULATION OF PATHOLOGICAL VASCULATURE OF MALIGNANT ASTROCYTOMAS BY ANGIOPOIETIN-1

G. Zadeh, R. Reti, K. Koushan, Q. Baoping, P. Shannon, and *A. Guha*; Neurosurgery Division, Toronto Western Hospital, Toronto, Canada

A histopathological hallmark of malignant astrocytomas is microvascular proliferation and formation of vascular entities referred to as "glomeruloid bodies." The significance of glomeruloid bodies and the molecular mechanisms driving the abnormal vascular architecture in human malignant astrocytomas are not known. Vascular endothelial growth factor-A (VEGF-A) is known to be the main angiogenic regulator of tumor angiogenesis in malignant astrocytomas. However, a direct link between VEGF-A and generation of glomeruloid bodies in tumor models, in particular astrocytomas, has not been established. Angiopoietins (Ang1 and Ang2) and their endothelial-cell (EC)-specific receptor-tyrosine-kinase Tie2, have also been implicated as important regulators of both normal and pathological tumor angiogenesis. In this study we have focused on the potential role of Ang1 as a regulator of glomeruloid bodies in malignant astrocytomas. U87 human GBM cell lines with constitutive or Tet-Off regulated overexpression of Ang1 and vector controls in subcutaneous or stereotactic intracranial xenograft models in Nod-Scid mice were used. Tie2 inhibition of the tumor vasculature was undertaken with a kinase-dead dominant-negative Tie2, containing only the extracellular domain, termed ExTek. Ex-Tek was purified from a baculovirus expression system, and intratumoral injections with a screw-guided system were undertaken. At necropsy, FactorVIII staining was undertaken to visualize the tumor microvasculature and computer-assisted image guided analysis undertaken. Overexpression of Ang1 by human malignant astrocytoma cell lines in subcutaneous or intracranial xenografts reproduce many of the vascular features of human malignant astrocytomas, including glomeruloid bodies. The association of glomeruloid bodies was dependent on Tet regulated levels of Ang1 expressed and was inhibited by blocking the Tie2 receptor by ExTek. Furthermore, although Ang1 mediated astrocytoma angiogenesis and growth also requires VEGF-A, induction of glomeruloid bodies by Ang1 was independent of VEGF-A. These results suggest that the pathological piling of EC and generation of glomeruloid bodies is regulated by Ang1.

286. IMINO SUGAR-BASED GANGLIOSIDE INHIBITORS DOWNREGULATE INVASIVE BEHAVIOR IN MALIGNANT GLIOMA IN VITRO

G. Pilkington, Y. Kyriazis, Y. Orphanos, M. Christidou, V. Drewett, and D. Schelshorn; Cellular and Molecular Neuro-oncology Group, School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK

Gangliosides are acidic glyco-sphingolipids characterized by the presence of one or more sialic acid residues. In neoplastic tissues simple gangliosides are overexpressed and their role in tumor-tumor cell interactions as well as the interactions between healthy cells and tumor cells is pivotal to tumor growth, development, and metastasis/invasion. Simple gangliosides (e.g., GM3 and GD3) modulate neoplastic cell adhesion as well as production of extracellular matrices such as laminin as well as certain degradative enzymes (metalloproteinases). The function of integrins, integral components of the cell-to-cell adhesion process, is also modulated by gangliosides. A crucial step in ganglioside biosynthesis is the glucosylation of the ceramide moiety that leads to the formation of glucosylceramide, the backbone of gangliosides. This reaction is catalyzed by glucosylceramide synthase, inhibition of which by the use of the imino sugar, *N*-butyl-deoxynojirimycin (NB-DNJ), and other structurally similar compounds (such as NB-DGJ), result in reduced or ablated ganglioside synthesis. By use of a highly invasive anaplastic astrocytoma culture (IPSB-18) and a cultured (IPTP) giant cell variant glioblastoma (a tumor which, although histologically similar to classical glioblastoma, shows a limited degree of infiltrative behavior, both in vivo and in vitro), we have examined the modulatory effect of 4 imino sugars with structural similarities to NB-DGJ on ganglioside expression (immunocytochemistry, flow cytometry, and HPLC) as well as on invasive behavior in glioma by the use of time-lapse microscopy and Transwell modified Boyden chamber invasion assays. We also assessed MMP-2 and -9 and TIMP gene expression (TaqMan RT-PCR) before and after treatment. All agents inhibited ganglioside expression (in particular GD3), albeit to different extents. Moreover, a differential downregulatory effect was seen on expression of MMPs/TIMPs as well as on invasion. Results were more marked in the invasive IPSB-18 line than in IPTP. These imino sugar agents not only show that targeting of ganglioside synthesis pathways may prove of benefit in reduction of glioma invasion but also may have a potential role to play in clinical neuro-oncology. This work was supported, in part, by Oxford Glycosciences.

287. ANGIOSUPPRESSIVE THERAPY TARGETING HIF-1/VEGF FOR MALIGNANT GLIOMAS IN VITRO AND IN VIVO

S. Takano, H. Kamiyama, K. Tsuboi, and A. Matsumura; Department of Neurosurgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba City, Japan

Hypoxia inducible factor-1 (HIF-1) is an important molecular target because it is a mediator of hypoxic condition, VEGF induction, and radiation/chemo resistance. We evaluated angiosuppressive action of CPT-11, YC-1, and 2ME (2-methoxyestradiol) for ACNU resistant gliomas. In vitro, active metabolite SN38, YC-1, and 2ME were evaluated for (1) endothelial cell proliferation with WST assay, (2) vascular tube formation with angiogenesis kit (Kurabo), and (3) VEGF and hypoxia inducible factor 1a (HIF1a) expression of U87 malignant glioma cells with quantitative RT-PCR, Western blot, and ELISA. In vivo, CPT-11 low (10 mg/kg) and high (40 mg/kg) dose was evaluated for (1) U87 subcutaneous growth and survival and (2) ACNU resistant U87 subcutaneous growth with immunohistochemical evaluation of angiogenesis (VEGF expression, vessel density, MIB-1, apoptotic index). In vitro, SN38 effectively inhibited the endothelial cell proliferation and vascular tube formation at non-toxic concentrations (less than 0.01 μM). Also, SN38 decreased HIF-1a and VEGF expression of glioma cells in a dose- and time-dependent manner under hypoxic condition. Although 2ME inhibited the HIF-1 and VEGF expression, the inhibi-

tory concentration was more than 2.5 μM. In vivo, CPT-11 even at low dose inhibited malignant glioma growth, inhibiting tumor cell proliferation and enhancing tumor cell apoptosis, in addition inhibiting angiogenesis, and decreased number of tumor vessels and decreased expression of VEGF, a most important angiogenic factor of gliomas. Based on the angiosuppressive effect of CPT-11, the metronomic and angiosuppressive scheduled CPT-11 chemotherapy may be promising for malignant glioma treatment.

288. FUNCTIONAL AND PHENOTYPICAL CHARACTERIZATION OF ENDOTHELIAL CELLS DERIVED FROM GLIOMAS OF DIFFERENT GRADES

S. Miebach, *S. Grau*, O. Schnell, S. Schichor, J. Tonn, and R. Goldbrunner; Neurosurgery, Universiteitsklinikum Grosshadern, Munchen, Germany

The ability to induce neo-angiogenesis is a key feature in glioma grading. Investigations of endothelial cells (EC) from various organs revealed significant differences between the populations depending on organ and dignity of the tissue of origin. For assessment of tumor-induced angiogenesis, use of organ- and tissue-specific EC appears mandatory. EC were isolated from 22 low-grade astrocytomas (LGEC), 11 malignant gliomas (HGEC), and 7 normal brain specimens (obtained from epilepsy surgery procedures). Isolation was performed by combining density gradient separation with magnetic bead sorting. Characterization of EC was performed by measuring the expression of EC-specific cell surface antigens. On mRNA level expression of MMP 2, 3, 7, 9 and TIMP 1, 2 and 3 was measured by real-time rt-PCR. Activity of MMP 2 and 9 was assessed by zymography. Functional ability of the cells was evaluated by means of proliferation, tube formation, and co-culture. A reproducible method for isolating EC from brain tumors could be established. There was no correlation seen between antigen expression pattern and cell morphology. Expression of EC-specific antigens was strongly influenced by cell culture conditions; surface antigens like Glut-1 and wWF were expressed only in primary cultures, while CD31 and VE-Cadherin could be shown present up to passage five. Regarding proliferation, there were major differences between EC subpopulations, with HGEC showing a fourfold higher proliferation rate than LGEC and NBEC. In MMP expression, mRNA for MMP 2, 3, and 7 was found in all EC; MMP 9, however, was exclusively expressed in HGEC. None of the EC isolated could form tubes in vitro, even in presence of various stimulating factors. Due to rapid changes in EC antigen expression the use of freshly isolated cells in very low passages should be obligatory. Some first functional assessments show clear differences between NBEC, LGEC, and HGEC, pointing toward a different biological behavior among these cell populations. Thus, for investigating glioma angiogenesis the use of glioma specific EC is mandatory. The EC differences shown above may reveal a contribution of endothelial cells to the malignant phenotype of the tumor.

289. NOVEL PROBES FOR MOLECULAR MAGNETIC RESONANCE IMAGING (MMRI) OF GLIOMAS

B.J. Bedell,[1] S. Assadian,[2] S. Mzengeza,[3] R.F. Del Maestro,[4] and A.C. Evans[3]; [1]Department of Pathology, [2]Brain Tumor Research Centre, [3] Department of Neurology & Neurosurgery, [4]Brain Tumor Research Centre and Department of Neurology & Neurosurgery, McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, Canada

Numerous factors, including hypoxia-inducible factor-1a (HIF-1a), vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs), are strongly associated with tumor angiogenesis and invasion. These proteins have become a central focus of novel targeted therapies. The current methods of evaluating the expression of these targets in tissue specimens, however, do not allow for a complete spatio-temporal picture of the changes occurring in the tumor. The ability to noninvasively monitor the efficacy of therapeutic intervention on the expression/activity of these molecular targets would greatly facilitate drug development, shortening the time from the bench to clinical use. The advantages of MRI over other modalities has made it a promising candidate for the imaging of molecular targets. The emergence of molecular MRI (mMRI), however, has been lagging primarily due to the low detectability of conventional MRI contrast agents. We have overcome this limitation by developing novel probes which modulate microvascular permeability, providing a sensitivity much higher than conventional paramagnetic contrast agents. Quantitative analyses of changes in local microvascular permeability following administration of a targeted probe provide an estimation of the relative tissue content of the molecular entity of interest. These probes were investigated for their ability to detect the expression of enzymes non-invasively, in vivo in a rat C6 glioma model. C6 glioma cells were transfected with a "secreted lacZ gene," which allows for the secretion of ,-galactosidase into the tumor interstitium, as a model tumor-localized enzyme system. Spheroids of the transfected cell line were implanted into cerebral hemispheres of male Sprague-Dawley rats. Animals implanted with nontransfected spheroids served as the control group. Two weeks post-implantation, the animals were imaged using a Siemens 1.5 T whole-body MRI scanner. T1-weighted images were acquired following intravenous administration of probe, and quantitative maps of enzyme expression were generated. Following completion of imaging, enzyme expression was determined on tissue sections. Quantitative MR maps successfully demonstrated enzyme expression within the transfected tumors, but not the control tumors. The image data was correlated with expression of the enzyme on tissue sections. These studies also demonstrated the ability of these probes to detect temporal changes in the molecular mediators of angiogenesis. These novel probes promise the successful MR imaging of the expression of various molecular targets localized to gliomas in vivo and should find numerous applications, including the evaluation of novel, targeted therapeutic agents.

290. TUMOR-PRODUCED EXTRACELLULAR MATRIX INFLUENCES BRAIN TUMOR TROPISM OF HUMAN NEURAL STEM CELLS

N.O. Schmidt,[1] M. Ziu,[2] T. Cargioli,[2] K. Aboody,[3] P. Black,[2] and R. Carroll[2]; [1]Department of Neurosurgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]Neurosurgical Oncology Laboratory, Neurosurgery, Brigham & Women's Hospital, Boston, Massachusetts, USA; [3]Divisions of Hematology/HCT, City of Hope National Medical Center and Beckman Research Institute, Duarte, California, USA

A major obstacle in the treatment of gliomas is the invasive capacity of the tumor cells. Previous studies have demonstrated the capability of neural stem cells (NSCs) to target infiltrated tumor cells and to deliver therapeutic gene products. However, the signals involved in the brain tumor tropism of NSCs and their interactions within the tumor environment are hardly defined. As gliomas progress and invade, an extensive modulation of the extracellular matrix (ECM) occurs. Tumor-ECM derived from 6 glioblastoma cell lines and purified ECM compounds known to be upregulated in the glioma environment were analyzed for their effects on NSC motility in vitro. We found that tumor-produced ECM was highly permissive for NSC migration. Laminin was the most permissive substrate for human NSCs migration, and tenascin-C the strongest inducer of a directed human NSC migration (haptotaxis). A positive correlation between the degree of adhesion and migration of NSCs on different ECM compounds exists, as for glioma cells. Our data indicate that the ECM of malignant gliomas is a significant modulator of NSC migration. ECM proteins preferentially expressed in areas of glioma cell invasion may serve as additional local guidance signal for NSC tropism to disseminated tumor cells.

291. INHIBITION OF HIF-1A BY SHORT HAIRPIN RNA TECHNOLOGY INHIBITS BOTH IN VITRO AND IN VIVO HUMAN GLIOMA CELL GROWTH

R. Jensen and D. Gillespie; Neurosurgery, Huntsman Cancer Institute, Salt Lake City, Utah, USA

Hypoxia-inducible factor-1a (HIF-1a) is the major regulator of vascular endothelial growth factor (VEGF). VEGF is thought to be the principal mediator of peritumoral edema and angiogenesis in malignant gliomas. Interruption of VEGF secretion could result in growth inhibition of these tumors. We examine the role of inhibition of HIF-1a using short hairpin RNA (shRNA) techniques in the growth of human gliomas. Malignant glioma cell lines were stably transfected with vectors expressing either shRNA directed against the HIF-1 gene or with control plasmids containing nonsense shRNAs. HIF-1 and VEGF expression was examined by immunohistochemistry, Western blot, and RT-PCR. In vitro and in vivo growth studies were carried out on these cells. Measures of proliferation, angiogenesis, tissue perfusion, and hypoxia were performed on cells in culture as well as on xenograft tumors. HIF-1a siRNA transfected cells demonstrate inhibition of both HIF-1a expression and VEGF secretion. In vitro growth is slightly decreased by shRNA directed toward HIF-1a, while vivo growth is significantly decreased in these cells compared to control cells. Interestingly, the plasmid expressing the anti-HIF-1 shRNA is still present in the xenograft cell after 75 days of implantation in the mouse. Cell proliferation and angiogenesis are decreased in the tumors containing the shRNA inhibiting HIF-1. Preliminary results of intratumoral and intravenous use of anti HIF-1 siRNAs in established xenograft human gliomas is discussed. Inhibition of HIF-1a results in inhibition of VEGF secretion, glioma tumor growth and angiogenesis. Further studies are necessary, but this study suggests potential clinical applications of targeting HIF-1a mediated growth for inhibition of glioma growth and angiogenesis.

292. TWIST: A NOVEL CANDIDATE OF CELL INVASION REGULATOR IN MALIGNANT GLIOMA

M. Noha[1], M. Nakane[1], M. Hirata[1], H. Nakayama[1], D. Yoshida[2], and A. Teramoto[2]; [1]Department of Neurosurgery, Teikyo University School of Medicine, Kawasaki; [2]Department of Neurosurgery, Nippon Medical School, Tokyo; Japan

Malignant glioma is hallmarked as one of the most invasive tumors in the human. According to recent literature, 18 months would be the best long-term median survival. Twist, which has been noted to play an essential role on carcinoma metastasis, expresses in malignant glioma cell lines also. However, that function is unclear. We hypothesized and investigated an interaction between TWIST and the invasiveness of malignant glioma. Malignant glioma cell lines U87MG and U251MG, which have already confirmed TWIST expression, were employed to evaluate the inhibitory effect on cell invasion after silence of the TWIST gene using specific siRNA, with zymogram and chemotaxis assay. In the zymogram assay, MMP-2 and MMP-9 were suppressed after gene silence. Furthermore, under the same conditions, the numbers of invading cells through Matrigel were significantly reduced, as compared with control cells using non-sense siRNA. In several recent reports, TWIST is highlighted as associated with metastasis of breast cancer and other malignant tumors. We reveal here that TWIST could associate with the cell invasion in malignant glioma through MMP-2 and MMP-9 expression, which is reported for the first time. This might pioneer a new horizon on the invasiveness of malignant glioma.

293. DOWNREGULATION OF ADAMTS-8 (METH-2) IN GLIOMAS AND OTHER BRAIN TUMORS

J. Dunn,[1] J. Reed,[1] D. du Plessis,[2] E. Shaw,[1] P. Reeves,[2] A. Gee,[1] P. Warnke,[2] and C. Walker[1]; [1]JK Douglas Laboratory, Clatterbridge Cancer Research Trust, Bebington; [2]Department of Neurological Science, University of Liverpool, Liverpool; UK

ADAMTS-8 (Meth-2) is a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family of proteases comprising 19 genes. ADAMTS-8 is a member of the family subgroup of aggrecanases (ADAMTS-1, -4, -5, -8 and -15) that are able to cleave the matrix proteoglycan aggrecan. Furthermore, ADAMTS-1 and ADAMTS-8 also have potent anti-angiogenic properties. ADAMTS-8 expression has been shown to be downregulated in breast carcinoma and NSCLC. In lung tumors this gene may be silenced by promoter hypermethylation. ADAMTS-8 has not yet been studied in brain tumors. Therefore, the aim of our study was to determine the importance of ADAMTS-8 in the pathogenesis of gliomas and other brain tumors. Expression of ADAMTS-8 in normal whole brain, cerebral cortex, frontal lobe, cerebellum, meninges and lung, 38 brain tumors (22 gliomas, 4 meningiomas, 7 metastastic carcinomas, 1 hemangioblastoma and 1 medulloblastoma) and 4 glioma cell lines (U373, Hs683, T98G and U87MG) was studied by using quantitative RT-PCR. The expression of the anti-angiogenic TSP-1 and the angiogenic VEGF was also investigated in a subset of 28 of the original 38 tumors. Promoter hypermethylation analysis of ADAMTS-8 by Methylation Specific-PCR was undertaken in 31 brain tumors, two normal brain samples, and the aforementioned four glioma cell lines. ADAMTS-8 was highly expressed at similar levels in normal whole brain, cerebral cortex, frontal lobe, cerebellum, meninges, and lung. Furthermore, analysis of ADAMTS-8 in the brain tumors and glioma cell lines indicated a decrease in mRNA levels of at least twofold in comparison to normal whole brain in all tumors and cell lines, and no detectable ADAMTS-8 transcript in 18/38 tumors. In contrast to ADAMTS-8, expression of TSP-1 was upregulated in the majority of tumors (54%), while VEGF was upregulated in less than half (43%). There was no correlation between the expression patterns of these genes. One metastatic carcinoma and three cell lines (U373, T98G and U87MG) showed hypermethylation of the ADAMTS-8 promoter region. These data suggest that downregulation of ADAMTS-8 may be important in the pathogenesis of a range of brain tumor types, whereas the regulation of expression of TSP-1 and VEGF may be more variable. Our results also suggest that an alternative, and as yet unknown, mechanism of silencing of ADAMTS-8 occurs in the majority of the brain tumors tested.

294. INTER ALPHA TRYPSIN INHIBITOR HEAVY CHAIN 2: A NOVEL INHIBITOR OF GLIOMA CELL INVASION IN THREE-DIMENSIONS

T.E. Werbowetski, N.Y.R. Agar, and R.F. Del Maestro; Neurology and Neurosurgery, Montreal Neurological Institute, Montreal, Canada

The highly infiltrative nature of malignant glioma makes surgical removal difficult. There are many positive regulators of malignant brain tumor growth and motility; however, little is known about inhibitory modulators of malignant glial cell invasion. Previous studies in our laboratory suggest the presence of repellents and/or inhibitors in the serum-containing conditioned medium from glioma cell lines (Werbowetski et al., J. Neurobiol. 60, 71, 2004). We have, therefore, developed and optimized a functional screening assay using protein purification to isolate potential inhibitors or repellents of glioma tumor invasion in three-dimensional collagen gels from both endogenous and serum-derived sources. Serum-containing conditioned medium from C6 rat astrocytoma spheroids from spinner culture was concentrated and applied to a Resource Q anion exchange column on an AKTA FPLC. Fractions were collected and applied to C6 spheroids implanted in a collagen matrix, and those that inhibited invasion were pooled and applied to a heparin sepharose affinity column. Fractions were again collected and subjected to the spheroid functional screening assay, and the most inhibitory fractions were sent for mass spectrometry. Mass spectrometry analysis of inhibitory fractions identified inter alpha trypsin inhibitor heavy chain-2 (ITI-H2). This protein is produced by the liver, secreted into serum, and acts alone or bound to the light-chain bikunin to stabilize the extracellular matrix and/or inhibit serine protease activity in the cumulus oocyte complex and in a variety of tumor cells in hyaluronic acid–rich environments to negatively regulate cell motility. An antibody raised against ITI-H2 and tested using Western blot analysis suggests that ITI-H2 is present as both a single protein and a bikunin-bound form in the inhibitory fractions. Stable cell lines of ITI-heavy chain and bikunin overexpressing glioma and HEK-293 cell lines are currently being tested to further validate our model both in vitro and in vivo. Our studies identify a role for inter alpha trypsin inhibitor in malignant glial cell invasion and warrant further study of serum proteins as readily available sources of invasion inhibitors. The identification of serum-derived inhibitors of invasion may have potential therapeutic value in a variety of infiltrative and metastatic tumors in addition to malignant glioma.

295. BACITRACIN INHIBITS GLIOMA CELL MIGRATION AND INVASION IN VITRO

D. Goplen,[1] J. Wang,[1] B.B. Tysnes,[1] A.J.A. Terzis,[4] O.D. Laerum,[2] and R. Bjerkvig[1,3]; [1]Department of Biomedicine, University of Bergen, Bergen, Norway; [2]The Gade Institute, University of Bergen, Bergen, Norway; [3]NorLux Neuro-Oncology, Bergen, Norway; NorLux Neuro-Oncology, Centre de Recherche Public Santé, Luxembourg

By serial transplantation of human glioblastoma biopsies in nude rats, two different tumor phenotypes are obtained, one that is highly invasive and one that is predominantly angiogenic. Using a global proteomics approach on the phenotypes, we identified a number of unique proteins that were differentially expressed in the invasive phenotype. The proteins were identified by peptide mass fingerprinting and bioinformatics and verified by Western blots and on immunostained biopsy specimens. Protein disulfide isomerase (PDI) A6 precursor was identified as one of the overexpressed proteins in the invasive phenotype. PDI is a chaperone protein that mediates integrin-dependent cell adhesion. It is present both in the cytosol and at the cell surface. Immunofluorescent staining of glioma spheroids *in vitro* showed that migrating cells expressed PDI. Since cell migration is a part of the tumor invasion process and PDI was stronger expressed in invasive glioma phenotype, we tested the effect of the PDI inhibitor bacitracin on migration of glioma cells in different invasion assays. Both tumor spheroids derived from human glioblastoma xenografts in the nude rat brain as well as cell line spheroids were used. The human glioma cell lines (U373, U251, D54, GaMg, and U87) expressed PDI. Bacitracin at concentrations as low as 5 mM inhibited tumor cell migration and invasion. The anti-invasive effect of bacitracin was reversible after withdrawal of the inhibitor, indicating a specific, non toxic effect. In conclusion, using a global proteomics approach, PDI was identified to play an important role in glioma cell invasion, and its action was effectively inhibited by bacitracin.

296. SLIT2-ROUNDABOUT 1(ROBO1) SIGNALING INHIBITS MEDULLOBLASTOMA BUT NOT GLIOMA CELL INVASION IN THREE-DIMENSIONS

T.E. Werbowetski,[1] M. Seyed Sadr,[1] N. Jabado,[3] A. Angers-Loustau,[1] R. Bjerkvig,[4] J. Antel,[2] D. Faury,[3] Y. Rao,[5] and R.F. Del Maestro[1]; [1]Neurology and Neurosurgery and [2]Neuroimmunology Unit, Montreal Neurological Institute, Montreal, Canada; [3]Department of Pediatrics, Montreal Children's Hospital Research Institute, Montreal, Canada; [4]Department of Anatomy and Cell Biology, University of Bergen, Bergen, Norway; [5]Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

Chemotropic cues such as the Slit, Netrin and Semaphorin families guide the migration of neuronal and glial cell precursors during neural development. Recently, Slit and its receptor Roundabout (Robo) have been implicated in tumor angiogenesis and leukocyte migration. It is not known if these molecules contribute to directing the invasion of brain tissue by

medulloblastoma and glioma cells. The biology of brain neoplasms such as medulloblastoma and malignant glioma has provided a link between tumorigenesis and neurodevelopment. Recent studies placing brain tumors in the context of neurodevelopment have led to the recognition of new tumor suppressors and oncogenes involved in tumor progression. The current study focuses on the expression and functional significance of Slit2 and Robo1 in medulloblastoma and glioma tumors. Invasion assays were carried by out using time-delayed co-culture, sodium alginate bead microencapsulation, time-lapse videomicroscopy, and confrontation co-culture with astrocyte aggregates in collagen type I matrices. RT-PCR was used to examine Slit2 and Robo1 expression levels in glioma and medulloblastoma cell lines and primary human tumors. Here, we provide evidence that medulloblastoma cells are inhibited, but not repelled, by a localized concentration of Slit2 in collagen gels as demonstrated by time-delayed coculture, sodium alginate bead microencapsulation, and confrontation coculture with astrocyte aggregates. Slit2 had no significant effect on glioma cell invasion in all models tested. Medulloblastoma and glioma cell lines express Slit2 and its receptor Robo1, and both functional blocking antibody and dominant negative Robo experiments demonstrate a 50% rescue of the invasive phenotype compared with Slit treatment alone. In addition, Slit2 had an inhibitory effect on medulloblastoma cell proliferation, but did not affect glioma cell doubling time. Studies are currently underway to assess the effect of RNAi knockdown of Robo1 in medulloblastoma cell lines with the goal of rescuing the inhibitory effect of Slit2. The signal transduction machinery downstream of Slit-Robo is also currently being dissected in medulloblastoma and glioma cell lines. Our findings indicate that the effect of Slit2 is not conserved for all types of brain tumors, and that manipulation of Slit-Robo signaling may serve as a potential treatment for medulloblastoma tumors.

297. EGFR-MEDIATED ACTIVATION OF PHOSPHOLIPASE C GAMMA IS ASSOCIATED WITH INCREASED GLIOBLASTOMA MOTILITY/INVASION

J. Uhm,[1] K. Aldape,[2] A. Yates,[3] J. Goble,[1] A. Nilson,[1] L. Frederick,[1] and C.D. James[1]; [1]Neurology, Mayo Clinic Rochester, Rochester, Minnesota; [2]M.D. Anderson Cancer Center, Houston, Texas; USA

EGFR alterations represent one of the most frequent gene alterations in glioblastoma. We have previously shown that EGFR activation in vitro stimulates glioma motility/invasion by way of increased PLC-gamma activation. Conversely, inhibition of PLC gamma by pharmacologic means (U73122) or by small interfering RNA (siRNA) abrogrates EGFR-induced motility. We further evaluated the relationship between EGFR and PLC phosphorylation in vivo at two levels. First, in both orthotopic (intracranial) and heterotopic (subcutaneous flank) xenografts, EGFR-amplified xenograft tumors demonstrated PLC-gamma activation, in contrast to tumors not amplified for EGFR. We also evaluated 89 human glioblastoma specimens and found a very significant correlation between EGFR amplification and PLC gamma phosphorylation: 16/31 EGFR amplified tumors versus 6/58 EGFR nonamplified tumors ($P < 0.0001$). Of the EGFR amplified tumors, those with amplification of EGFRvIII (deleted for codons 6–273) showed a higher association with PLC gamma phosphorylation (8/10) than tumors with wild-type EGFR amplification (8/21). Taken together, our results support that a relationship exists between high-level, amplification-associated expression of EGF receptor and PLC gamma activation and that PLC-gamma activity may be an important mediator of glioblastoma cell invasion. Hence, EGFR (especially the vIII form) as well as PLC gamma may represent viable targets for anti-invasive therapeutics.

298. REGULATION OF GLIOMA INVASION BY A PAIR OF INTERACTING AND FUNCTIONALLY OPPOSING PROTEINS IGFBP2 AND IIP45

W. Zhang, G. Fuller, S. Song, and G. Wang; Pathology, M.D. Anderson Cancer Center, Houston, Texas, USA

Cancer cell invasion, often a first step in cancer metastasis, is a major factor contributing to the morbidity and mortality of cancer. In diffuse gliomas, the high mortality rate is primarily a result of local invasion. Thus, a detailed understanding of how tumor invasion is regulated is critical. Using genomic and proteomic approaches, we discovered that insulin-like growth factor binding protein 2 (IGFBP2) is overexpressed in 80% of glioblastoma. Cell biology studies and animal model experiments showed that IGFBP2 enhances glioma invasion through activation of integrin signal transduction pathways. Using yeast two-hybrid system, we discovered a new protein that binds to IGFBP2. Our in vitro assays and xenograft mouse model experiments functionally characterized this novel gene as an inhibitor of glioma invasion (Song et al., Proc. Natl. Acad. Sci. USA 100, 13970, 2003). We named this gene *IIp45*, which stands for Invasion Inhibitory protein with a molecular mass of 45 kDa. In recent studies, we found that the *IIp45* gene is alternatively spliced in a tumor-specific manner. The alternatively spliced transcript produces an isoform, IIp45S, which has a distinct C-terminal region due to a frame-shift. The tumor specific isoform IIp45S is unstable and is rapidly degraded by the uniquitin-proteasome degradation pathway. Thus, we have revealed two interacting and functionally opposing proteins, IGFBP2 and IIp45, which play an important role in regulation of invasion. Elevation of IGFBP2 and inactivation of IIp45 are two key events that contribute to the highly invasive phenotype of glioblastoma.

299. CELL INVASION OF HUMAN PITUITARY ADENOMA CELL LINE, HP-75 IN HYPOXIA

D. Yoshida, K. Kim, M. Noha, and A. Teramoto; Neurosurgery, Nippon Medical School, Tokyo, Japan

Pituitary adenoma tissue is known as hypovascular, and concomitantly the partial oxygen pressure is lower than the surrounding normal organs likely in the other cancer tissues. In this study, we investigated whether hypoxia influences the cell invasiveness of the human nonfunctioning pituitary adenoma cell line, HP-75. HP-75cells were exposed to hypoxia (1% for 24 h). The subsequent mRNA expression of genes was examined by cDNA microarray. The results were verified by real-time RT-PCR. Gelatin zymogram and reverse zymogram were employed to determine enzyme activities of MMP and TIMP. Cell motility, chemotaxis, and haptoinvasion were studied with Boiden chamber. Cell adhesion assay and cell-to-cell adhesion were further studied. Cyclic DNA microarray and real-time RT-PCR indicated that laminin β-2 chain mRNA was specifically upregulated by hypoxia (4.16-fold), but not in the other genes relating to cell motility and invasion involving extracellular matrix, cell adhesion molecules, and MMP families. Immunofluorescent study also demonstrated the increased expression of laminin β-2 chain at the protein level followed by hypoxic induction. Gelatin zymogram and reverse zymogram, showing MMP and TIMP activities, were not particularly changed. Haptoinvasion and chamotaxis or cell adhesion to collagen type IV were elevated (12.8-, 6.8-, or 8.8-fold). Meanwhile, cell adhesion and chemotaxis to laminin, collagen type I, and fibronectin were not modulated by hypoxia. Cell motility was not amended by hypoxia. Cell-to-cell adhesion was significantly elevated (9.6-fold). These results highly suggest that hypoxia induces elevated cell invasion and cell to cell mediated by elevated transcription of laminin β-2 molecule that is specifically bound to collagen type IV.

300. HEMANGIOGENIC PHENOTYPES AS SURROGATE BIOMARKERS IN BRAIN TUMOR TREATMENT

P. Gutin,[1] D. Jin,[2] P. Lau,[2] L. Vincent,[2] K. Shido,[2] P. Salerno,[1] and S. Rafii[2]; [1]Neurosurgery Service, Memorial Sloan-Kettering Cancer Center; [2]Department of Genetic Medicine, Weill Medical College of Cornell University, New York, New York; USA

No biomarkers are currently available to indicate the angiogenic propensity of brain tumors nor to evaluate their response to therapy. Nevertheless, many different antiangiogenic drugs are currently being evaluated in clinical trials. Here we describe a novel in vitro functional assay, HUVEC-based angiogenic scale (HBAS), to assess the overall angiogenic activity in the plasma of patients with glioma and meningioma. Analysis of a cohort of 50 consecutive patients with these tumors showed that the majority had higher HBAS at baseline prior to surgical resections than age-matched normal subjects. The plasma HBAS also correlated with increased plasma and cellular vascular endothelial growth factor-A (VEGF-A) levels by ELISA. The cell lysate fraction from platelets was found to have the highest level of pro-angiogenic activity. In addition, comparison of hemangiogenic cellular markers indicated a switch toward increased circulating $CD133^{+}VEGFR2^{+}$ endothelial progenitor cells (by flow cytometry) and circulating hematopoietic stem/progenitor cells (by methylcellulose colony forming assays) in subsets of patients with active glioma or meningioma. This "hemangiogenic switch" also correlated with an increased plasma level of stromal-derived factor-1 alpha (SDF-1a), suggesting that mobilization of these hemangiogenic progenitors from the bone marrow to the brain tumor neo-angiogenic niche may be at least partially mediated by the chemokinetic SDF-1a/CXCR4 pathway. Work is underway to study the correlation between the hemangiogenic phenotypes and the clinical outcome of these patients. Taken together, collective assessment of hemangiogenic biomarkers is a promising tool to provide valid end points for future clinical trials for patients with glioma and meningioma.

301. VEGF DOES NOT PROTECT ENDOTHELIAL CELLS, OR GBM-CELLS, AGAINST IRRADIATION

M. Donker,[1] W. van Furth,[2] K. Hovinga,[2] C. van Bree,[1] D. Bosch,[2] and L. Stalpers[1]; [1]Radiation Therapy; [2]Neurosurgery, Academic Medical Centre, Amsterdam, The Netherlands

In a number of recent publications, VEGF has been implicated not only as a stimulator of angiogenesis, but also as a survival factor for both endothelial cells and GBM cells after irradiation. GBM is a highly resistant tumor with a high VEGF secretion. We hypothesize that VEGF protects endothelial cells and GBM cells against damage of ionizing radiation. Four different types of endothelial cells were used, commercially available HUVECs (from Clonetics), a HUVEC cell line (EC-RF24), primary HUVECs and bovine retina endothelial cells (BRC), and two GBM cell lines (Gli-06 and U87). Cells were irradiated with 0, 2, or 5 Gy under low-serum conditions with three different concentrations of VEGF added to the medium. Cell survival after single dose irradiation was measured by the XTT proliferation assay after 96 h. In addition, four human GBM cell lines (U87, Gli-06, U251, and U251-NG2) were irradiated with various single dose fractions between 0 Gy and 20 Gy. The VEGF concentration in the medium was measured by ELISA at 0, 24, 48 and 72 h after gamma-irradiation. All cells tested showed already inhibited cell proliferation after single dose irradiation of 2 Gy. Although VEGF stimulated endothelial cell proliferation in a dose dependent manner, the cell-killing effect of irradiation was not affected. The variation in radiosensitivity of endothelial cells was smaller than that of GBM-cells, with U87 being the most radioresistant. Interestingly, in all GBM cell lines we found a dose-dependent increase in the VEGF-secretion after ionizing radiation, with U87 having a 8-times higher VEGF secretion than the other tested cell lines, with a twofold increase in VEGF secretion 72 h after a single dose of 20 Gy. We could not demonstrate a protective effect of VEGF on cell death after ionizing irradiation of endothelial cells, nor GBM cells. We used four different types of endothelial cells to model brain tumor endothelial cells. The endothelial cells showed proliferative effects of VEGF, suggesting an active VEGF-R1 system. The discrepancy with the literature will be discussed. We conclude that VEGF is not a survival factor for endothelial or GBM cells.

302. ANTITUMOR EFFECTS OF A COMBINATION THERAPY CONSISTING OF IRRADIATION AND COX-II INHIBITION IN AN ORTHOTOPIC, SYNGENEIC MURINE GLIOMA MODEL

M. Wagemakers,[1] G. van der Wal,[2] J. Mooij,[1] and G. Molema[2]; [1]Neurosurgery, Groningen University Hospital, Groningen; [2]Pathology and Laboratory Medicine, Medical Biology section, Groningen University Institute for Drug Exploration and University Hospital Groningen, Groningen; The Netherlands

COX-II inhibitors and irradiation have been demonstrated to act synergistically in rodent tumor models, among others in a subcutaneous glioma model. Controversy exists regarding the molecular basis for this phenomenon and whether the tumor cells, tumor vasculature, host immune cells, or a combination of these is primarily affected. We initiated a study to investigate the presence of synergistic effects of COX-II inhibition and ionizing radiation treatment of intracranial glioma, and to analyze the possible role of tumor endothelial cells in this process. Syngeneic, COX-II negative, GL261 tumor cells were injected intracranially into C57bl6 mice. Administration of a COX-II inhibitor or vehicle control was started 15 days after tumor cell inoculation. Local fractionated irradiation (5×3 Gy) or sham procedures were performed starting at day 22. Animals were terminated 4 weeks after inoculation of tumor cells. Tumor volume was determined, and cryostat cut sections were immunohistochemically stained for endothelial cells activation markers, and leucocytes. Tumor and brain tissues were furthermore prepared for quantitative RT-PCR analysis regarding mRNA expression levels of VEGF, VEGF receptor, angiopoietin-1 and -2, Tie-2, and av integrin. Preliminary results show a trend toward a radiosensitizing effect of COX-II inhibitor treatment. COX-II inhibition alone was devoid of an antitumor effect. All tumors intensively expressed ICAM-I. Irradiation affected leucocyte infiltration into the tumors. Analysis of the mRNA expression levels of the above-mentioned genes is ongoing. There is a trend toward synergy between COX-II inhibition and irradiation in the treatment of intracranial glioma in this model. Experiments are ongoing to further validate these first observations of a radiosensitizing effect of COX-II inhibitors, and to analyze the molecular basis of this effect in in vivo and in vitro studies.

303. SYNERGISTIC EFFECTS OF THE VEGF-R TYROSINE KINASE INHIBITOR ZD6474 AND RADIOTHERAPY ON TUMOR GROWTH IN THE INTRACEREBRAL BT4C RAT GLIOMA MODEL

M. Sandström,[1] P. Bergström,[1] M. Johansson,[1] and R. Henriksson[1,2]; [1]University Hospital Umeå, Umeå; [2]AstraZeneca AB; Sweden

Malignant glioma is characterized by extensive pathological neovascularization. Vascular endothelial growth factor (VEGF) is commonly believed to be the key positive regulator of glioma angiogenesis. ZD6474 is a potent, orally active, low-molecular-weight inhibitor of VEGF receptor tyrosine kinase activity with additional inhibitory effects on the epidermal growth factor (EGF) receptor tyrosine kinase. ZD6474 has previously been reported to inhibit tumor growth and neovascularization in a broad panel of tumor xenografts. In a previous study we show that ZD6474 significantly inhibits tumor growth in an orthotopic intracerebral glioma model. In the present study we have investigated if ZD6474 in combination with radiotherapy has any synergistic effects on tumor growth in an intracerebral rat glioma model. The effects of ZD6474 and radiotherapy upon tumor growth were investigated in the intracerebral BT4C rat glioma model. Animals were randomized into four groups, with 7 animals in each group. One group was untreated. A second group received ZD6474 30 mg/kg daily as an oral gavage for 14 days, starting day 6 after tumor implantation. A third group received 12 Gy single fraction radiotherapy at day 6. Finally, the fourth group was treated with ZD6474 30 mg/kg for 14 days and radiotherapy 12 Gy at day 6, in combination. Animals were sacrificed on day 20 and tumor size was measured. ZD6474 30 mg/kg in combination with radiotherapy significantly decreased tumor area from 16 mm^2 (range, 4–28) to 6 mm^2 (range, 2–10) ($P < 0.05$) when compared to untreated controls. The orally available VEGFR2 receptor tyrosine kinase inhibitor ZD6474 inhibits tumor growth in an intracerebral rat glioma model. Combination with radiotherapy results in more than additive effects. These results reported justify further experimental investigations on the effects of ZD6474 in malignant glioma.

304. ASSESSING U251 CLONAL POPULATIONS AT THE CELLULAR AND MOLECULAR LEVELS

D. Maret and R.F. Del Maestro; Neurology and Neurosurgery, Montreal Neurological Institute, Montreal, Canada

Malignant gliomas are highly invasive tumors that exhibit heterogeneity of morphology and behavior. We have isolated clonal populations of the U251 cell line and analyzed these clones at the cellular and molecular levels. Clonal populations of wild-type U251 were isolated and expanded. Spheroids composed of individual clones were implanted into a type I collagen gel to assess invasion in three dimensions. Time-lapse microscopy was used to monitor the directional invasion and mitosis of individual cells. The mitotic activity observed was compared with Ki-67 staining and doubling times of monolayer cultures. Clonal populations exhibiting differences in invasion are being studied at the molecular level, by differential protein expression profiling. We have determined that the U251 clones fall into three categories of invasiveness: hypo-, hyper-, and intermediate invasion. We found that the U251 clones do not defer cell proliferation for invasion in a type I collagen matrix. Individual cells were found to invade a clonal-dependent distance regardless of the number of divisions taking place. This is contrary to the "Go or Grow" hypothesis, which proposes that cell division and cell migration are temporally exclusive events and that tumor cells defer cell division to migrate. Plasma membrane proteins have been isolated from hyper- and hypo- invasive clones, and the enrichment and purity have been assessed. Multiple clonal populations are present in glioma cell lines harboring distinct invasive and mitotic characteristics. Invasion studies carried out by using glioma cell lines are the combined results of heterogeneous populations and do not accurately reflect the individual clonal populations present. Our differential protein expression analysis will identify key proteins involved in invasion, which have the potential to serve as therapeutic targets.

305. MAP2K3 AND P38 ARE DRIVERS OF IN VITRO GLIOMA INVASION

T. Demuth,[1] J. Rennert,[1] D. Hoelzinger,[1] R. Reavie,[1] L. Mariani,[2] and M. Berens[1]; [1]Brain Tumor Unit, Neurogenomics Department, TGen, Phoenix, Arizona, USA; [2]Department of Neurosurgery, Inselspital, Bern, Switzerland

The early and pervasive tendency of glioma cells to invade into peritumoral normal brain underlies the poor prognosis for patients with glial tumors. This malignant dispersion of glioma prevents complete surgical resection, positions tumor cells behind an intact blood-brain barrier, and sequesters tumor cells outside the fields of focal radiation; each of these

leads to heightened (almost certain) tumor recurrence. A three-dimensional spheroid invasion assay was employed to determine the transcriptome of invasive glioma cells (U87WT and U87*f*EGFR) compared to their non-invading counterparts from the spheroid center. Cells from invasive rim and spheroid core were collected by laser capture microdissection as three biological replicates representing each cell line; mRNA was isolated and underwent oligonucleotide microarray analysis. Mitogen-activated protein kinase kinase 3 (MAP2K3), a member of the MAP-kinase family, was identified to be significantly upregulated in invasive cells. MAP2K3 is involved in stress signaling, tumor cell invasion, and apoptosis resistance; MAP2K3 activates p38 by phosphorylation. Immunofluoresence on sections from paraffin-embedded spheroids in the 3D invasion assay revealed increased levels of MAP2K3 and phosphorylated p38 in invasive cells. Inhibition of these genes by siRNA and small molecules decreased invasiveness of spheroids in vitro while sensitizing them to apoptosis induction, confirming significance of this pathway for glioma invasion. Glioma invasion tissue microarray staining revealed strong intensity for MAP2K3 and phosphorylated p38 in 100% of invasive glioma cells, while cells from the core exhibited weak or no staining; noncancerous brain revealed no staining for MAP2K3. This data suggests that MAP2K3 and p38 are potential targets for anti-invasive therapies in combination with cytotoxic agents.

306. MICROARRAY-BASED COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS OF ASTROCYTIC TUMORS OCCURRING IN A LI-FRAUMENI-LIKE SYNDROME FAMILY

R.M. Reis,[1] B. Carvalho,[2] J. Amorim,[3] S.A. Ribeiro,[1] F. Pardal,[4] R. Almeida,[5] and B. Ylstra[6]; [1]Life and Health Sciences Research Institute (ICVS), Health Sciences School, University of Minho, Braga, Portugal; [2]Department of Pathology, Free University Medical Center, Amsterdam, The Netherlands; Departments of [3]Internal Medicine, [4]Pathology, and [5]Neurosurgery, Hospital S. Marcos, Braga, Portugal; [6]Microarray Core Facility, Free University Medical Center, Amsterdam, The Netherlands

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant disease. It is characterized by a familiar clustering of a wide range of cancers, predominantly breast cancer, sarcomas, brain tumors, and adrenal cortex cancer, diagnosed before the age of 45 years. Two distinct forms of LFS can be recognized, a classic LFS and a Li-Fraumeni-like syndrome (LFL). In the majority of the LFS families, the underlying genetic defect is a germ line mutation in *TP53* gene. Here, we describe a LFL family characterized by the presence of brain tumors in two siblings, a 26-year-old man with three metachronous astrocytic tumors (two diffuse astrocytomas and one glioblastoma, which occurred within a 4-year interval) and his 30-year-old sister with one diffuse astrocytoma. The advent of microarray-based comparative genomic hybridization (array-CGH) technology allows the analysis of whole chromosomal aberrations in a single experiment with high spatial resolution. The aim of this study was to assess the presence of *TP53* germ line mutations in this LFL family and to relate it with the chromosomal aberrations of the astrocytic tumors. *TP53* germ line mutation analysis of exons 5–8 was performed by PCR followed by direct sequencing of DNA isolated from blood of affected siblings. From the formalin-fixed, paraffin-embedded histological sections of the four astrocytic tumors, DNA was isolated and used for microarray-CGH analysis. The array-CGH consisted of about 5000 BAC clones with an average resolution of 1 Mb. Identical germ line *TP53* exon 8, G871A, Arg290His mutation was identified in both siblings. The microarray-CGH analysis of the three metachronous tumors of the male has as follows: The first diffuse astrocytoma did not show clear chromosomal alterations; the second diffuse astrocytoma showed some chromosomal alterations, including losses on chromosome 6q, 10q, and 13 and gain on 5q region; the glioblastoma showed the aberrations detected in the previous astrocytoma and contained additional abnormalities, including losses on 2p, 3p, 5p, 10q, 11p, 13p, 14q, 16q, and 22, as well as gain on 5p and 10p. The microarray-CGH analysis of the diffuse astrocytoma of the female showed no clear chromosomal aberrations. This study reports a *TP53* germ line mutation in an LFL family associated with brain tumors in young adults. Microarray-CGH analysis of tumors shows the presence of chromosomal alterations, such as loss on 2p, 3p, and 16q and gain on 5q regions that are not usually present in sporadic astrocytic tumors.

307. CHANGES IN PROTEIN EXPRESSION FOLLOWING RADIOTHERAPY IN EXPERIMENTAL MALIGNANT GLIOMA

C. Wibom,[1] F. Pettersson,[2] M. Johansson,[1] R. Henriksson,[1] and A.T. Bergenheim[3]; [1]Oncology; [2]Organic Chemistry; [3]Neurosurgery, University Hospital Umeå, Umeå, Sweden

The outcome of modern treatment for glioblastoma (GBM) has so far been disappointing. In order to develop new GBM treatment modalities, as well as to improve present ones, a more detailed understanding of the biological effects of different treatments must be acquired, together with new tools for a more rapid assessment of these effects. Today, radiotherapy is one of the mainstays of GBM treatment. This study aims to characterize differences in protein expression patterns in brain tumor tissue following radiotherapy in an experimental rat glioma model. BT4C-cells were stereotactically implanted into the right nucleus caudatus of 24 BD IX-rats. One group received radiotherapy delivered as a 12-Gy single fraction on day 12 after tumor implantation. On day 1, 5, 7, and 12 after irradiation, three animals from each group were sacrificed, and tumor tissue from each animal was analyzed with regard to protein expression using surface-enhanced laser desorption/ionization–time of flight–mass spectrometry (SELDI-TOF-MS). Mass spectrometric data was analyzed with principle components analysis (PCA) to detect differences between the groups as well as possible temporal changes. Using PCA, regions of interest within mass spectrograms of 2.5–50 kDa were identified and further characterized through comparisons of mean peak intensities between the groups. Univariate F-test statistics revealed several peaks whose intensity significantly changed after radiotherapy. The prompt changes in the protein expression are a novel observation and might be of value to understand biological events following irradiation. The SELDI-TOF-MS technique in conjunction with PCA seems to be a well suited tool to study these changes and can detect specific proteins displaying differentiated expression levels. In a further perspective these findings may prove to be useful in the development of new GBM treatment schedules.

308. MGMT METHYLATION STATUS AND EXPRESSION LEVEL DO NOT CORRELATE WITH SENSITIVITY TO CCNU IN SHORT-TERM CULTURES DERIVED FROM MALIGNANT ASTROCYTOMA

T. Warr,[1] R. Poh,[1] B. Suarez-Merino,[1] S. Ward,[1] P. Warren,[3] J. Darling,[3] and D. Thomas[2]; [1]Department of Molecular Neuroscience and [2]Division of Neurosurgery, Institute of Neurology, University College London, London; [3]University of Wolverhampton, Research Institute of Healthcare Science, Wolverhampton; UK

Adjuvant chemotherapy using DNA-damaging agents has largely failed to make a significant impact on the outcome of patients with malignant astrocytoma. One of the primary mechanisms of resistance to nitrosureas such as CCNU is mediated through O^6-methylguanine-DNA methyltransferase (MGMT). This DNA repair enzyme removes the cytotoxic alkyl adducts from O^6-guanine, and hence the level of MGMT activity in tumor cells is related to their sensitivity to nitroureas. It has been proposed that functional inactivation of MGMT through hypermethylation of the gene promotor region could be predictive of chemosensitivity. We have previously reported differential sensitivity to CCNU in a panel of 17 short-term cultures derived from malignant astrocytoma. In this study, we determined the methylation status of *MGMT* using methylation-specific PCR in these 17 cultures. We also assessed the amounts of MGMT mRNA and protein present in each culture using real-time quantitative PCR and immunohistochemistry with a commercial antibody against MGMT. There was good correlation between *MGMT* promotor methylation and presence of MGMT mRNA and protein in all but 2 cases. In both these cultures, mRNA and protein were not detected even though the *MGMT* promotor was unmethylated. However, there was no correlation between sensitivity to CCNU and MGMT status. In the 2 most resistant cultures, the *MGMT* gene was methylated and was not expressed. Similarly, in 4/5 of the most sensitive cultures, *MGMT* was unmethylated, and in 2 of these cases, there was commensurate MGMT expression. However, in the remaining 2 cultures, MGMT expression was not detected, indicating that an alternative mechanism to gene methylation is responsible for MGMT inactivation. This study highlights that the resistance of malignant astrocytoma to nitroureas may be more complex than simple reliance on MGMT activity and prediction of response to such agents by *MGMT* methylation status should be used with caution.

309. SHARED EPIGENETIC MECHANISMS OF GENE INACTIVATION IN HUMAN AND MOUSE GLIOMAS

J.F. Costello,[1] C. Hong,[1] P. Jun,[1] A. Maunakea,[1] W.A. Weiss,[3] and A.W. Bollen[2]; [1]Neurological Surgery, [2]Pathology, and [3]Neurology, University of California San Francisco, San Francisco, California, USA

Human tumors arise from the deleterious effects of genetic and epigenetic mechanisms on gene expression. In several mouse models of human tumors, the tumorigenic phenotype is reversible, suggesting that epigenetic mechanisms also contribute significantly to tumorigenesis in mice. It is not known whether these are the same epigenetic mechanisms in human and mouse tumors, or whether they affect homologous genes. Using an integrated approach for genome-wide methylation (epigenetic) and copy number (genetic) analyses, we identified *SLC5A8* on chromosome 12q23.1

and *PRKWNK2* on chromosome 9q22.31 that were affected primarily by aberrant methylation in human astrocytomas and oligodendrogliomas. *SLC5A8* encodes a sodium/monocarboxylate cotransporter, and *PRKWNK2* encodes a putative serine/threonine kinase. Both genes are highly expressed in normal brain but significantly downregulated in primary gliomas. Bisulfite sequencing analysis showed that their promoter CpG islands were unmethylated in normal brain, but extensively methylated in brain tumors, consistent with the tumor-specific loss of gene expression. In glioma cell lines *SLC5A8* and *PRKWNK2* expression was also suppressed but could be reactivated with a methylation inhibitor. Expression of exogenous *SLC5A8* or *PRKWNK2* in glioma cells inhibited colony formation, suggesting they may function as growth suppressors in vitro. Remarkably, 9 of 10 murine oligodendroglial tumors (from *p53+/-* or *ink4a/arf+/-* animals transgenic for S100b-*v-erbB*) demonstrated a similar tumor-specific downregulation of m*SCL5A8*, the highly conserved mouse homologue of *SLC5A8*. Similarly, the murine *PRKWNK2* was also methylated and downregulated in a proportion of the mouse gliomas. Taken together, these data suggest that *SLC5A8* and *PRKWNK2* function as growth suppressors in vitro and that epigenetic mechanisms are their primary cause of gene silencing in human gliomas. The shared epigenetic inactivation of both *SCL5A8* and *PRKWNK2* in mouse and human gliomas indicates an additional degree of commonality in the origin and/or pathway to tumorigenesis between primary human tumors and these mouse models of gliomas.

310. GENETIC ALTERATIONS IN DESMOPLASTIC MEDULLOBLASTOMAS: EVIDENCE FOR MONOCLONAL TUMOR ORIGIN AND IDENTIFICATION OF NOVEL AMPLIFIED AND OVEREXPRESSED PROTO-ONCOGENES

A. Ehrbrecht,[1] U. Mueller,[2] M. Wolter,[3] A. Hoischen,[1] B. Radlwimmer,[2] T. Pietsch,[4] P. Lichter,[2] G. Reifenberger,[3] and R. Weber[1]; [1]Institute of Human Genetics, Bonn; [2]German Cancer Research Center, Rheinische Friedrich-Wilhelms-University, Heidelberg; [3]Department of Neuropathology, Heinrich Heine University, Duesseldorf; [4]Institute of Neuropathology, Rheinische Friedrich-Wilhelms-University, Bonn; Germany

Desmoplastic medulloblastomas (dMBs) are histologically characterized by two distinct tumor components, the so-called pale islands and the desmoplastic areas. Previous molecular studies have shown that dMBs frequently carry *PTCH* mutations. However, little is known about other genetic and chromosomal aberrations associated with these tumors. We investigated total tumor DNA of 23 sporadic dMBs using comparative genomic hybridization (CGH). Chromosomal imbalances were identified in 17 tumors (74%). The number of aberrations detected per tumor varied from 1 to 12, with an average of 4.61 ± 0.73 (mean ± SEM). Recurrent chromosomal gains were detected on chromosomes 3 and 9 (6/23); 2 and 20 (5/23); 6, 7, 17, and 22 (4/23 each); and 1 (3/23). Recurrent losses were found on chromosomes X (8/23); Y (6/13 male patients); 9 and 12 (4/23 each); as well as 10, 13, and 17 (3/23 each). Amplifications were detected in 4 tumors and mapped to 1p22, 5p15, 9p, 12p13, 13q33-q34, and 17q22-q24. To address the question of clonality of the two components in dMBs, we performed CGH analysis on microdissected pale islands and desmoplastic areas. In 5/6 informative tumors both histological components shared common chromosomal imbalances, indicating an origin from a single progenitor cell. We additionally characterized the amplicons detected on 5p15, 9p, and 17q22-q24 in 2 dMBs using matrix-CGH on genomic arrays of 6.000 large insert clones. Subsequent molecular analyses of amplified candidate genes identified by matrix-CGH confirmed amplification of several genes on 17q23 in three dMBs and the *JMJD2C* gene on 9p24 in one dMB, respectively. Expression analysis suggested *RPS6KB1* as the most important target on 17q23, which was found to be markedly overexpressed in 10/11 medulloblastomas investigated. Taken together, our study provides strong genetic evidence for a monoclonal origin of dMBs and implicates *RPS6KB1* and *JMJD2C* as novel proto-oncogenes that are aberrantly activated in these tumors.

311. AMPLIFICATIONS AND DELETIONS IN THE GLIOBLASTOMA GENOME: FROM NOVEL LOCI TO CANDIDATE GENE AND NON-GENE TARGETS

C. Brennan,[1] B. Feng,[2] D. Louis,[3] P.M. Black,[4] and L. Chin[2]; [1]Neurosurgery, Memorial Sloan-Kettering Cancer Center, New York, New York; [2]Medical Oncology, Dana Farber Cancer Institute, Boston, Massachusetts; [3]Pathology, Massachusetts General Hospital, Boston, Massachusetts; [4]Neurosurgery, Brigham and Women's Hospital, Boston, Massachusetts; USA

Array-based comparative genomic hybridization (ACGH) offers increasing resolution of amplification and deletion events in the tumor genome. The targets of less common events, along with the significance of these events, are the subject of intense investigation. We present the combined analysis of ACGH and expression profiling of 40 primary glioblastomas and 20 cell lines in an effort to identify narrow, high-copy-number aberrations which point to a limited number candidate gene and non-gene targets, such as micro-RNAs. Profiles were generated using cDNA microarrays (Agilent) providing an interval resolution of approximately 100 kb. A changepoint algorithm (circular binary segmentation) was used to identify discrete copy number aberrations (CNAs) and their boundaries. Locus boundaries are systematically defined by grouping CNAs across multiple profiles. Within each locus, one or more minimal common regions (MCRs) of overlapping alteration are automatically identified, each potentially harboring a distinct cancer-relevant target. Twenty cell lines and 14 tumors were additionally profiled for RNA expression (Affymetrix). Expression data were mapped to genome position, and each gene was tested for copy-number-driven expression by calculating the shift in expression in samples with CNA. Significance was estimated by permutation testing. One hundred sixty-four discrete autosomal loci were identified, 111 present in more than one sample. Many MCRs were of low-level copy number alteration. Fifty-five MCRs met high-confidence criteria: high-level alteration and/or high recurrence. Average size of this subset was 3.5 Mb, spanning an average of 36 genes. Fifteen of these MCRs were present only in cell lines; 40 were identified in tumors as well (21 amplifications, 19 deletions). Supporting the validity of the approach, all common CNAs previously described in high-grade gliomas were identified, including amplifications of PDGFRA, EGFR, MDM2, and CDK4 and deletions of p16(Ink4a) and PTEN. Aside from these, the majority of loci were novel, either not previously described in glioma or without characterized target genes. Eighteen loci spanned <1 Mb, containing only 20 genes. Each of these smaller loci was subject to quantitative PCR validation. We identified several candidate targets, including TERT, not previously identified as a target of amplification. Genes within the 52 loci were evaluated for expression, and 520 out of 1740 were found to show significant effect of copy number on gene expression. Results are contrasted with identical analysis of 300 samples of 4 other non-glioma tumor types. High-grade gliomas harbor infrequent novel chromosomal copy aberrations which mark potential cancer-relevant genes. Expression profiling allows further narrowing of this list of targets by characterizing the response to gene dosage.

312. COMPREHENSIVE GENETIC CHARACTERIZATION OF PLEOMORPHIC XANTHOASTROCYTOMAS

A. Hoischen,[1] M. Ehrler,[1] K. Kaulich,[3] B. Blaschke,[3] P. Zipper,[3] A. Becker,[2] B. Radlwimmer,[4] O. Wiestler,[4] P. Lichter,[4] G. Reifenberger,[3] and R. Weber[1]; [1]Institute of Human Genetics and [2]Department of Neuropathology, Rheinische Friedrich-Wilhelms-University, Bonn; [3]Department of Neuropathology, Heinrich-Heine-University, Duesseldorf; [4]Department of Molecular Genetics, German Cancer Research Center, Heidelberg; Germany

Pleomorphic xanthoastrocytomas (PXAs) are rare astrocytic neoplasms corresponding histologically to WHO grade II. They usually show circumscribed growth and favorable prognosis despite exhibiting a high degree of cellular pleomorphism. PXAs mainly affect children and young adults. Here we present genomic profiling experiments of 50 PXAs. Chromosomal-CGH revealed a distinct pattern of chromosomal imbalances. The hallmark alteration detected in 50% of PXAs was loss on chromosome 9. Less common recurrent losses affected chromosomes 17 (10%); 8, 18 and 22 (4% each); and the Y chromosome in 7.7% of tumors from male patients. Recurrent gains were identified on chromosome X (16%); 7, 9q, and 20 (8% each); and 4, 5, and 19 (4% each). Amplifications were found in 2 tumors and mapped to 2p23-p25, 4p15, 12q13, 12q21, 21q21, and 21q22. To achieve a higher resolution, 7 PXAs were analyzed with a whole genome microarray of 6.000 large insert clones resulting in a resolution of at least 1Mb. In each of these cases, the results obtained by chromosomal-CGH could also be detected by the matrix-CGH experiments. In 3 of the 7 PXAs, additional aberrations were found by matrix-CGH. Imbalances detected by matrix-CGH were verified by interphase-FISH on tumor tissue sections. In particular, breakpoints were confirmed in one case with partial deletions on 9p and 18p. Molecular genetic analysis of selected candidate genes revealed *TP53* mutations in only 3 of 62 (5%) PXAs analyzed. The *CDKN2A*, *CDKN2B*, and *p14^{ARF}* genes on 9p21 did not show homozygous deletion, mutation, promoter hypermethylation, or complete loss of mRNA expression. None of the tumors showed mutation of the *PTCH* or *TSC1* genes or amplification of the *EGFR*, *CDK4*, or *MDM2* genes. Taken together, our study provides a comprehensive overview of genetic alterations in PXAs and indicates that chromosome 9 carries one or more not yet identified tumor suppressor gene(s) with relevance to the molecular pathogenesis of these tumors.

313. ALTERATIONS OF p53 AND p73 OCCUR INDEPENDENTLY OF ONE ANOTHER IN ALL GRADES OF PEDIATRIC DIFFUSE ASTROCYTOMA

S. Ward,[1] B. Suarez-Merino,[1] Y. Ding,[1] R. Hayward,[2] W. Harkness,[2] D. Thompson,[2] K. Phipps,[2] D. Thomas,[3] and T. Warr[1]; [1]Department of Molecular Neuroscience, Institute of Neurology; [2]Department of Neurosurgery, Great Ormond Hospital for Sick Children NHS Trust; [3]Division of Neurosurgery, Institute of Neurology, London; UK

The *p53* tumor suppressor gene plays an essential role in cell cycle arrest, response to DNA damage, and apoptosis, and abnormalities of p53 are present in many tumor types. Also, *p73* shows structural and functional similarity to *p53* and is the potential gene target of 1p36.3 deletion in human cancer. Inactivation of *p53* through mutation, allelic loss, or hypermethylation of CpG islands in the promotor region occurs in approximately 30% of all three grades of adult astrocytoma and is strongly associated with the development of secondary GBM; *p73* methylation has also been reported in malignant astrocytoma in adults. However, the roles of *p53* and *p73* in pediatric astrocytoma remain equivocal. In this study, we determined the frequency of *p53* mutations by direct sequencing of exons 4 to 7 in 34 pediatric astrocytoma comprising 13 WHO grade II, 3 WHO grade III, and 18 WHO grade IV tumors. We also investigated the methylation status of both *p53* and *p73* using methylation-specific PCR. Mutations of *p53* were present in 2 samples of high-grade astrocytoma only. One grade III astrocytoma showed a mutation at codon 271 (GTG to GAG, Val to Glu), while the second mutation was observed at codon 282 (CGG to TGG, Arg to Trp) in a grade IV tumor. Methylation of *p53* was detected in 8 cases (24%) comprising 3 grade II (23%), 1 grade III (33%), and 4 grade IV (22%) tumors. Eleven (32%) samples showed methylation of *p73*, comprising 6 grade II (46%) and 5 grade IV (27%) astrocytoma. Methylation of *p73* occurred independently of both mutation and methylation of *p53*. Overall, alterations of *p53* or *p73* were present in 58% of tumors. This data indicates that although mutations of *p53* are rare in pediatric astrocytoma, methylation of the promotor region may offer an alternative mechanism of p53 inactivation. This contrasts with adult astrocytoma in which mutation of p53 is much more common than hypermethylation. Similarly, in adult astrocytoma, methylation of *p73* has been observed predominantly in grade IV tumors and there is no correlation with p53 status. The findings in the present study that *p73* methylation occurred only in tumors with no alterations of *p53* and at similar frequencies in low-grade and malignant tumors provides further evidence of a different genetic pathogenesis between pediatric and adult astrocytoma.

314. INTERSTITIAL LOSS AND GAIN OF SEQUENCES ON CHROMOSOME 22 IN MENINGIOMAS WITH NORMAL KARYOTYPE

A. Prowald,[1] S. Urbschat,[2] S. Wemmert,[1] R. Ketter,[1] E. Meese,[2] K.D. Zang,[3] and W.-I. Steudel[1]; [1]Neurochirurgie, Neuroonkologie, Universitätskliniken des Saarlandes, Homburg/Saar; [2]Institut fur Humangenetik, Universität des Saarlandes, Saarbrücken; [3]IGD Saar, Institut für genetische Diagnostik, Homburg/Saar; Germany

In nearly half of sporadic low-grade meningiomas, no chromosome aberration can be detected. In the majority of the other half, chromosome 22 is lost. In higher grade meningiomas, this loss is followed by characteristic secondary chromosome aberrations. Regarding the molecular findings in schwannomas, homozygous loss or mutation of the NF2 gene located on chromosome 22 was supposed also to be the primary event in meningioma development. However, in nearly all high-grade but in only a minority of low-grade meningiomas, the loss of the NF2 protein is observed. Therefore, the hypothetical combined heterozygous loss of or inactivation of two or more tumor suppressor genes (at least one of them located on chromosome 22) as well as the homozygous loss of a regulatory gene on chromosome 22 different from NF2 was discussed. In a search for microdeletions or/and structural recombinations of chromosome 22, we investigated primary cell cultures of 43 meningiomas by conventional G-banding (26 without, 17 with loss of chromosome 22). Twenty-seven tumors were analyzed with spectral karyotyping (SKY) and 16 with fluorescence in situ hybridization (FISH) with DNA probes for the chromosomal regions of 22q11.2, 22q11.23q12.1, 22q12.1, and 22q13.3. SKY analysis confirmed G-banding data for chromosome 22 and could specify marker chromosomes and translocations containing material from chromosome(s) 22. Confirming our assumption, microdeletions on chromosome 22 were detected by FISH in 6/8 cytogenetically nonaberrant meningiomas. Surprisingly, in 2/8 cases we observed gains of the 22q13.3 region and in 2/8, gains of the 22q12.1 region. Here we present first evidence for an uncommon mechanism during early meningioma development at least for a meningioma subgroup: (i) duplication and translocation of sequences from chromosome 22 to different chromosomes, (ii) deletion of the original sequences on chromosome 22, resulting in disomy again (only visible as translocation in metaphase FISH), and (iii) loss of chromosome 22.

315. CORRELATING GENE EXPRESSION TO DNA COPY NUMBER IDENTIFIES GENES INVOLVED IN THE PATHOGENESIS OF HUMAN GLIOBLASTOMA

J. Nigro,[1,2] A. Misra,[1,2] I. Smirnov,[1,2] L. Prestegarden,[6] L. Wanyu,[7] R. Yeh,[3] K. Aldape,[8] S. VandenBerg,[2,4] and B. Feuerstein,[1,2,5]; [1]Cancer Center and Departments of [2]Neurological Surgery, [3]Epidemiology and Statistics, [4]Pathology, and [5]Laboratory Medicine, University of California, San Francisco, San Francisco, California, USA; [6]Biomedicine, University of Bergen, Bergen, Norway; [7]Neurosurgery, University of Lausanne, Lausanne, Switzerland; [8]M.D. Anderson Cancer Center, Houston, Texas, USA

Tumor behavior is related to molecular changes that occur during neoplastic development. In this study, we used expression and genomic array analysis to reveal molecular profiles associated with patient survival in human glioblastomas. Cases fell into two genetic subgroups with poorer survivors (<2 years) associated with chromosome 7 gain and 10 loss. Expression profiles of the same tumors were strongly associated with patient survival. We correlated these two data sets to explore how gene expression is influenced by underlying genomic changes. We found that gene expression was correlated with DNA copy number, but that loss of chromosome 10 was a much stronger influence on global gene expression. One homeobox gene, Meox2, that is expressed during mesoderm induction and required for normal muscle development, was strongly associated with both patient survival and chromosome 10 loss. The expression array data showed that in cases with chromosome 10 loss, Meox2 was fourfold overexpressed relative to those where chromosome 10 was intact and greater than eightfold overexpressed relative to non-neoplastic brain. Expression array results were confirmed by real-time reverse transcriptase PCR. By immunohistochemistry, we localized the protein to the nuclei of primary human glioblastomas, xenografts, and tumor cell lines. We are using biological and statistical models to identify genes regulated coordinately with Meox2 expression. The results indicate that molecular approaches can identify biologically distinct groups of human glioblastomas and genes that may contribute to the pathogenesis of human gliomas. This work was supported by the NIH (PO1 NS42927 and CA 85799) and the National Brain Tumor Foundation.

316. POSSIBLE RELATIONSHIP BETWEEN P27/KIP.1 AND CYCLIN D1 DEGRADATION AND THE IMMUNOPROTEASOME IN GLIOBLASTOMA

D. Schiffer,[1,2] M. Piccinini,[3] A. Anselmino,[3] V. Fiano,[2] C. Ghimenti,[2] M. Rinaudo[3]; [1]Neuroscience, University of Turin, Turin; [2]Research Centre for Neuro-bio-pathology Oncology, Foundation Policlinico di Monza, Vercelli; [3]Medicine Experimental Oncology, Section Biochemistry, University of Turin, Turin; Italy

The cell cycle is regulated by the cyclin/kinase complexes with p27/Kip.1 functioning as CDK inhibitor at the checkpoint G1-S. Cyclin D1 and p27/Kip.1 are under the control of PKB/Akt pathway, which is a crosspoint between Ras-MAPK from PTKR and PTEN pathway. Phosphorylated Akt activates cyclin D1 through AKT/FRAP mTOR and inhibits p27/Kip.1 through AFX/FKHR. In gliomas, cyclin D, E, A, and B1 and CDK4-6-2 and cdc2 expression increases with malignancy, whereas that of p27/Kip.1 decreases in parallel with the increase of its F-box targeting protein Skp2. Among many other proteins, cyclins and p27/Kip.1 are degraded into the ATP-dependent ubiquitin-proteasome system. Ubiquitin ligation with proteins targeted for degradation is accomplished by an enzymatic complex, E1-E2-E3, and the ubiquitinated protein is then substrate for the 26S proteasome, which cleaves the proteins into small peptides by means of its catalytic core (20S proteasome). The 20S proteasome contains a- and β- constitutive subunits with proteolytic activity. Under cytokine stimulation, constitutive subunits β1, β2, and β5 are replaced by the inducible subunits LMP2, MECL-1, and LMP7, transforming the proteasome into the immunoproteasome. Based on the previous study of cyclins and p27/Kip.1 in a series of gliomas, 18 glioblastomas were studied by immunohistochemistry for inducible subunits and evaluated for peptidase activity and subunit composition by spectrofluorimetric assay and Western- immunoblotting. In malignant gliomas, it has been observed that the inducible subunits are more expressed, but their proteolytic activity is strongly reduced in comparison with controls. Preliminary results indicate that the 26S proteasome does occur in glioblastomas. Post-translational modifications of the 20S proteasome can be responsible for the discrepancy, and the finding is consistent with the increased expression of cyclin D1 in glioblastomas, but not with the reduction of p27/Kip, which is a proteasome substrate. It may be that in glioblastomas the immunoproteasome expression represents a more adequate way for the degradation of p27/Kip.1.

317. DISSECTING GLIOMA SYSTEMS BY GENOMICS, PROTEOMICS, AND MODELING

W. Zhang, I. Shmulevich, R. Jiang, C. Mircean, and G. Fuller; Pathology, M.D. Anderson Cancer Center, Houston, Texas, USA

Cancer systems are overwhelmingly complex. They, like healthy cells, are regulated by numerous genes and proteins in a highly coordinated fashion. The main difference is that in cancer cells, the rules of regulation have been altered by events that cause the underlying complex dynamical system governing cellular activity to behave in an aberrant manner. The complex nature of a cancer system, with its many unrevealed components and levels of complexity, calls for a more systematic measurement of gene and proteins and the development of suitable representational models that can ultimately be used to guide cancer researchers in their pursuit of understanding cancer and finding effective treatment. Using microarray technology, we have profiled gene expression of different grades of gliomas. In addition to some highly interesting individual markers such as insulin-like growth factor binding protein 2 (IGFBP2), we have developed a mathematical model called Probabilistic Boolean Network (PBN) to construct a network based on expression of 600 genes that are functionally well characterized. The network has revealed novel relationships among genes, some of which have subsequently been confirmed by experiments. For example, the PBN model revealed a relationship between IGFBP2 and NFkappaB, which was experimentally confirmed. To extend our understanding, we have recently constructed a reverse-phase protein lysate array with 90 different glioma tissues and assayed for the expression of 50 signaling proteins. To gain a quantitative insight, each sample is serially diluted and in triplicate on the array. Mathematical analyses have revealed key protein expression and posttranslational modification events during glioma progression. For example, akt phosphorylation is most dominant in glioblastomas. In summary, we begin to be able to dissect the glioma systems with genomics, proteomics, and bioinformatics.

318. TELOMERASE AND ESTROGEN RECEPTOR ROLES IN GLIOMAS

N. Cruickshanks,[1] A. Shervington,[1] R. Lea,[1] L. Shervington,[1] and G. Roberts[2]; [1]Biological Sciences, University of Central Lancashire, Preston; [2]Department of Neurosurgery, Royal Preston Hospital, Preston; UK

The most common primary brain tumors are glioma. Glioma cells that respond to chemotherapy have shown a decline in the activity of a specific enzyme called telomerase. This enzyme is usually only active in undifferentiated cells. However, it has been detected in 94% of neuroblastoma and 100% of oligodendroglioma. Numerous studies have concentrated on the induction level of hTERT, the major subunit of telomerase in cancer tissue, but few workers have correlated the level of telomerase with hsp90 and p23 in cancer cells and glioma tissue. Hsp90 and p23 both function as a co-chaperone for the telomerase. It is proposed here that if there is high constitutive expression level of hTERT, then there may also be a high expression level of hsp90 and a lower expression level of p23, since this is a cancer suppressor. Furthermore, a high brain concentration of telomerase protein may offer an alternative and direct indicator of malignancy. In this study, the level of telomerase in cell samples has been quantitatively measured by several methods. Results indicate an elevated constitutive expression of telomerase in cancerous cell lines with respect to control cells. The cells lines used in this study namely are the following: U-87-MG (glioblastoma astrocytoma), 1321N1 (astrocytoma), IPDDC-A2 (astrocytoma; grade II), GOS-3 (astrocytoma/oligodendroglioma), JCRB0068 (brain, embryonic), and NHA (normal astrocytes). Unfixed tumor biopsy material will be obtained from Royal Preston Hospital. Handling of the tissue followed university code of practice. Total RNA was isolated from biopsy tissues, primary cells, and cell lines using RNA capture kit (Roche). RNA will be used to generate cDNA using a Reverse Transcription System (Roche). A potential application for this work is to use it as a diagnostic tool for glioma cancer by finding the right marker.

319. HYPERDIPLOIDY DEFINES A DISTINCT CYTOGENETIC ENTITY OF AGGRESSIVE MENINGIOMAS

R. Ketter,[1] Y.-J. Kim,[2] S. Storck,[3] W.-I. Steudel,[1] K.D. Zang,[4] and W. Henn[3]; [1]Neurosurgery and Neurooncology and [2]Neuropathology, Kliniken des Saarlandes, Homburg/Saar; [3]Institute of Human Genetics, Universität des Saarlandes, Saarbrücken; [4]Institut für Genetische Diagnostik, IGD, Homburg/Saar; Germany

The most common chromosomal aberration found in meningiomas of all grades is monosomy 22. Progression and recurrence of meningiomas is usually associated with stronger hypodiploidy, i.e., monosomy of further autosomes and, most frequently, heterozygous loss of chromosome 1p. Rarely, however, hyperdiploid karyotypes occur; the objective of this study was to explore the cytogenetic and histopathologic patterns as well as the clinical significance of hyperdiploidy in meningiomas. A consecutive series of over 400 meningiomas were cultured in vitro and cytogenetically characterized by using standard banding techniques and, in one structurally aberrant case, spectral karyotyping (SKY). In patients with hyperdiploid meningiomas, clinical and histomorphological data as well as results of long-term postoperative survey were compared with data from patients with cytogenetically typical meningiomas. We identified a subgroup comprising about 4% of all meningiomas that do not display the common chromosome losses but instead a strikingly uniform pattern of hyperdiploidy. Mostly in the absence of structural chromosome rearrangements, these meningiomas each have between 49 and 56 chromosomes, with trisomy 12 (14/16 cases), trisomy 20 (13/16 cases), trisomy 5 (12/16 cases), and trisomy 17 (10/16 cases), along with variable trisomies of all other autosomes except #1, #2, and #21. Chromosome losses are rare, affecting #22 in 2/16, and #7 and Y each in only 1/16 cases. Histomorphologically, the hyperdiploid meningiomas show intermediate differentiation with patternless growth and/or microcystic degeneration. However, a loss of chromosome 22 may be associated with a persistent fibrous growth pattern. The proliferative potential in terms of increased mitotic activity and Ki-67 labeling index is significantly elevated; all investigated hyperdiploid meningiomas were assigned to WHO grade II. Fourteen patients in whom tumor resections were determined to be simpson grade I or II and 2 patients with simpson grade III could be followed up after tumor extirpation. In 2 patients, recurrences were documented and 3 patients died during the period of observation. We conclude that hyperdiploidy constitutes a small but clinically relevant entity of biologically aggressive, histopathologically atypical meningiomas (WHO grade II), which are cytogenetically distinguishable from the majority of common-type meningiomas.

320. PHARMACOLOGICAL REVERSAL OF GENE SILENCING IN MALIGNANT GLIOMA: A WHOLE GENOME MICROARRAY ANALYSIS

G. Foltz and A. Madan; Neurosurgery, University of Iowa College of Medicine, Iowa City, Iowa, USA

Promoter methylation and histone modification play a critical role in transcriptional regulation. We explore the role of histone deacetylation in aberrant gene silencing in malignant glioma. Histone deacetylase inhibitors induce growth arrest and apoptotic cell death in malignant astrocytes. We use a whole-genome microarray analysis to identify genes differentially expressed in malignant astrocytes in response to treatment with Trichostatin A (TSA), a specific inhibitor of histone deacetylase activity. ChIP analysis of histone modifications identified specific promoter region alterations in selected differentially regulated genes. Microarray studies were performed on 11 primary glioblastoma cell lines (UI series), the T98 and U87 cell lines, and normal human astrocytes. Three experimental paradigms were used: (1) a time-course analysis of T98 cells treated with 1 μM TSA for 6, 12, 24, 36 and 48 h, (2) a dose-response analysis of T98 cells treated with increasing doses of TSA (300 nM to 5 μM), and (3) a threshold analysis requiring response in 8 of 11 primary lines and U87 cells treated with 1 μM TSA for 24 h. Biological replicates were performed independently in triplicate for each condition. A cohort of TSA regulated genes was selected as an intersection of the three experimental paradigms after statistical analysis using Significance of Microarrays (SAM) and ANOVA/Q-Value (Storey) with maximum stringency ($q < 0.001$). GBM specific responses were identified by comparison to normal human astrocytes treated with 1 μM TSA for 24 h. Differentially expressed genes were confirmed with real-time PCR and evaluated for promoter region histone modification using ChIP analysis with antibodies to K4-methylation and K9/14 acetylation. Promoter regions of select genes were also analyzed with bisulfite sequencing. Two selected genes were transfected into T98 and U87 cells resulting in suppression of cell growth in a colony focus assay. Over 800 genes were upregulated at least 2-fold across all conditions. Fifty selected genes were confirmed by real-time PCR. Eight of 10 selected genes had increased acetylation of promoter region K9/14 acetylation, independent of promoter methylation and K4-methylation status. Two genes demonstrated suppression of cell growth after plasmid-mediated transfection in U87 and T98 cells. GBM specific gene expression profiles resulting from histone deacetylase inhibition provides insight into the mechanisms of aberrant transcriptional regulation during malignant astrocyte transformation. The identification of epigenetic silenced genes represents a potential entry point for biomarker development and therapeutic intervention.

321. MOLECULAR CLASSIFICATION OF GLIOMAS BY COMPARATIVE GENOMIC HYBRIDIZATION ARRAY IS CORRELATED WITH PROGNOSIS

A. Idbaih,[1] Y. Marie,[2] A. Aurias,[1] P. Pierron,[1] K. Mokhtari,[2] M. Kujas,[2] J. Delattre,[2] M. Sanson,[2] K. Hoang-Xuan,[2] and O. Delattre[1]; [1]Institut Curie, Unité INSERM U509, Paris; [2]Hôpital Pitié-Salpêtrière, Unité INSERM U495, Paris; France

The purpose of our study was to screen gliomas for genomic imbalances by the comparative genomic hybridization array technique (CGHa). DNA from 100 unselected diffuse gliomas were investigated by a genome-wide array-based CGHa technique (a total of 3342 mapped and amplified BAC DNA were spotted in an array format on glass, providing an average resolution of 1 Mb across the human genome). The genomic profiles of all tumors were classified by using clustering software. The results were correlated with the outcome of the patients. The most frequent alterations were loss of 1p, 19q, 9p (P16/CDKN2A locus), and 10q; gain of chromosome 7; and amplification of EGFR. In addition, CGHa detected less well-documented recurrent abnormalities (loss of 4, gain of 19, amplification of PDGFRa, MDM4, MDM2 and CDK4 locus). The results were concordant with those obtained in the same series of tumors with other conventional techniques (LOH and real-time PCR techniques). Moreover, CGHa allowed us to detect small interstitial deletions that would be unlikely detected by other methods, so that we could narrow considerably the candidate region on 1p. Lastly, gliomas could be classified according to their CGHa genetic profile into several groups demonstrating distinct clinical outcome. CGHa is a reliable and powerful method to investigate genomic imbalances in gliomas, to narrow candidate regions and identify novel genetic alterations, and to classify the tumors into distinct prognostic subgroups.

322. ANALYSIS OF "MEDIUM THROUGHPUT" QUANTITATIVE LOH IN ANAPLASTIC OLIGODENDROGLIOMA

R.A. Betensky,[1] E.A. Houseman,[1] B.A. Coull,[1] T. Mikkelson,[2] L. Tahash,[2] J.G. Cairncross,[3] D.N. Louis,[4] and O. Bögler[2]; [1]Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA; [2]Neurosurgery, Henry Ford Hospital, Detroit, Michigan, USA; [3]Foothills Medical Centre, University of Calgary, Canada

Allelic losses on chromosomes 1p and 19q are known to occur frequently in anaplastic oligodendrogliomas. Current assessment of LOH in oligodendrogliomas is based on 3-4 distally located CA-repeat polymorphism markers. A call of LOH is made if LOH is detected at all informative markers. We recently used capillary electrophoresis for assessing LOH at 15 markers on 1p and 4 markers on 19q in 93 tumors. We derived meaningful thresholds for the quantitative LOH through the use of a latent mixture model that leveraged the measurements from normal specimens to gain information about the non-LOH distribution. We applied latent class analysis to cluster subjects according to patterns of LOH and found three distinct LOH profiles among our samples: a group with LOH at all markers, a group with moderate LOH, and a group with low LOH. The survival outcomes of the high-LOH group were significantly more favorable than those of the other groups, which were not different from each other. We used the latent class model to multiply impute values for the noninformative LOH outcomes and thereby increased the power of the survival analyses. Last, we developed constrained estimation techniques to enable complete joint modeling of all 19 markers while avoiding overfitting the data. These techniques can be applied more generally to the analysis of other genomic assays, including array comparative genomic hybridization (aCGH).

323. IDENTIFICATION OF NOVEL GLIOBLASTOMA TUMOR SUPPRESSOR GENE CANDIDATES ON CHROMOSOME 10q24-qTER

G. Reifenberger, M. Wolter, B. Köhler, and C. Knobbe; Neuropathology, Heinrich-Heine-University, Duesseldorf, Germany

Allelic losses on chromosome 10 are common genetic alterations in glioblastomas that are found in approximately 60% to 90% of the cases. Molecular genetic analyses suggested the presence of up to 3 distinct tumor suppressor gene loci at 10p, 10q23, and 10q24-qter, respectively. The *PTEN* tumor suppressor gene has been identified as the relevant gene on 10q23. The *KLF6* gene at 10p15 had been suggested as another glioblastoma suppressor gene, but does not appear to carry frequent alterations in these tumors. Thus, the target genes on 10p and 10q24-qter are not known yet. We performed real time-RT PCR expression analysis of 17 genes located on 10q24-qter in a series of 34 primary glioblastomas and 12 secondary glioblastomas to identify novel candidate tumor suppressor genes that are downregulated in glioblastomas. The genes were either selected from the NCBI Human Genome Browser (www.ncbi.nlm.nih.gov) or from microarray expression profiling data of glioma cell lines treated with the demethylating agent 5´-aza-deoxycytidine and the histone deacetylase inhibitor trichostatin A. So far, we identified 3 genes (*ADD3*, *EMX2*, and *OAT*) that show markedly reduced mRNA levels relative to non-neoplastic brain tissue in substantial fractions of glioblastomas. Furthermore, treatment of glioma cell lines with 5´-aza-deoxycytidine and trichostatin A resulted in increased expression of these genes, suggesting that their transcriptional downregulation may be due to promoter hypermethylation. These three genes represent interesting novel tumor suppressor candidates that are presently further investigated for promoter hypermethylation and coding region mutations.

324. GENES THAT PROMOTE CELL GROWTH ARE ABERRANTLY EXPRESSED IN PILOCYTIC ASTROCYTOMA

N. Potter,[1] R. Poh,[1] S. Ward,[1] B. Suarez-Merino,[1] W. Harkness,[2] R. Hayward,[2] D. Thompson,[2] K. Phipps,[2] J. Rees,[1] D. Thomas,[3] and T. Warr[1]; [1]Institute of Neurology, UCL, London; [2]Department of Neurosurgery, Great Ormond Street Hospital for Sick Children NHS Trust, London; [3]Division of Neurosurgery, Institute of Neurology, London; UK

Astrocytoma are the most common brain tumors occurring in children. The majority of these are low-grade pilocytic (grade I) and diffuse (grade II) astrocytoma comprising 80% of cases. Little is known about the genetic basis underlying the development of pediatric astrocytoma. Previous cytogenetic studies have revealed that the majority of low-grade pediatric astrocytoma appear to be karyotypically normal. It is therefore possible that abnormal gene expression rather than chromosomal abnormalities are involved in the development of these tumors. The oligonucleotide Affymetrix Human Genome U133 Array representing 33,000 genes was used to generate expression profiles of 14 grade I pediatric astrocytoma biopsy samples and three normal brain controls. Genespring version 6.1 was used for array data analysis including the completion of 1-way ANOVA statistical tests. In total 1340 genes were differentially expressed in tumor samples compared to normal brain controls; 1063 were upregulated and 277 were downregulated. Those genes overexpressed included components of the Wnt signaling pathway, *Wnt5A*, *FDZ6*, *PKC*, *CKIa*, *LRP6*, *cyclin D1*, *v-jun*, and *TCF*, as well as genes associated with the Notch and TGF-b signaling pathways, *Bmi-1*, *JAG1*, *BMP2*, *Hey-1*, *Id4*, and *Bcl-2*. Growth promoters *PDGFRa*, *PDGFC*, and *FGF2* were also upregulated. The downregulation of *SMAD7* and *SMURF1* may increase TGF-b pathway signalling, and the downregulation of tumor suppressor genes *p57, p19*, and *MTUS1* may also contribute to tumor growth. In contrast, the tumor suppressor gene *p53* was significantly upregulated. Differential gene expression in pilocytic astrocytoma indicates increased activity of pathways involved in cell growth.

325. GENE EXPRESSION PROFILING OF GLIOMAS

J. Takahashi,[1] K. Koizumi,[2] S. Saito,[2] S. Oba,[3] S. Ishii,[3] K. Kato,[2] N. Hashimoto,[1] and *M. Shirahata*[1]; [1]Neurosurgery, Kyoto University, Kyoto; [2]Functional Genomics, Research Institute, Osaka Medical Center, Osaka; Theoretical Life Science, [3]Nara Institute of Science and Technology, Nara; Japan

We analyzed the expression level of 3456 genes in 109 glioma using adaptor-tagged competitive PCR(ATAC-PCR), a high-throughput reverse transcription-PCR technique. The purpose of this study is to investigate the molecular features of glioma through a large-scale gene expression profiling. A total of 109 gliomas specimens including 80 glioblastomas, 10 anaplastic astrocytomas, 12 diffuse astrocytomas, and 7 anaplastic oligodendrogliomas were obtained from surgical resection. We first surveyed the genes actually expressed in glioma using expressed sequence tag (EST) sequencing. A 3'-end cDNA library was constructed by using a mixture of RNA from 12 gliomas, and we randomly selected 3036 genes from this EST collection. We then designed PCR primers for 3456 genes including 420 genes known to be expressed in glioma from previous literature. The expression level of these genes in sample RNAs derived from 109 gliomas was assayed by the ATAC-PCR technique. ATAC-PCR is an advanced version of quantitative competitive PCR, characterized by the addition of unique adaptors for different cDNAs, measuring the relative expression of samples against the control. In this assay, using seven adaptors, 4 samples and 3 known amounts of controls were processed in a single reaction. The PCR amplification was performed using an adaptor-primer, the sequence of which was from the common part of adaptors, and a primer specific to the gene of interest, the sequence of which was included in the 3' end fragment. Amplified fragments were separated by denaturing polyacrylamide gel electrophoresis, and the amount of fragments was measured by an automated sequencer. We analyzed the expression levels of 3456 genes in 109 gliomas. The unsupervised hierarchical cluster analysis revealed that different types and grades of glioma had a unique gene expression pattern of a specific group of genes. In addition, glioblastomas were divided into

molecular groups, each of which possessed a distinct gene expression signature. Our gene expression study showed that each histological type and grade of glioma has a distinct gene expression signature. Our results may lead to molecular classification of gliomas.

326. NAVIGATING GLOBAL TRANSCRIPTOMICS TO THERAPEUTIC TARGETS ON INVASIVE GLIOMA CELLS

J. Rennert,[1] T. Demuth,[1] D. Hoelzinger,[1] L. Reavie,[1] L. Mariani,[2] K. Pan,[3] C. Lih,[3] R. Lin,[3] S. Cohen,[3] and M. Berens[3]; [1]Department of Neurogenomics, Translational Genomics Research Institute, Phoenix, Arizona, USA; [2]Department of Neurosurgery, Inselspital, Bern, Switzerland; [3]Department of Genetics, Stanford University School of Medicine, Stanford, USA

Gliomas are the most common primary brain tumors, they exhibit highly invasive behavior, which is a major cause of the morbidity and prompt lethality of the disease. A better understanding of the determinants of glioma migration and invasion may explain the ubiquitous recurrence of glioblastoma and may reveal novel therapeutic targets aimed at invasive cells. Motile and stationary cell populations from seven human glioma cell lines and three primary glioblastoma cultures were isolated from a monolayer radial migration assay for gene expression analysis using oligonucleotide microarrays. Differentially expressed genes between these two populations across all cell lines were identified using a pattern recognition approach that integrates a priori knowledge in conjunction with expression data as implemented in GABRIEL (Genetic Analysis by Rules Incorporating Expert Logic), a Web-based application designed for rule-based analysis) (Proc. Natl. Acad. Sci. USA 99, 2118, 2002). Principal component analysis (PCA) is a method for reducing high dimensional data into a few components that represent the majority of variation within the data. PCA of the differential expression data revealed two discriminating patterns, a global downregulation profile and a global upregulation profile. Two genes (AK098354 and Cyr61 [cystein rich 61]) following these profiles were used in GABRIEL's proband rule-based function to find subsets of genes with similar expression patterns. Differential expression of eight candidates (four from each subset) was validated by QRT-PCR. Of these eight, Cyr61 and CTGF (connective tissue growth factor) are secreted extracellular proteins in the CCN family of growth factors, (Cyr61, CTGF, and Nov) whose properties include tumorigenesis and cell migration. Immunofluorescence confirmed increased protein expression between motile and stationary cells in a migration assay. Immunochemistry on glioma invasion tissue microarrays revealed expression of Cyr61 and CTGF in association with invasion. siRNA knockdown of Cyr61 showed a reduction in mRNA expression. Its effect on motility will be examined in a migration assay. These findings provide strong evidence that Cyr61 and CTGF play important roles in glioma invasion potentially leading to new anti-invasion therapies.

327. THE TRANSCRIPTION FACTOR FOXG1 IN GLIOMAS

A.B. Lassman,[1] Q. Wang,[2] J. Massague,[2] and E.C. Holland[1,2,3]; [1]Neurology, [2]Cancer Biology & Genetics, and [3]Surgery (Neurosurgery), Memorial Sloan-Kettering Cancer Center, New York, New York, USA

Foxg1 is a winged helix/forkhead transcription factor previously called brain factor 1 (BF-1). It is critical for the normal development of the brain as demonstrated by the lack of cerebral hemispheres in knockout mice. In addition, the chicken ortholog (Qin) is an oncogene that was originally isolated from avian sarcoma virus 31; it both transforms cultured chicken embryonic fibroblasts and induces fibrosarcomas in birds. Evidence also suggests that Foxg1 contributes to deregulated proliferation by repressing transcription of the tumor suppressor p21CIP. Finally, Foxg1 is overexpressed in low- and high-grade human gliomas as well as the U87MG glioblastoma cell line relative to normal human brain. As many parallels exist between normal brain development and gliomagenesis, these features of Foxg1 function imply a role in glioma biology not described previously. We modeled the pathways driving human glioma growth both in vitro and in vivo to explore the role of Foxg1 in glioma formation. We first transformed primary mouse glial progenitors in culture with activated forms of Ras, Akt, Ras + Akt, or PDGF by somatic cell gene transfer of the various oncogenes. We then performed anti-Foxg1 Western blot analysis on protein lysates from the transformed glia. We also pharmacologically silenced Ras signaling in the Ras transformed glia with a MEK inhibitor to analyze the impact on Foxg1 expression. We then used our established mouse glioma models, including astrocytomas by Ras or Ras+Akt and oligodendrogliomas induced by PDGF, to evaluate the impact on Foxg1 expression in vivo. Finally, we tested the ability of Foxg1 to induce gliomas both alone and combined with other oncogenic abnormalities. Glia transformed by Ras and Ras+Akt exhibited elevated Foxg1 expression. Pharmacologic inhibition of Ras signaling reduced Foxg1 expression. These results suggest that Ras activity induces Foxg1 expression or selects cells expressing Foxg1. Experiments to evaluate the impact of Ras, Akt, and PDGF on Foxg1 in modeled gliomas in vivo are ongoing as are experiments to determine the ability of Foxg1 to cause gliomas. The forkhead transcription factor Foxg1 is critical for normal brain development and is overexpressed in both low- and high-grade human gliomas. By modeling the pathways driving human glioma growth, we demonstrated that Ras signaling may be particularly important for the expression of Foxg1 in glia. We are validating these results in vivo with mouse glioma models and are also testing the gliomagenic capacity of Foxg1. The project described was made possible in part by grant number CA009512 from the National Cancer Institute and by the America Brain Tumor Association.

328. IDENTIFICATION OF PROTEIN MARKERS FOR BRAIN CANCER USING EXPRESSION PROTEOMICS

A. Khalil,[1,4] O. Persson,[2] L. Salford,[2] B. Widegren,[3] and P. James[1]; [1]Department of Protein Technology; [2]Department of Neurosurgery and The Rausing Laboratory, and [3]Division of Tumor Immunology, Lund University, Sweden; [4]Department of Protein Research, Mubarak City for Science, Alexandria, Egypt

Gliomas comprise nearly one-half of primary brain tumors and one-fifth of all primary spinal cord tumors. Combined, grade III and IV gliomas represent about 40% of all primary brain tumors in patients aged 40 to 49 years, and 60% in patients older than 60 years. In most clinical series, grade III tumors comprise approximately 10% and grade IV 90% of the total number of high-grade, malignant primary brain tumors. We have carried out DIGE (Difference gel electrophoresis) analysis on glioma biopsies. The DIGE technology enables different samples to be run on the same gel by pre-labeling the samples with three different cyanine dyes. Each sample is covalently labeled with a different dye from mass and charge-matched set of fluorescent CyDyes, cyanine 2 (Cy2), cyanine 3 (Cy3), and cyanine 5 (Cy5). Effectively, gels can be standardized by using one sample as an internal standard (pool sample). The pool sample is prepared by mixing equal amounts of protein from each individual homogenate. Proteomics promises the discovery of biomarkers and tumor markers for early detection and diagnosis, novel protein-based drug targets for anticancer therapy, and new end points for the assessment of therapeutic efficacy and toxicity. The focus is using the DIGE 2D system to enable large-scale analyses of samples to generate data sets that can be used to differentiate between the various brain cancer stages and types. Fifty human brain tissues were run in duplicate, together with an internal pool sample on each gel. Protein was extracted with buffer I (2% ASB-16, 8 M urea, 5 mM magnesium acetate, 20 mM Tris-base) and rehydrated in buffer II (4% CHAPS, 7 M urea, 2 M thiourea, 5 mM magnesium acetate, 1.2 mM Destreak, 1% IPG buffer, 20 mM Tris-base pH 8.5). The standard sample is ideally a pool comprising equal amounts of each of the 50 brain tissues being compared. The 150 images were acquired, and then spot detection and alignment was carried out using the Decyder software. In addition, the gels for picking protein spots were run. The spot cut list was generated covering proteins whose expression levels were changing, as well as set of marker proteins that were constant, throughout the DIGE experiment. The proteins of interest were automatically picked, in-gel digested, and then finally fingerprinted by MALDI-TOF. Reproducible DIGE spot maps of brain tumor proteins were obtained. The protein spots were occasionally distributed over the whole gel but more concentrated in the pH range 4–7. The protein analyses gave high confidence identifications for 118 spots cut from the preparative gel. One main protein that was clearly dramatically upregulated was astrocyte glial fibrillary acidic protein. This marker used by histologists to determine whether a tumor is a glioma or not. A low-sulfate fragment of the chondroitin sulfate proteoglycan called brevican was also identified which known to be excreted by tumor cells of patients with brain tumors. A series of proteins were identified in this preliminary analysis that include known generic tumor markers such as 78 kDa glucose-regulated protein, annexin I, and several carbonic anhydrase isoforms. Other proteins that were thought to be tissue-specific markers were found, and a number of interesting finds will be presented and further discussed. The day will come when people will be screened for hundreds of diseases through a simple blood test if our vision is fulfilled. Through "proteomics," conditions like human cancer will be diagnosed at a stage early enough so that there will be a good chance they can be treated and cured.

329. IDENTIFICATION OF A NOVEL HOMOZYGOUS DELETION REGION AT 6Q23.1 IN MEDULLOBLASTOMAS USING HIGH-RESOLUTION ARRAY CGH ANALYSIS

H. Ng,[1] A. Hui,[1] H. Takano,[2] K. Lo,[1] W. Kuo,[2] C. Lam,[1] C. Tong,[1] C. Chang,[1] and J. Gray[2]; [1]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China; [2]Laboratory Medicine, University of California, San Francisco, California, USA

We used a high-resolution array comparative genomic hybridization analysis for whole-genome analysis of medulloblastomas. Although there have been many genomic surveys of medulloblastomas in the literature, such an approach has not been performed before. We identified several consistent chromosomal aberrations. These included consistent chromosomal gains of 2p23-p25 (52.63%), 7 (57.89%), 9q34 (47.37%), and 17q11-q25 (89.47%), as well as losses of 3q25.33-q26.32 (57.9%), 4q31-33 (42.1%), 6q22-27 (57.9%), 8p21.3-23 (78.95%), 10q22-26 (57.9%), 16q22-q24 (63.16%), and 17p13 (31.6%). This information will lead to a better understanding of medulloblastoma tumorigenesis and identify new molecular markers for therapeutic intervention and drug discovery. One of the most notable findings is homozygous deletion on chromosome 6q23. Homozygous deletion of this region was found on cell line DAOY, whereas copy loss was detected on 30% primary medulloblastomas. Further study using 30 pairs of STS markers confined a 0.887 Mb minimal region of homozygous deletion at 6q23.1 which was flanked by markers SHGC-14149 (6q22.33) and SHGC-110551 (6q23.1). Quantitative RT-PCR analysis showed complete loss of expression of 2 genes located at 6q23.1, AK091351 (hypothetical protein FLJ34032) and KIAA1913, on cell line DAOY. mRNA level of these genes was reduced in cell lines D283 and D384, as well as 50% (AK091351) and 70% (KIAA1913) of 10 primary tumors. Frequent detection of reduced expression of AK091351 and KIAA1913 implicated that these 2 genes may play a critical role in medulloblastoma tumorigenesis.

330. MOLECULAR CLASSIFICATION OF HUMAN GLIOMAS USING MATRIX-BASED COMPARATIVE GENOMIC HYBRIDIZATION

P. Roerig,[1] M. Nessling,[2] B. Radlwimmer,[2] S. Joos,[2] G. Wrobel,[2] C. Schwaenen,[3] *G. Reifenberger*,[1] and P. Lichter[2]; [1]Neuropathology, Heinrich-Heine-University, Duesseldorf; [2]Division of Molecular Genetics, Deutsches Krebsforschungszentrum, Heidelberg; [3]Department of Internal Medicine, University of Ulm, Ulm; Germany

Gliomas are morphologically, biologically, and clinically heterogeneous brain tumors whose classification is based on histological features. However, evidence is increasing that the different glioma types are associated with distinct genetic aberrations, which may provide useful information for tumor classification as well as prediction of prognosis. To facilitate the molecular classification of gliomas, we established a microarray that consists of genomic clones representing tumor suppressor genes, proto-oncogenes and chromosomal regions frequently gained or lost in gliomas, as well as reference clones distributed evenly throughout the genome in approximately 15 Mbp intervals. These microarrays were used for matrix-based comparative genomic hybridization (matrix CGH) analysis of 70 gliomas. Matrix CGH results were validated by molecular genetic analyses of candidate genes, loss of heterozygosity studies, and chromosomal CGH. Our results indicate that matrix CGH allows for the sensitive and specific detection of gene amplifications, as well as low-level copy number gains and losses in gliomas. Furthermore, molecular classification based on matrix CGH data closely paralleled histological classification, e.g., enabled clinically important differential diagnoses, such as diffuse astrocytoma versus oligodendroglioma, anaplastic astrocytoma versus anaplastic oligodendroglioma, anaplastic oligodendroglioma versus glioblastoma, as well as primary versus secondary glioblastoma. Thus, matrix CGH is a powerful technique that allows for an automated genomic profiling of gliomas and represents a promising tool for their molecular classification.

331. DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF TRANSFORMING GROWTH FACTOR-BETA (TGF-BETA) SIGNALING AND THE IMPACT OF A SPECIFIC LOW-MOLECULAR-WEIGHT TGF-BETA INHIBITOR IN HUMAN MALIGNANT GLIOMA CELL LINES

O. Alzate,[1] C. Osorio,[1] M. Herbstreith,[1] M. Hjelmeland,[2] R. Buechler,[1] M. Ehlers,[1] and *J. Rich*[3]; Departments of [1]Neurobiology, [2]Surgery, and [3]Medicine, Duke University Medical Center, Durham, North Carolina, USA

Malignant gliomas remain among the most lethal forms of cancer, with resistance to radiation and chemotherapy. The identification and targeting of pathways critical to the phenotype of cancers offer new hopes in the treatment of many patients. The multifunctional cytokine transforming growth factor-β (TGFβ) is expressed by grade III/IV gliomas and promotes tumor angiogenesis, invasion, and immune escape. We have recently demonstrated that a novel low-molecular-weight inhibitor of type I TGFβ receptor kinase activity, SB-431542, blocks TGFβ signal transduction and TGFβ -mediated expression of VEGF and invasion. We have noted that human glioma cell lines respond differentially to both TGFβ and SB-431542. We now seek to determine markers of cellular response to SB-431542 through protein expression differences using two-dimensional differential in gel electrophoresis (2D-DIGE) proteomics. This is a powerful technique that allows for the identification of protein expressional changes between control and target samples. We calculated the relative expression of proteins undergoing different treatments with DeCyder software (Amersham), designed for analysis in DIGE proteomics, assuming a threshold of 1.67-fold (2 standard deviations). We treated a human glioma cell line that is responsive to SB-431542 with vehicle control, SB-431542 (1 μM), TGFβ (100 pM), or the combination of these two agents. We found that SB-431542 modestly impacted protein expression when administered alone, whereas TGFβ significantly induced the expression of 11 proteins and repressed the levels of 5 proteins. Of these proteins, SB-431542 treatment blocked the TGFβ-induced expression of 5 proteins. The identities of these proteins are currently under confirmation and validation. In addition, these studies will be extended to other glioma cell lines with variable responses to both TGFβ and SB-431542. These results suggest that we can identify potential markers of response to low-molecular-weight signal transduction inhibitors as well as discover proteins downstream of specific signal transduction pathways both dependent and independent of specific kinases. This work was supported in part by funds from the Pediatric Brain Tumor Foundation of the United States, Accelerate Brain Cancer Cure, and NIH grant NS047409 (J.N.R.). J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

332. PSEUDOPODIAL PROTEOMICS OF GLIOMA CELLS REVEAL THE SIGNALING AND ENERGY PATHWAYS ACTIVATED IN MIGRATION

M.E. Beckner,[1] X. Chen,[3] J. An,[3] B.W. Day,[3] and I.F. Pollack[2]; [1]Pathology, [2]Neurological Surgery, and [3]The Genomics and Proteomics Core Laboratories, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

The invasive migration of malignant gliomas (astrocytomas) leads to treatment failure. In vitro, U87 glioma cells exhibited impressive migration. Although LN229, LN18, U373, C6, and T98G glioma cells migrated to hepatocyte growth factor (HGF) with fetal bovine serum (FBS) present, U87 cells migrated at comparable levels with only FBS present under normoxia and hypoxia. All of the migratory glioma cell types expressed Met. Invasive cell migration is initiated by extension of pseudopodia into interstitial spaces. In this study, U87 cells formed pseudopodia in vitro as cells pushed through 3-μm pores of polycarbonate membranes. Harvesting pseudopodia in a novel 2-step method provided material for proteomic analysis. Differences in the protein profiles of pseudopodia and whole cells were found by using differential gel electrophoresis (DIGE) and immunoblotting. Proteins from 2-dimensional (2D) gels were identified by peptide mass fingerprinting analysis (PMF) using mass spectrometry. For DIGE, lysates of pseudopodia and whole cells were each labeled with electrophilic forms of fluorescent dyes, Cy3 or Cy5, and analyzed as mixtures. Analysis was repeated with reciprocal labeling. Differences in protein distributions were detected by manual inspection and computer analysis. Pseudopodial proteins in Coomassie-stained 2D gels included isoforms of glycolytic enzymes as the largest group, 7 of 24 proteins. PMF analysis of DIGE gels demonstrated increased isoforms of annexin (Anx) I, AnxII, enolase, pyruvate kinase, and aldolase, and decreased mitochondrial manganese superoxide dismutase and transketolase in pseudopodia. Specific antibodies showed localization of the HGF a chain (activated form) to pseudopodia and increased pseudopodial Met, actin, and total AnxI (Beckner et al., Lab Invest, in press). Phosphorylated epidermal growth factor receptor was also increased in pseudopodia. The proteome of U87 glioma cell pseudopodia includes activated signaling pathways for migration growth factors/cytokines, potential downstream targets, and components of the glycolytic pathway to energize the cytoskeleton. Pseudopodia of other glioma cell lines are being analyzed for comparison. Identification of pseudopodial proteins offers targets for suppression of tumor invasion. This work was supported by The Nick Eric Wichman Foundation, Ellicott City, Maryland, USA, and The Pittsburgh Foundation's Walter L. Copeland Fund for Cranial Research, Pittsburgh, Pennsylvania, USA.

333. MICRO-GENOMICS OF SCHWANN CELLS IN PERIPHERAL NERVE SHEATH TUMORS (PNST)

A. Pandita,[1] L. Bereskin,[1] J. Wiley,[1] Z. Karim,[1] A. Okhawat,[1] P. Shannon,[1] and *A. Guha*[1,2]; [1]Neurosurgery Division, Toronto Western Hospital; [2]Neurosurgery Division, Labatts Brain Tumor Centre, HSC, Toronto, Canada

Neurofibroma (nfib) subtypes have varying biology with dermal nfibs remaining benign, plexiform nfibs having an ~0% risk of becoming malignant termed MPNST. Although consisting of many cell types, Schwann cells are the primary transformed cells in all subtypes of nfibs. Schwann cell transformation is a result of common bi-allelic inactivation of *Nf1* and neurofibromin expression, in addition to alterations at the genomic level which likely confer their varying biological properties. Limited conventional CGH studies on nfibs have not yet led to an understanding of these additional genetic alterations in the Schwann cells of these nfib subtypes. Schwann and endothelial (control) cells were isolated using laser capture microdissection to create a high-resolution genetic alteration map using array-CGH analysis on BAC and cDNA arrays. In total to date, 8 plexiform nfibs and 8 MPNSTs have been analyzed. Genetic losses were more prevalent in the plexiform nfibs, compared to MPNSTs, where gains were more common. Loss of regions on chromosomes 1, 2, 10, 13, 17, and 18 were common in both benign and malignant tumors. Gains on chromosomal arms 4q, 5p, 6q, 8q, 10q, 11q, 13q, and 17q were commonly found in MPNSTs. Genes located in regions lost in both plexiform nfibs and MPNSTs are those potentially involved in initiating nfibs. Similarly, regions of gain found in MPNSTs are likely involved in progression from plexiform nfibs to MPNSTs. These array-CGH results will be confirmed using FISH and/or real-time PCR. FISH analysis using BAC probes for the *Nf1* gene corroborated the findings of the array analysis. Currently, we are utilizing the available NCBI databases to screen for potential novel oncogenes and tumor suppressor genes that would be present in these chromosomal regions. These candidate genes will be further utilized to screen for the frequency of the alteration in a larger cohort of tumors. Analysis of dermal nfibs is ongoing.

334. REAL-TIME QUANTITATIVE PCR REVEALS LOSS AT 22q12.3-13.3 IN TWO THIRDS OF PEDIATRIC EPENDYMOMA AND DEFINES A MINIMUM DELETION OF 3.4MB

B. Suarez-Merino,[1] R. Hayward,[2] W. Harkness,[2] D. Thompson,[2] K. Phipps,[2] D. Thomas,[3] and *T. Warr*[1]; [1]Department of Molecular Neuroscience, Institute of Neurology, London; [2]Department of Neurosurgery, Great Ormond Street Hospital for Sick Children NHS Trust, London; [3]Institute of Neurology, Division of Neurosurgery, London; UK

Ependymomas are glial cell–derived tumors which arise from the ependymal lining of the ventricular system of the central nervous system. They manifest preferentially in childhood, where they account for up to 10% of intracranial tumors. At present, the genetic events that contribute to the pathogenesis of this pediatric malignancy remain essentially unknown, with up to 50% of tumors appearing karyotypically normal. One of the most common chromosomal aberrations reported in intracranial ependymoma is monosomy of 22 or structural abnormalities of 22q, suggesting the presence of a tumor suppressor gene on that chromosome. Previously, by expression microarray analysis and real-time quantitative PCR (Q-PCR) we identified an interstitial deletion at 22q12.3-13.3 in 6 pediatric samples. In this study we used Q-PCR based deletion analysis to investigate seven genes mapping to 22q11.3-22q13.3 in a series of 34 pediatric ependymoma and 32 normal blood controls. The seven genes analyzed were phosphatidylinositol 4-kinase (*PIK4CA*) mapping to 22q11.21, *FBX7* (22q12.3), which may be implicated in phosphorylation-dependent ubiquitination, the RNA binding protein *RBM9* (22q12.3), *MFNG* (22q13.1), which may be involved in the Notch pathway, the ,-catenin interacting protein coding gene *C22orf2* (22q13.1), the chromobox homolog *CBX7* (22q13.1), and the SET binding factor *SBF1* (22q13.3). Overall, we observed loss of 22q in 65% of cases, higher than previously reported in pediatric ependymoma using other methodologies. The most common region of loss maps between loci *RBM9* and *CBX7*, spanning 3.4 Mb of chromosome 22. Mutation analysis of two candidate genes mapping to this region (*C22orf2* and *CBX7*) did not reveal any mutations. Further analysis of the promoter region of *CBX7* in tumors showing underexpression of the *CBX7* transcript but no allele loss revealed that this promoter is not methylated and therefore other mechanisms, such as histone deacetylation, may be responsible for the silencing of the *CBX7* gene in our cohort. Our results provide further evidence that one or more tumor suppressor genes critical for ependymoma development are located at 22q12.3-13.1. Further analysis of this region is necessary to identify candidate transcripts.

335. WHOLE GENOME CDNA MICROARRAY ANALYSIS OF GLIOMAS: A SUBSET OF GENES DIFFERENTIATES INVASIVE FROM LESS OR NON-INVASIVE LOW-GRADE LESIONS

L. Mariani,[1] M. Primig,[2] C. Wiederkehr,[2] and *M. Schobesberger*[1]; [1]Universitätsklinik für Neurochirurgie, Inselspital, Bern; [2]Swiss Institute of Bioinformatics, Biozentrum, Basel; Switzerland

Diffuse gliomas WHO grade II-IV are generally not amenable to cure. Their ability to invade the surrounding normal parenchyma diffusely and eventually regrow the tumor bulk is unique among cancer types and is probably the main obstacle for therapeutic intervention. The invasive potential of these tumors is proportional to the tumor grade. However, magnetic resonance imaging (MRI) and surgery can reveal striking differences in the invasion of the brain in tumors sharing the same histopathological characteristics and grade, e.g., in WHO grade II gliomas. The aim of this large-scale microarray study was to identify genes differentiating invasive from less- or non-invasive tumors. Tissue samples from 18 gliomas (WHO grades I to IV), 2 brain metastases, and 4 "normal" brains were collected during surgery and immediately transferred into RNA later for storage. Tumors were graded for their invasive behavior according to MRI (T2-weighted and FLAIR-sequence). Total RNA was isolated from the samples and processed according to standard protocols for use in the GeneChip Human Genome U133 Plus 2.0 Array from Affymetrix which allows analysis of the expression level of over 47,000 transcripts and variants. The data were analyzed by hierarchical and two-way clustering algorithms. Five-hundred eight genes with a maximum intensity >5 across all the samples (19,572 genes) and a P value <0.01 after a permutation test were identified and selected. These probesets allowed clustering of the samples according to histological diagnosis and WHO grade of malignancy. Invasive and non- or less-invasive low-grade gliomas could be differentiated by hierarchical and coupled two-way clustering. Whole-genome expression analysis of low-grade gliomas identifies subsets of genes differentially expressed in highly and non- or less invasive tumors.

336. FUNCTIONAL ASSESSMENT OF GBM-DERIVED COMPOSITE BAC CLONES IDENTIFIED USING END SEQUENCE PROFILING

G. Hodgson,[1] S. Volik,[2] and C. Collins[2]; [1]Neurosurgery; [2]University of California San Francisco, Cancer Research Institute, San Francisco, California, USA

End sequence profiling (ESP) is a novel technique that reveals structural alterations in tumor genomes such as translocations, inversions, deletions, and amplifications. To accomplish this, both ends of a number of BAC clones from a tumor genome–derived BAC library are sequenced and mapped onto the assembled normal human genome sequence. Recent ESP studies on breast, brain, and prostate tumors and cell lines have identified a number of structurally aberrant genomic clones. While the functional significance of these clones remains to be determined, one provocative hypothesis is that they encode novel composite transcripts important for tumor development, akin to the BCR-ABL fusion gene. Indeed, fusion transcripts have been identified by transcript ESP in breast cancer cell line MCF7. To test whether glioblastoma (GBM)-derived composite BACs are functionally significant for GBM formation, we are testing their effects on proliferation, apoptosis, and motility when transfected into immortalized human astrocytes and brain tumor cell lines. Phenotypic alterations in response to transfection are being measured by using a combination of flow cytometry and high-content automated imaging microscopy. A total of 5 BACs are currently under investigation, each of which has been draft sequenced and fingerprinted in respective collaborations with the Joint Genome Institute and the Genome Sciences Centre at UBC to confirm their composite nature. Results from these studies will be presented at the conference. These studies take an important first step toward discovery of novel tumorigenic mechanisms in brain cancer and potential development of tumor specific markers and therapeutics.

337. DNA HYPERMETHYLATION OF MULTIPLE TUMOR SUPPRESSOR GENES IN DIFFERENT TYPES OF MENINGIOMAS

J. Kim, H. Kim, and Y. Shim; Department of Neurological Surgery, Asian Medical Center, Seoul, Korea

Aberrant methylation of CpG islands in promoter of human genes is known as an alternative mechanism of gene silencing that contributes to tumorigenesis in various human tumors. We have examined methylation status of 5 tumor suppressor genes in 81 human meningiomas (61 benign, 12 atypical, and 8 malignant meningiomas) by methylation-specific polymerase chain reaction to determine roles for DNA methylation during

transformation to meningioma. Five tumor suppressor genes including *p16*, *p14*, *RB*, *RASSF1A*, and *E-cadherin* showed different profile of methylation status on CpG islands in their promoter regions depending on stages of meningioma. Methylation frequencies of tumor suppressor genes in benign, atypical and malignant meningiomas were 42.6%, 66.7%, and 50.0% for *p16*; 16.4%, 50.0%, and 25.0% for *p14*; 42.6%, 41.7%, and 37.5% for *RB*; 1.0%, 8.0%, and 12.5% for *RASSF1A*; 41.0%, 66.7%, and 12.5% for *E-cadherin*, respectively. Except *RASSF1A*, other tumor suppressor genes showed relatively high methylation status in meningioma. Forty-six of 61 (75.4%) benign meningiomas, 7 out of 8 (87.5%) malignant meningiomas, and all 12 cases (100%) of atypical meningiomas showed hypermethylation in at least one of the test genes. Surprisingly, DNA was methylated more frequently in atypical meningiomas than in any other tumor stages: 66.7% for *p16*, 50% for *p14*, 41.7% for *RB*, and 66.7% for *E-cadherin*. Our results suggest that DNA methylation is a frequent and an early event in tumorigenesis of meningiomas, and it appears to be a major molecular mechanism for meningiomas, especially for atypical type.

338. THE P15INK4B/P16INK4A/RB1 PATHWAY IS FREQUENTLY DEREGULATED IN HUMAN PITUITARY ADENOMAS

A. Ogino, Y. Katayama, A. Yoshino, T. Watanabe, T. Fukushima, T. Yokoyama, C. Komine, and T. Ota; Neurosurgery, Nihon University School of Medicine, Tokyo, Japan

Pituitary adenomas are common benign intracranial neoplasms. However, their tumorigenesis is not yet clearly defined. Inactivation of genes involved in the negative cell cycle regulatory $p15^{INK4b}$-$p16^{INK4a}$-cyclin D/CDK4-RB1–mediated pathway (RB1 pathway) is one of the most common and important mechanisms in the growth advantage of tumor cells. Recently, much attention has been focused on the importance of alternative mechanisms of gene inactivation, particularly promoter hypermethylation in the transcriptional silencing of such tumor suppressor genes. Based on the rare occurrence of inactivation by gene mutations and deletions of the RB1 pathway in pituitary adenomas, we investigated the deregulation of the RB1 pathway in 42 sporadic human pituitary adenomas, especially focusing on the methylation status of this pathway as determined by a methylation-specific PCR assay. Homozygous deletion of the $p15^{INK4b}$ or $p16^{INK4a}$ gene was detected in one adenoma each. Amplification of the *CDK4* gene was not apparent in any of the pituitary adenomas presently examined. Promoter hypermethylation of the $p15^{INK4b}$, $p16^{INK4a}$, and *RB1* genes was detected in 15 (35.7%), 30 (71.4%), and 12 (28.6%) of the adenomas, respectively. Promoter hypermethylation of the $p15^{INK4b}$ gene coincided with $p16^{INK4a}$ alteration and/or *RB1* methylation, whereas $p16^{INK4a}$ and *RB1* methylations tended to be mutually exclusive ($I = 0.019$). Thus, the vast majority of the adenomas (38/42, 90.5%) displayed alterations of the RB1 pathway. None of the clinicopathological features including the proliferation cell index was significantly correlated with any particular methylation status. Our results suggest that inactivation of the RB1 pathway may play a causal role in pituitary tumorigenesis, with hypermethylation of the $p16^{INK4a}$ gene being the most common deregulation, and further provide evidence that *RB1* and $p16^{INK4a}$ methylations tend to be mutually exclusive but occasionally coincide with $p15^{INK4b}$ methylation.

339. COMPREHENSIVE GENE EXPRESSION ANALYSIS OF HUMAN MALIGNANT GLIOMA USING CDNA MICROARRAY FOR THERAPEUTIC STRATEGY

H. Takahashi,[1] J. Kouno,[1] and A. Teramoto[2]; [1]Neurosurgery, Nippon Medical School Dai-ni Hospital, Kanagawa; [2]Neurosurgery, Nippon Medical School Hospital, Tokyo; Japan

The treatment of malignant brain tumors, especially malignant gliomas, is still extremely difficult, and there are many indistinct points in the molecular mechanisms participating in growth and development of malignant brain tumors. Thus we tried to extract related genes by comparing a gene expression profile of malignant glioma according to malignancy grade and to apply such results to a clinical treatment. We extracted mRNA from 22 surgical specimens, comprising 8 diffuse astrocytomas (grade II), 6 anaplastic astrocytomas (grade III), 8 glioblastomas (grade IV), by a conventional method, and the microarray analysis of complementary DNA (cDNA) using commercial human brain total RNA (Clontech company) as control was done. The interferon (IFN) effect prediction tip on which 775 genes were selected (MBC Company) was used for analysis of human malignant glioma as the basis for building a therapeutic strategy. Manifest changes were recognized by 32 genes when having compared grade IV with grade II, and significant variations were recognized by 12 out of 32 genes. There was restraint tendency of gene expression level in grade IV generally, and in particular expression of interferon reference genes were restrained. We were able to identify the gene cluster which varied with malignancy grade by analyzing a gene expression profile with cDNA microarray. This study revealed that the building of a therapeutic strategy against grade IV astrocytomas, which are very difficult to treat, is enabled on the basis of this gene information.

340. COMBINED IMMUNOCHEMOTHERAPY WITH REDUCED DOSE WHOLE BRAIN RADIOTHERAPY (WBRT) FOR NEWLY DIAGNOSED PATIENTS (PTS) WITH PRIMARY CNS LYMPHOMA (PCNSL)

G. Shah,[1] R. Lai,[2] J. Yahalom,[1] D. Correa,[1] L. DeAngelis,[1] J. Raizer,[3] D. Schiff,[4] R. LaRocco,[5] B. Grant,[6] and L. Abrey[1]; [1]Memorial-Sloan Kettering Cancer Center, New York, New York, USA; [2]McMaster University, Hamilton, Ontario, Canada; [3]Neurology, Northwestern University, Chicago, Illinois, USA; [4]Neurology, University of Virginia Medical Center, Charlottesville, Virginia, USA; [5]Kentuckiana Cancer Institute PLLC, Louisville, Kentucky, USA; [6]University of Vermont, Burlington, Vermont, USA

High-dose methotrexate (MTX)-based chemotherapy in combination with WBRT for pts with newly diagnosed PCNSL has been shown to improve disease control and survival. However, disease recurrence is common, and treatment-related neurotoxicity is an increasingly recognized complication. The addition of rituximab has improved survival and disease control in systemic non-Hodgkin's lymphoma. In a phase 2 trial we added rituximab to MTX, procarbazine, and vincristine (R-MPV) and decreased the dose of WBRT in pts who achieved a complete response (CR) after chemotherapy to diminish the risk of treatment-related neurotoxicity. Twenty-seven (12 men; median age 57, range 32–71; median KPS 80%) of a planned 30 pts were enrolled from August 2002 to October 2004. Three patients deemed ineligible for the study were not treated. Each pt received rituximab 500 mg/m^2 on day 1 and MTX 3.5 g/ m^2 with vincristine 1.4 mg/m^2 on day 2. Procarbazine 100 mg/m^2 a day was given for seven days during odd-numbered cycles. Pts achieving a CR after 5 cycles received dose-reduced WBRT (2340 cGy), while pts with less than a CR received 2 more cycles, after which they received standard WBRT if a CR was still not achieved. All pts received two cycles of Ara-C 3 g/m^2 after WBRT. Sixteen pts were followed with prospective neuropsychological evaluations. CSF + serum levels of rituximab were assayed in pts with an Ommaya reservoir. Fifteen of the 23 treated pts have been assessed for response after R-MPV (five pts are still on study, two pts were taken off study for toxicity including grade 3 nephrotoxicity and death from neutropenic sepsis in one pt each, and data on one pt is still being gathered). Eleven of the fifteen (73%) had a CR, two (13%) a partial response (PR) for an overall response rate of 93%, and two (13%) progressed. The median number of cycles of R-MPV received was five, with four patients needing seven cycles to achieve a CR. The eleven pts with a CR received 2340 cGy of WBRT, while one pt with a PR received 4500 cGy and one received 3640 cGy. The most common grade 3 or 4 toxicities were granulocytopenia (34%), hyponatremia (24%), and hyperglycemia (19%) followed by hepatotoxicity, thrombocytopenia, coagulopathy, hypophosphatemia, hypokalemia, and anemia (10%–15% each). No grade 3 or greater neurotoxicity has been reported to date. The safety and efficacy of R-MPV is comparable to MPV. Further investigation of this regimen in PCNSL is warranted.

341. VACCINATION OF RECURRENT GLIOMA PATIENTS WITH TUMOR LYSATE-PULSED DENDRITIC CELLS ELICITS IMMUNE RESPONSES: RESULTS OF A CLINICAL PHASE 1/2 TRIAL

J. Homa,[1] R. Yamanaka,[1] T. Abe,[2] M. Narita,[2] M. Takahashi,[2] and R. Tanaka[1]; [1]Department of Neurosurgery, Brain Research Institute, Niigata University; [2]Department of Hematology, Niigata University School of Medicine, Niigata University, Niigata; Japan

The objective of this study was to investigate the safety and immunological responses of dendritic cells therapy pulsed with autologous tumor lysate in patients with malignant glioma. Twenty-two patients with malignant glioma (16 and 6 patients with grade 4 and grade 3 gliomas, respectively) entered in the phase 1/2 clinical study of dendritic cell vaccination. All patients were recurrent malignant glioma patients who were resistant to the standard maximum therapy. Dendritic cells were seven days maturation in GM-CSF (1000 IU/ml) and IL-4 (500 IU/ml), RPMI-1640 supplemented with 1% autologous serum, pulsed with autologous tumor cell lysate and activated with OK 432 (0.1KE/ml). The mean numbers of vaccinations of tumor lysate-pulsed dendritic cells were 6.2 times intradermally close to a cervical lymph node and 4.7 times intratumorally via an Ommaya reservoir. Clinical responses were 1 partial response, 2 minor responses, and 8 cases of no change evaluated by radiological findings. Dendritic cell vaccination

elicited T-cell-mediated antitumor activity, as evaluated by ELISPOT assay after vaccination in 6 of 14 tested patients. This protocol was generally well tolerated. This study demonstrated the safety and antitumor effects of autologous tumor lysate-pulsed dendritic cell therapy for patients with malignant glioma.

342. BEVACIZUMAB AND CPT-11 IN THE TREATMENT OF RELAPSED MALIGNANT GLIOMA

V. Stark-Vance; Neurosciences, Presbyterian Hospital, Dallas, Texas, USA

Bevacizumab (Avastin) is a monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF). CPT-11 is a semisynthetic camptothecin that binds to and inhibits DNA topoisomerase I, an enzyme necessary for DNA replication. This combination of agents has shown clinical benefit in a clinical trial of metastatic colorectal cancer, while bevacizumab alone was associated with inferior survival in the same population. In phase 2 clinical trials of CPT-11 in patients with recurrent glioma, 4/59 pts demonstrated tumor regression. Some malignant gliomas overexpress VEGF, suggesting that bevacizumab may have clinical activity in this population, possibly increasing the response rate over CPT-11 alone. Between March and December 2004, 21 patients with malignant glioma treated with this combination (BC) were evaluated for treatment response and toxicity. A 6-week cycle consisted of bevacizumab, 5 mg/kg, every other week × 2, and CPT-11, 125 mg/m^2 every week × 4, followed by a 2-week rest. MRI scans were obtained after each cycle of treatment. Bevacizumab was not dose-reduced, but CPT-11 doses were reduced for grade 3 or 4 myelosuppression. Of the 21 pts, 11 were GBM and 10 were other high-grade gliomas; median age was 42 (30–73) and median number of prior treatments was 3 (2–10). Toxicities included neutropenia, diarrhea, epistaxis, emesis, and asthenia. One pt died of an intracranial hemorrhage and one pt died of complications of gastrointestinal perforation; both are previously reported toxicities of bevacizumab. Four additional pts died of non-treatment-related complications. Of the 21 pts who could be evaluated for response, there were 1 CR, 8 PRs, and 11 SD. Thirteen pts remain on treatment, 5 after more than 4 cycles. In all pts, radiographic responses were accompanied by reduction in both peritumoral edema and contrast enhancement; most patients who did not meet the criteria for partial response did show clinical and/or radiographic improvement. These early results suggest an improvement in response rate and duration of response over treatment with CPT-11 alone and a possible role for bevacizumab in primary therapy for high-grade glioma.

343. THE FAS/FAS LIGAND PATHWAY FAILS TO INDUCE APOPTOSIS IN EXPERIMENTAL GLIOMA CELLS AND COULD BE RESPONSIBLE FOR THE APOPTOSIS OF T-CELL SURROUNDING MALIGNANT GLIOMAS IN VIVO

F. Legnani,[1] G. Pradilla,[2] Q. Thai,[2] B. Tyler,[2] G. Zamora,[2] S. Gaini,[3] F. DiMeco,[1] H. Brem,[2] and A. Olivi[2]; [1]Neurosurgery, Istituto Neurologico C. Besta, Milan, Italy; [2]Neurosurgery, Johns Hopkins University, Baltimore, Maryland, USA; [3]Neurosurgery, Università degli Studi di Milano, Milan, Italy

Human and experimental gliomas have been shown to express the Fas-receptor (FasR). Activation of this receptor by direct agonists could trigger caspase-3/8-mediated apoptosis. FasR-agonist-mediated apoptosis of tumor invading lymphocytes has also been demonstrated. Therefore, the role of Fas-ligand (Fas-L) as an anti-tumor pathway remains controversial. In this study we tested the ability of recombinant-FasL and of a FasR-agonist-antibody (Ab) to induce apoptosis in 9L cells, and we explain the tumor immune-evasion in the 9L-gliosarcoma model. FasR expression was determined in 9L, F98, U257, and U373 cells by Western blot. Cytotoxicity of recombinant-FasL and FasR-agonist-Ab was evaluated in vitro against 9L. Cells were treated with 50, 100, 250, and 500 ng/ml of FasL and FasR-Agonist-Ab. Percentage of cell viability was established by using the MTT assay. Statistical analysis was done using the Student *t* test ($P < 0.05$ was considered significant). For the in vivo studies, 33 Fischer 344 rats were intracranially implanted with 9L and perfused/fixed on day 10. Tumor samples were processed for immunohistochemical staining and confocal microscopy analysis. Monoclonal antibodies were used against CD3 (Mouse monoclonal, Serotec), Fas-L (Rabbit polyclonal, Santa Cruz Biotechnology), GFAP (Mouse monoclonal, NeoMarkers; Rabbit polyclonal, DAKO), and caspase-3 (Rabbit polyclonal) to identify lymphocytes, Fas-L, astrocytes, and apoptotic lymphocytes. A robust band at 48 kDa confirmed strong FasR expression in all cell lines tested. Treatment with recombinant-FasL at 50 ng/ml and 500 ng/ml decreased cell viability to 98% ± 0.03% and 95% ± 0.03%, respectively. Similarly, treatment with FasR-agonist-Ab at 50 ng/ml and 500 ng/ml decreased cell viability to 93% ± 5% and 96% ± 1%, respectively. Percentage of growth was statistically insignificant for the tested concentrations. Compared to controls, animals intracranially challenged with 9L exhibited significantly higher expression of FasL, caspase 3, and CD3+ cells. Apoptotic CD3+ cells were identified by co-localization of CD3 and caspase 3. CD3+ cells were identified peri- and intra-tumorally by distinctive clustering patterns in the center of the tumor. Although FasR was strongly expressed in all cell lines tested, the treatment with FasL and FasR-agonist-Ab failed to induce apoptosis. Under these experimental conditions, both FasR-agonist-Ab and recombinant-FasL are ineffective in treating brain tumors. Rats intracranially implanted with 9L gliosarcoma exhibit marked upregulation of Fas-ligand in GFAP+ tumor cells. The presence of apoptotic CD3+ cells suggests that a Fas-ligand-mediated immune-evasion mechanism is responsible for T-lymphocyte apoptosis in the 9L gliosarcoma model.

344. GPNMB: A NEW TARGET FOR HUMAN HIGH-GRADE GLIOMAS (HGL) IMMUNOTHERAPY

C. Kuan,[1] K. Wakiya,[1] J.M. Dowell,[2] J. Herndon,[2] G.J. Riggins,[3] M. Graner,[1] C.J. Wikstrand,[1] and D.D. Bigner[1]; [1]Pathology and [2]Cancer Center Biostatistics, Duke University Medical Center, Durham, North Carolina; [3]Neurosurgery, Johns Hopkins University, Baltimore, Maryland; USA

Targeting neoplastic HGL cells for treatment with monoclonal antibody (MAb) constructs requires markers expressed on the cell surface of gliomas but not normal brain. We here identified promising new HGL tumor targets, human transmembrane glycoprotein nmb ($GPNMB_{wt}$), and a splice variant form ($GPNMB_{sv}$) leading to an in-frame insertion of 12 amino acids in the extracellular domain (ecd). We have performed genetic and immunohistochemical (IHC) evaluation of human gliomas to determine incidence, distribution, and pattern of localization of GPNMB antigens in brain tumors as well as survival analyses. We have generated anti-GPNMB antibodies by immunizing rabbits and mice with plasmid DNA coding the ecd of GPNMB, followed by boost with corresponding recombinant proteins. To determine the GPNMB mRNA transcript and protein expression levels in glioma samples, we have performed quantitative RT-PCR (49 cases) and IHC (140 cases) in HGL patient cases and glioma GPNMB transfected cell lines. We also characterized five monoclonal antibodies using Western blotting, BIAcore, Scatchard, and FACs analyses. Survival analyses were also conducted based upon IHC information from 80 newly diagnosed patients (63 glioblastoma multiforme [GBM] and 17 anaplastic astrocytomas [AA]) and the RNA expression data from 29 newly diagnosed patients (26 GBM and 3 AA). The real-time PCR results from 49 HGL biopsies indicated 31/36 (86%) GBM, 3/6 (50%) AA, and 3/7 (43%) anaplastic oligodendrogliomas (AO) were positive for gpnmb RNA transcripts ($gpnmb_{wt}$ plus $gpnmb_{sv}$). $gpnmb_{sv}$ was detected in 16/36 (44%) GBM and 4/6 (67%) AA. In normal brain samples, little or no expression was noted for $GPNMB_{wt}$ and $GPNMB_{sv}$ mRNA. We have obtained and characterized anti-GPNMB polyclonal rabbit antiserum #2640 as well as two IgG_1 MAbs, G11 and A3, and three IgG_{2b} MAbs, U2, U4, and U5. The binding affinity constant K_A of MAbs to GPNMB was 2.7 to 96 × 10^7 M^{-1} and 7.6 to 47 × 10^7 M^{-1} by BIAcore and Scatchard analysis, respectively. A larger HGL study (n = 140) revealed detectable GPNMB by IHC with antiserum #2640 and MAb G11 in a membranous and cytoplasmic pattern, with occasional focal perivascular reactivity in 21/32 (66%) AA and 75/108 (70%) GBM. Quantitative flow cytometric analysis of 11 GPNMB positive GBM fresh biopsy specimens revealed GPNMB cell surface molecular density at 1.1 to 7.8 × 10^4 molecules/cell, levels sufficient for MAb targeting. The survival analysis demonstrated newly diagnosed AA/GBM patients over the age of 45 have a higher risk of death (hazard ratio of 2.5). Significantly, in this population (AA/GBM or GBM alone) univariate analyses show that patients with relative mRNA transcript levels greater than 3-fold over normal brain also have a higher risk of death (hazard ratio of 5 to 7). Increased mRNA levels correlated with higher survival risk and elevated protein expression in HGL biopsy samples, combined with the detection of surface membrane proteins in glioma cells, indicates that GPNMB is a valuable tumor-associated antigen in immunotherapeutic approaches for malignant gliomas.

345. IMMUNIZATION WITH AUTOLOGOUS GLIOMA CELLS TRANSFECTED WITH IFN-G GENE SIGNIFICANTLY PROLONGS SURVIVAL IN GBM-PATIENTS OLDER THAN 50 YEARS

L. Salford,[1,2] E. Ask,[3] P. Siesjö,[1,2] G. Skagerberg,[1,2] C. Bauréus-Koch,[2,4] C. Blennow,[1,2] A. Darabi,[1,2] C. Elfgren,[5] E. Englund,[6] S. Janelidze,[1,2] E.-M. Larsson,[7] Å. Lilja,[1] B.R.R. Persson,[2,4] A. Rydelius,[1,3] S. Strömblad,[1,2] E. Visse,[1,2] and B. Widegren[7]; [1]Department of Neurosurgery, [2]The Rausing Laboratory, and Departments of [3]Neurology; [4]Medical Radiation Physics, [5]Neuropsychology, [6]Neuropathology, [7]Neuroradiology, and [8]Tumor Immunology, Lund University, Lund, Sweden

Based upon earlier experimental work by our group, we have completed the first part of a human immuno-gene therapy study of patients who undergo an operation for a glioblastoma multiforme. The goal is to ascertain whether immunization with autologous tumor cells expressing gene sequences for human IFN-g is safe for the patients, gives rise to an immunological response, and adds any beneficial effect to conventional therapy. This report is based upon the completed treatment of our first nine patients. Patients aged 50 to 69 years, in which at least 80% of the GBM is resected during neurosurgery, can be included in the study. The tumor cells are cultured in a special Clinical Transduction Laboratory. When at least 40 million cells, karyotyped as malignant, are collected, the immunizations are given 3-weekly, repeated at least 4 and at most 10 times. The immunization takes place in the dermis of the upper arm. Seven days after each immunization, a skin biopsy is taken from the centre of one of the injection sites. The composition of the cellular infiltration in the skin is studied by markers for T lymphocytes (CD3); helper cells, subset of T cells (CD4); cytolytic T-cells, subset of T cells (CD8); natural killer cells (CD16); and B lymphocytes, B cells (CD20). Also the expression of cytokines for functional T cell subsets is studied: IL-2, IL-4, IL-10, IL-12, IL-18, TNF-a, TNF-, IFN-g and TGF-b 1, 2 and 3. Peripheral blood is sampled both before and after operation and also after each immunization event. Co-culture of this blood with tumor cells from the patient allows for a selection of T-cells that can recognize tumor-specific antigens. The patients are followed regularly with neurological, neuropsychological, and MRI examinations. Results from the first human treatments show that the method is safe for the patients and that it gives rise to positive DTH reactions and an increase of infiltrative CD8+ and CD4+ T-cells at the site of immunization. Seven patients received 10 immunizations, and 1 had eight and 1 six immunizations. The material is small, but out of 9 patients, 3 survived for 19.5, 22, and 26.5 months and one is still alive after 23 months with a minor tumor burden. The mean survival time of these 9 patients (mean age 62 years) is 16.2 months to be compared to the 10.4-month survival ($P = 0.03$, Mann-Whitney one-sided test) of the 13 patients (mean age 60 years) included in the study but where the cells did not grow sufficiently well in the cultures to make immunization possible.

346. PRE-CLINICAL EVALUATION OF D2C7, A MONOCLONAL ANTIBODY REACTIVE FOR BOTH THE WILD TYPE AND VARIANT III MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR, FOR RADIOIMMUNOTHERAPY OF MALIGNANT GLIOMAS

A. Boskovitz,[1] C. Pegram,[1] K. Peixoto,[2] M.R. Zalutsky,[2] and D.D. Bigner[1]; Departments of [1]Pathology and [2]Radiology, Duke University Medical Center, Durham North Carolina, USA

The epidermal growth factor receptor (EGFR) is expressed by normal epithelial cells in most tissues but overexpressed in 71%–94% and 60%–90% of anaplastic astrocytomas and glioblastomas (GBM), respectively. In addition, 58%–61% of GBM also express the EGFR variant III mutant (EGFRvIII), which is not found on normal tissues. Monoclonal antibodies (MAbs) targeting either the wild-type EGFR (wt) or EGFRvIII have been developed, and one of them (MAb 425) has been introduced in radioimmunotherapeutic trials for patients with malignant gliomas. Although EGFRvIII is a preferred target because of its higher tumor specificity, some glioma cells may express only wt. Herein, we evaluated the biologic properties of D2C7, a novel MAb specific for both wt and EGFRvIII, with the hypothesis that it could serve as a more generally applicable radionuclide carrier for the targeted radiotherapy of patients with malignant gliomas. D2C7 was generated by using hybridomas reacting with the extracellular domain (ecd) of wt or EGFRvIII. The affinity for wt and EGFRvIII was determined by surface plasmon resonance. Paired-label experiments compared the tissue distribution of ^{125}I-labeled D2C7 and ^{131}I-labeled anti-wt MAb EGFR1 (Exp. 1), ^{131}I-labeled control MAb P588 (Exp. 2 and 4), or ^{131}I-labeled anti-EGFRvIII MAb L8A4 (Exp. 3) in a subcutaneous xenograft model expressing selectively wt (U87-wt; Exp. 1 and 2) or EGFRvIII (NR6M; Exp. 3 and 4) in athymic mice. The binding affinity (K_d) of D2C7 against the wt and EGFRvIII ecd was 1.93×10^{-10} M^{-1} and 2.8×10^{-10} M^{-1} respectively. In vivo, wt-expressing tumor uptake of ^{125}I-labeled D2C7 was 30.8 ± 3.0 (Exp. 1) and 34.3 ± 9.6 (Exp. 2) percent injected dose (ID)/g at 24 h and declined thereafter. The tumor localization index of D2C7 versus EGFR1 and P588 was 3.4 ± 0.9 and 3.3 ± 0.4, respectively, at 24 h and increased thereafter. ^{125}I-labeled D2C7 uptake at 24 h in EGFRvIII-expressing tumors was 37.0 ± 4.2 (Exp. 3) and 41.7 ± 17.6 (Exp. 4) percent ID/g and increased to peak at 52.5 ± 14.0 and 81.8 ± 8.6 percent ID/g, respectively, 72 h after injection. The tumor localization index of D2C7 versus L8A4, an EGFRvIII specific MAb, and P588 was 2.0 ± 0.1 and 2.9 ± 1.0 at 24 h and 3.0 ± 0.9 and 5.8 ± 1.0 at 72 h, respectively. In vitro, D2C7 demonstrated strong affinity and delayed off-rate for both the wt and EGFRvIII molecules. In vivo, tumor localization of D2C7 in tumors expressing wt or EGFRvIII was significantly higher than that of established specific MAbs. In correlation with the in vitro data, uptake of D2C7 by EGFRvIII-expressing tumors was higher compared to that of wt-expressing lesions. Further investigations of the D2C7 MAb are in progress to assess its therapeutic potential as a radiolabeled targeting agent for EGFRvIII-expressing malignant gliomas. Because of the presence of wt on some normal tissues, compartmental administration is preferred in order to reduce potential targeting of normal tissues.

347. EXTERNALLY APPLIED MONOCYTES INFILTRATE GLIOMA SPHEROIDS AND RAT GLIOMAS

H. Strik,[1] P. Erdlenbruch,[2] P. Hülper,[2] J. Maier,[1] A. Kowalewski,[1] B. Hemmerlein,[3] R. Gold,[4] and M. Bahr[1]; [1]Neurology, [2]Pediatrics, [3]Pathology, [4]MS Research, Georg August University, Göttingen, Germany

One prominent feature of human gliomas is the lack of cellular anti-tumor immune response despite extensive infiltration with mononuclear cells. We established a model of monocytic invasion into glioma cell spheroids in vitro and showed that monocytes injected into the peripheral blood infiltrate rat gliomas in vivo. Monocytes were isolated from human blood and added to precultured spheroids of U118 or A172 glioma cells. After three days, the spheroids were collected, fixed and examined immunohistochemically. Peritoneal macrophages were stained with the fluorescent marker PKH2, collected after 24 h, and injected into RG2-glioma-bearing Fischer rats intravenously or into the carotid artery. After 24 h, the rats were sacrificed and frozen sections of the brains searched for fluorescent macrophages. Spheroids were infiltrated regularly by monocytes in vitro. The monocytes expressed MRP8, MAC387 (MRP14), and 25F9. In the rat glioma model, accumulation of the marked monocytes was observed 24 h after injection into the peripheral blood. The experimental models presented here allow us to analyze interactions between monocytes and glioma cells both in vitro and in vivo. A better understanding of these interactions may help to overcome the inactivation of the monocytic anti-tumor response by glioma cells.

348. EXPRESSION OF 8 TUMOR ANTIGENS BY QUANTITATIVE-POLYMERASE CHAIN REACTION IN A SERIES OF 50 GLIOBLASTOMAS

V. Quillien,[1] B. Collet,[1] S. Saikali,[2] T. Avril,[3] and Y. Guegan[2]; [1]Biology, Anti-cancer Center Eugene Marquis, Rennes; [2]Neuro-Oncology University Hospital, Rennes; [3]UPRES EA 3889, Medical School, Rennes; France

The prognosis of glioblastoma (GBM) remains particularly poor and needs development of new therapeutic strategies. Recent pilot studies have shown that immunotherapy could be of interest in GBM. Currently, protocols use whole autologous tumor as source of antigenic peptides (intact tumor cells, tumor lysates or peptides eluted from autologous tumors) because no definitive glioma-specific antigens have been identified to date. This approach has drawbacks among which are the potential of initiating an auto-immune response against brain normal antigens, the delay required to have enough tumor cells after a possible step of culture, and the difficulties to analyze specific anti-tumor immune responses. In a series of 50 GBMs, we have tested mRNA expression of 8 tumor-associated antigens, ALK (anaplastic lymphoma kinase), IL13Rα2, gp100, Galt3, tyrosinase, TRP-2, NA-17A, and MAGE-A3, by Q-PCR (quantitative-polymerase chain reaction). All these antigens have previously been identified in brain tumor, often in small series of various histologies. In addition, they all contain HLA-A2-restricted CD8+ T-cell epitopes in their sequence. All GBMs were classified according to the WHO. Samples were stored at –80°C until RNA isolation. Quantitative gene expression was performed on a Taq Man ABI PRISM 7000 using predesigned probe and primer sets (Applied Biosystems). *GAPDH* was used as internal housekeeping gene. RNA sample expression was normalized to the expression obtained from an RNA pool of 4 normal brain tissues. Ratios (sample RNA level/normal brain RNA level) above 2 were considered as positive. Frequencies of expression were observed as follows: ALK, 8%; NA-17A, 8%; MAGE-A3, 12%; TRP-2, 38%; IL13Rα2, 60%, and gp100, 76%. No expression was found for tyrosinase and Galt3.

Gp100, IL13Rα2, and TRP-2 were simultaneously expressed in 24% of the GBMs; 38% of GBMs expressed two of them, and 84% expressed one of them. Mean ratios (minimum ratio – maximum ratio) were 7 (2–40) for gp100, 7 (2–9) for IL13Rα2, and 16 (2–155) for TRP-2. More than 70% of the samples had an expression ratio less than 10. Gp100, IL13Rα2, and TRP-2 may represent useful targets for specific immunotherapy. We are now testing their capacities to elicit a specific cytotoxic immune response in vitro in peripheral blood lymphocytes from patients with GBM.

349. THE STUDY OF INDUCING ANTI-GLIOMA ACTIVITY IN VITRO WITH HUMAN AUTOLOGOUS DENDRITIC CELL-BASED FUSION CELLS

Z. Chen and H. Shi; Neurosurgery/Neuro-oncology, Cancer Center, Guangzhou, China

As effective antigen-presenting cells, dendritic cell (DC)-based hybrid vaccine has shown much promise in cancer therapy. But there have been few approaches in human glioma treatment, and it is an area of research to be addressed in our country. In this study, DC-glioma fusion cells (FC) were prepared by the fusion of human glioma cells and the DC derived from peripheral blood mononuclear cells of the same patients. In vitro anti-glioma activity induced by FC was studied. The FC were prepared in the presence of polyethylene glycol, the proliferation capacity of a patient's T cells in vitro stimulated by FC was evaluated by autologous mixed leukocyte reaction, and the cytotoxicity against autologous glioma cells by the patient's T cells primed with FC was also assessed by lactate dehydrogenase (LDH) assay. The FC-preparation method was constructed. The patient's T cells primed with irradiated (1.5 Gy) FC induced a more remarkable glioma killing than the T cells primed with DC or autologous glioma cells ($P < 0.01$), and the cytolytic effects were up from 28% to 90% as the effector-target ratio increased. But irradiated FC primed T cells only induced about 10% MCF7 lysis, which was significantly lower than glioma killing ($P < 0.01$). When stimulated, the FC primed T cells showed remarkably high proliferation compared with DC or autologous glioma cells primed T cells ($P < 0.05$), and those primed T cells stimulated with autologous DC loaded with glioma lysate showed significantly enhanced proliferation compared with those stimulated with autologous glioma cells or DC alone ($P < 0.01$). The irradiated (1.5 Gy) FC prepared in this protocol could effectively prime a patient's T cells, and the primed T cells could be proliferated in vitro as stimulated with autologous DC loaded with glioma lysate. The primed T cells showed a specific tumor cytolysis, and the killing activities were up as the effector-target ratio increased. These studies may have implications for the clinical trials on human gliomas.

350. CULTURING OF HUMAN TUMOR CELLS FOR USE IN IMMUNE GENE TUMOR THERAPY

B. Widegren,[1,4] C. Baureus-Koch,[5] A. Rydelius,[2] G. Nyberg,[2] S. Järnum,[1] A. Darabi,[2] P. Siesjö,[2] E. Visse,[2] S. Strömblad,[2] C. Blennow,[2] G. Skagerberg,[2] X. Fan,[1] N. Mandahl,[3] H.-O. Sjögren,[1] B.R.R. Persson,[5] and L.G. Salford[2]; [1]Tumor Immunology, [2]Division of Neurosurgery, The Rausing Laboratory, and Departments of [3]Clinical Genetics, [4]Molecular Genetics, and [5]Medical Radiation Physics, University Hospital, Lund, Sweden

The study concerns the culturing of tumor cells from brain tumor specimens to provide a source of tumor antigens for immunization purposes. It is important here to be able to discriminate normal cells from malignant cells in glioma cell cultures effectively. We outline a protocol for enhancing primary tumor cell growth in vitro from tissue obtained during surgery from glioblastoma multiforme. Cancer immune therapy utilizing autologous human tumor cells, genetically modified to enhance their immunogenicity, is a method currently under development and successful clinical results have been published. Ongoing and planned clinical trials involve immunization with irradiated tumor cells as a method of choice, especially when the actual tumor antigens have not yet been identified. For immunotherapy using autologous human tumor cells to be successful, several million tumor cells are needed. The time elapsing between tumor biopsy and treatment should be as short as possible. Using tumor material directly will involve normal cells that increase the risk of autoimmunity. For many tumors, including human gliomas, however, the establishment of in vitro growth is not always possible. We have investigated the in vitro growth potential of human glioma biopsies from more than 30 patients. This involved testing different matrices (laminin, collagen, and fibronectin), supplemented by different growth factors (either platelet-derived growth factor [PDGF] or epidermal growth factor [EGF]), and using different batches of sera. The effect of steroids was also studied. To investigate the ratio of normal to malignant cells in the tissue cultures from the tumor biopsies, chromosomal analysis of the glioma cell cultures was performed. Since for most tumors, no tumor-protein marker exists that is able to adequately discriminate a glioma cell from a normal cell, high-resolution chromosomal analysis is the safer method. Establishing adherent human glioma cell cultures from surgical biopsies was successful in about 75% of the cases. Enhanced growth could sometimes be observed when cells were supplemented with EGF or PDGF, whereas the growing of cells in flasks coated with matrix proteins only rarely produced substantial improvements in growth. High-resolution chromosomal analysis of the cultures demonstrated the presence of normal cells in amounts ranging from 0% to almost 100%. The general tendency is for malignant cells to be present in cultures in large numbers initially but rather for normal cells to outnumber the malignant cells quickly. Later on, the normal cells cease to grow, and the tumor cells become dominant. The time elapsing between biopsy and development of a cell culture in which the majority of the cells are malignant varies from a few weeks to more than six months. The low frequency of tumor cells in cultures at certain stages makes the cultures unsuitable at such points for immunogene therapy with use of autologous tumor cells. We also demonstrate efficient transfection results with the use of adenovirus expressing human interferon gamma and green fluorescent protein.

351. TRAIL (APO2L) AND TRAIL-RECEPTOR EXPRESSION IN RELATION TO SURVIVAL OF PATIENTS WITH GLIOBLASTOMA MULTIFORME

J.M.A. Kuijlen,[1] J.J.A. Mooij,[1] H. Hollema,[2] E.W. Hoving,[1] W.T.A. van der Graaf,[3] and W.F.A. den Dunnen[2]; [1]Neurosurgery, [2]Pathology and Laboratory Medicine and [3]Internal Medicine, Groningen University Hospital, Groningen, The Netherlands

In order to improve survival of patients with a GBM, new therapeutic strategies must be developed. The use of a death-inducing ligand such as TRAIL (TNF-related apoptosis-inducing ligand) seems a promising innovative therapy. The aim of this study was to quantify the expression of the death regulating receptors TRAIL-R1 and TRAIL-R2, and the TRAIL ligand on primary GBM tumor specimens and to correlate this expression with survival. Sixty-two tumor specimens were taken from patients who had had a craniotomy with debulking of a primary GBM. Expression of TRAIL and TRAIL receptors was assessed by immunohistochemistry, both quantitatively (% of positive tumor cells) and semi-quantitatively (staining intensity) within both perinecrotic and intermediate tumor zones. RT-PCR of GBM tumor tissue and controls (other astrocytic tumors and normal brain) was performed to show expression of TRAIL receptor mRNA. Immunohistochemistry showed a slight diffuse intracytoplasmic and a stronger membranous staining for TRAIL and TRAIL receptors in tumor cells. Semi-quantitative expression of TRAIL showed a significantly higher expression of TRAIL in the perinecrotic area than in the intermediate zone of the tumor ($P = 0.0001$). A mean of 13% TRAIL positive tumor cells was found. A positive correlation was found between TRAIL expression (semi-quantitative) and survival ($P = 0.008$). Mean tumor cell positivity for TRAIL-R1 was 19%. A positive correlation was found between survival and the percent of TRAIL-R1 positive tumor cells ($P = 0.049$). Mean TRAIL-R2 expression was 27%. TRAIL-R2 expression was significantly higher expressed than TRAIL-R1 ($P = 0.005$). A positive correlation was found between TRAIL-R1 and TRAIL-R2 expression ($P = 0.002$). RT-PCR analysis detected a negative relation between the amount of TRAIL-R1 mRNA and the WHO grade of astrocytic tumors ($P = 0.005$). Immunohistochemistry and RT-PCR show a positive correlation between TRAIL-R1 expression and survival, and therefore TRAIL-R1 expression seems to be a prognostic indicator. TRAIL-R2 is significantly more expressed within tumor tissue than TRAIL-R1. Therefore TRAIL-R2 is of more importance as a target for future TRAIL therapy than TRAIL-R1. However, the way the death-inducing signal through the TRAIL-R2 receptor is regulated differs from TRAIL-R1 signaling. This has to be taken into account when developing new therapeutic strategies using TRAIL.

352. BCL-2 FAMILY MEMBERS, NFKAPPAB, AND DESFEROXAMINE PROTECT T-CELL FROM GBM-MEDIATED APOPTOSIS

A. Chahlavi,[1] M. Thornton,[2] C. Tannenbaum,[2] P. Rayman,[2] M. Vogelbaum,[1] G. Barnett,[1] and J. Finke[2]; [1]Neurosurgery and [2]Immunology, Cleveland Clinic Foundation, Cleveland, Ohio, USA

Prior studies have shown that glioblastoma multiforme (GBM) cells and possibly tumor-derived products may be responsible for the T cell dysfunction observed in GBM patients, thus impairing T-cell based immunotherapy. This notion is supported by the observation that GBM tumor cells, when co-cultured with T cells, can induce apoptosis of the lymphocytes. Established GBM lines are known to express gangliosides as well as death-inducing ligands such as CD70 that can induce apoptosis in T cells. We wanted to determine whether overexpression of Bcl-2 family members, Bcl-2 and Bfl-1, and RelA, a subunit of NFkappaB, will provide protection

to T cells from GBM-induced apoptosis. Also, we wanted to assess if cyclosporine A, bongkrekic acid (BA), and desferoxamine, which protect against mitochondrial damage, can protect T lymphocytes from cell death induced following co-culture with GBM lines or GBM gangliosides. The Jurkat T cell line was transfected with plasmids expressing the gene for Bcl-2, Bfl-1, or RelA, and stably transfected clones were established and confirmed by quantitative PCR and Western and Northern blot analysis. Five GBM cell lines were co-cultured with wild-type Jurkat cells and Jurkat cells overexpressing the various transgenes. Jurkat cell apoptosis was measured by trypan blue and TUNEL. The expression of Bcl-2, Bfl-1, and RelA was measured by quantitative PCR and Western blot in wild-type Jurkat cells after co-culturing with GBM cell lines. Four of five GBM cell lines induced high apoptosis of wild-type Jurkat cells and resting or activated T-cells in co-culture experiments. The expression levels of Bcl-2, Bfl-1, and RelA were subsequently reduced in the wild-type Jurkat cells following co-culture with the apoptogenic GBM cell lines. Overexpression of Bcl-2, Bfl-1, and RelA, however, conferred protection by at least 60% against the four apoptogenic GBM cell lines. As far as drug protection, all three achieved some protection. However, desferoxamine, an iron chelator, was most effective at blocking T-cell killing by GBM cell-lines and their purified gangliosides. Bcl-2, Bfl-1, and RelA overexpression is an effective means of protecting T lymphocytes from apoptosis mediated by GBM. Furthermore, the antioxidant desferoxamine may be useful in vivo as an adjunct drug in GBM immunotherapy for promoting T-cell survival and function.

353. CYTOKINE EXPRESSION IN RG2 GLIOMAS FOLLOWING TREATEMENT WITH CPG DNA, INTERFERON-GAMMA, AND INTERFERON WITH LPS

J. Schartner, L. Zhang, M. Van Handel, G. Allen, M. Curet, and B. Badie; Neurological Surgery, University of Wisconsin, Madison, Wisconsin, USA

The efficacy of immunotherapies against malignant gliomas is likely limited by the immunosuppressive tumor environment. Our lab has previously shown that intracranial RG2 gliomas demonstrate higher expression of both immuno-stimulatory (IL-1b and IFN-g) and immunosuppressive cytokines (IL-10 and TGF-b1) mRNA as compared to normal brain tissue. To investigate changes in baseline cytokine expression following immune stimulation, normal tissue and RG2 tumor tissue were analyzed by semi-quantitative real-time PCR 24 h after injections with CpG DNA, interferon-g (IFN-g), or IFN-g with lipopolysaccharide (IFN/LPS). CpG-injected normal brain tissue demonstrated a 21-fold increase in IFN-g and IL-1b and an 1160-fold increase in IL-10 mRNA as compared to sham-treated normal brain. TGF-b1 or TGF-b2 expression did not change in these samples. Although CpG-treated RG2 tumors also demonstrated an increase in IL-10 (363-fold), IFN-g (48-fold) and IL-b1 (only 8-fold) mRNA levels, they also demonstrated an increase in TGF-b2 expression (10-fold). Treatment of normal brain or RG2 tumors with IFN-g did not change the expression of any cytokines in normal brain, but induced a 5.6-fold increase in TGF-B2 mRNA in RG2 tumors. IFN/LPS treatment increased the expression of IFN-g, IL-1b, and IL-10 in normal brain (13.9-, 34-, and 15-fold increases over sham-treated normal brain). However, these effects were limited in RG2 tumors, with no change in IFN-g expression and only a 6-fold change in IL-1B expression compared to sham-treated tumors. Overall, these observations suggest that treatment of RG2 tumors with classical immune activating agents may result in increased expression of pro-inflammatory cytokines, but that the concomitant upregulation of immuno-suppressive cytokines may limit the efficacy of such treatment. Future therapeutic strategies will need to address pervasive immunosuppressive environment present in these tumors.

354. IMMUNE REJECTION OF GLIOMAS IN A MURINE MODEL

S. Jeyapalan,[1] M. Pasternack,[2] D. Knipe,[4] F.H. Hochberg,[5] and K. Reilly[3]; [1]Neurology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; [2]Pediatrics, Massachusetts General Hospital, Boston, Massachusetts; [3]National Institutes of Health, National Cancer Institute, Bethesda, Maryland; [4]Microbiology, Harvard Medical School, Boston, Massachusetts; [5]Neurology, Massachusetts General Hospital, Boston, Massachusetts, USA

Viral vectors encoding therapeutic genes are being investigated as novel agents for glioma therapy. These candidate therapeutic genes may modulate the immunogenicity of viral vectors and affect vector efficacy to inhibit tumor growth independent of their enzymatic activities. We investigated the immunogenicity of an exogenous gene product in a murine model using ovalbumin (OVA) to study whether an exogenous antigen promotes immune-mediated lysis of glioma cells. OVA-expressing glioma lines were established by transfection and cloning of KR-158 glioma cells (H-2b); OVA expression was confirmed by immunofluorescence and Western blotting. The tumorigenicity of wild-type, low- and high-expressing OVA-transfected glioma cells to induce subcutaneous tumors in syngeneic C57Bl/6 mice was compared. Although as few as 10^6 wild-type KR-158 glioma cells generated ultimately lethal bulky flank tumors, low- and high-expressing OVA transfectants ($>6 \times 10^6$) were not tumorigenic in C57Bl/6 mice. These transfectants remained tumorigenic in nu/nu recipients and pointed to immune rejection of OVA-transfected gliomas. The role of the host immune response in the rejection of OVA-expressing gliomas was explored through the use of C57Bl/6 mutants deficient in specific components of the T-cell response. $Beta_2$-microglobulin null mice did not reject OVA-expressing KR-158 glioma cells, pointing to an essential role of MHC class I–specific CD8+ cytotoxic T cell function in glioma rejection. Surprisingly, the OVA-transfectant gliomas were not tumorigenic in perforin null recipients, suggesting that Fas-mediated lysis, and not cytotoxic granule-mediated killing, was required for glioma rejection. The growth of OVA-expressing KR-158 glioma cells in gld/gld congenic mice, which lack Fas ligand on immune effector cells, confirmed the importance of Fas-mediated tumor cell lysis. Flow cytometric analysis of cultured KR-158 demonstrated subpopulations expressing Fas (~20%) or Fas ligand (15%). These data demonstrate that exogenous transgenes, modeled here by ovalbumin, are potentially immunogenic antigens, and may enhance immune-mediated oncolysis. As new candidate therapeutic genes are investigated, their impact on the immunogenicity of therapeutic viral vectors and their intrinsic effects on tumor growth in vivo should be explored.

355. INCREASED NUMBER OF LEUKOCYTES SECRETING IFN-GAMMA IN PATIENTS IMMUNIZED WITH AUTOLOGOUS IFN-GAMMA SECRETING GLIOMA CELLS CORRELATE WITH PROLONGED SURVIVAL

E. Visse, A. Darabi, K. Enell, S. Janelidze, M. Larsson, B. Widegren, G. Skagerberg, P. Siesjö, and L. Salford; Clinical Medicine, Neurosurgery, The Rausing Laboratory, Lund, Sweden

In a clinical study glioma patients were immunized intradermally in the right arm with autologous tumor cells secreting IFN-gamma. Peripheral blood leukocytes (PBL) and immune infiltration at the immunization site were studied to monitor the patients' immune response. Before and during the 18- to 30-week immunization schedule, blood samples were collected, and PBL were isolated and frozen. After thawing, PBL were restimulated with tumor cells in vitro for 5 days. The restimulated cells were assayed for lymphocyte subset distribution by flow cytometry, cytokine production by flow cytometry, and ELIspot and proliferation by thymidine incorporation. Immunohistochemically stained tissue sections from the immunization site were analyzed for leukocyte infiltration. Blood samples from 5 patients were analyzed and compared with survival data, immune infiltration, and DTH reactions at the vaccination site. No correlation or discernable pattern could be observed from proliferation and lymphocyte subset analyses. Increased IFN-gamma levels measured by ELIspot were seen in 2/5 patients with a prolonged survival. A decrease in IFN-gamma production was observed during tumor recurrence. Detectable levels of IL-10 were observed displaying a weak association with shorter survival. Lymphocyte cytokine production did not correlate with lymphocyte infiltration or DTH reactions at the immunization site. Compared to control patients, immunized patients had a mean prolonged survival of 170 days. No clear correlation between immune cell influx at the immunization site and recall lymphocyte activity or prolonged survival could be found. The mean survival time of patients immunized with IFN-gamma secreting tumor cells is prolonged by circa 4 months. The two patients with the longest survival time also had an increased number of IFN-gamma producing leukocytes.

356. IL-13 RECEPTOR ALPHA 2 PEPTIDE ANALOGUES INDUCE HIGHER LEVELS OF IL-13 RECEPTER ALPHA 2 (345-353)-SPECIFIC CTL RESPONSES IN BOTH GLIOMA PATIENT-DERIVED CD8+ CELLS AND HLA-A2 TRANSGENIC MICE

J. Eguchi,[1] M. Hatano, J. Dusak,[1] F. Lieberman,[1] W. Storkus,[2] I. Pollack,[1] and H. Okada[1]; [1]Neurosurgery; [2]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Restricted and high-level expression of interleukin-13 receptor-alpha 2 (IL-13Rα2) in a majority of human malignant gliomas makes this protein an attractive vaccine target. We have previously described identification of IL-13Rα2 (345-353) peptide as an HLA-A2-restricted cytotoxic T lymphocyte (CTL) epitope. However, as it remains unclear how efficiently peptide-based vaccines can induce specific CTLs in HLA-A2+ patients with malignant gliomas, we have examined whether peptide-analogues can be used for optimal expansion and activation of IL-13Rα2 specific CTL. We synthesized three IL-13Rα2 (345-353)-analogue peptides by substitutions of the carboxyl terminal isoleucine (I) for valine (V) and the amino terminal tryptophan (W) for either alanine (A), glutamic acid (E) or non-substituted

(W) (designated as IL-13Rα2-1A9V, -1E9V, and -9V, respectively). Among these, the modified peptides IL-13Rα2-9V and -1A9V displayed higher binding affinity to HLA-A2 than the native IL-13Rα2 (345-353) epitope. Stimulation of HLA-A2+ glioma patient-derived CD8+ T cells with autologous dendritic cells (DCs) loaded with IL-13Rα2-9V and -1A9V induced higher levels of lytic activity against T2 cells loaded with low concentrations (0.1–10 nM) of native IL-13Rα2 (345-353) peptide than other peptides including the native IL-13Rα2 (345-353). These CTL lines induced with IL-13Rα2-9V or -1A9V peptides lysed HLA-A2+ glioma cells that express IL-13Rα2 more efficiently than CTL induced by the wild-type peptide. Both IL-13Rα2-9V and -1A9V demonstrated superior immunogenicity to the native peptide at equal levels in most cases. However, we have observed, in multiple HLA-A2+ glioma patients, that IL-13Rα2-9V is the most consistent and strongest inducer of CTL responses against IL-13Rα2+ glioma cells. In order to examine whether immunization with IL-13Rα2 (345-353) and/or its peptide analogues can elicit CTL responses in vivo, we employed HLA-A2 transgenic mice (HHD mice). Immunization of HHD mice with IL-13Rα2-9V or -1A9V peptides induced higher levels of CTL reactivity in their splenocytes against the EL4-HHD cells pulsed with IL-13Rα2 (345-353) peptide as well as the EL4-HHD cells endogenously expressing IL-13Rα2 (EL4-HHD-IL-13Rα2) in comparison to IL-13Rα2 (345-353). These findings indicate that highly antigenic IL-13Rα2 peptide-analogues may be useful for the development of vaccines capable of effectively expanding IL-13Rα2 specific CTL in glioma patients. We are currently evaluating the anti-tumor effect of immunizations with IL-13Rα2-9V or -1A9V peptides in HHD mice bearing EL4-HHD-IL-13Rα2 tumors.

357. ENHANCED ANTI-TUMOR IMMUNE RESPONSE AFTER SELECTIVE INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN RATS CARRYING INTRACEREBRAL TUMORS AND IMMUNIZED WITH IFN-GAMMA SECRETING GLIOMA CELLS

W. Badn, P. Hegardt, M. Fellert, A. Darabi, M. Esbjörnsson, E. Visse, S. Janelidze, L. Salford, and P. Siesjö; Clinical Neurosurgery, Neurosurgery, Lund, Sweden

In this study we aimed to clarify whether IFNg could enhance anti-tumor immune responses. We investigated both an iNOS selective, L-N^6-(1-iminoethyl)-L-lysine (L-NIL), and a non selective, *N*-nitro-L-arginine methyl ester (L-NAME), inhibitor of NOS. Both L-NIL and L-NAME were able to inhibit NO production and enhanced the proliferation and production of IFN-g from polyclonally activated spleen cells in vivo. However, L-NIL had a broader window of efficacy and a lower minimal effective dose. In correlation to in vivo results, only spleen cells from rats treated with L-NIL, and not L-NAME, had an enhanced proliferation as well as IFN-g production in response to tumor cells. Furthermore, L-NIL was able to prolong the survival of rats with intracerebral tumors after therapeutic immunizations with tumor cells secreting IFN-g. These results imply that selective inhibition of iNOS can enhance anti-tumor immune responses evoked by IFN-g treatment.

358. ALTERATION IN THE TH1 AND TH2 CYTOKINE PROFILE IN PATIENTS WITH BRAIN TUMOURS

D. Kamdar,[1] R. Kumar,[2] J. Greenman,[1] D. O'Brien,[2] and L. Madden[1]; [1]Medical Research Division, University of Hull; [2]Department of Neurosurgery, Hull Royal Infirmary, Kingston Upon Hull, UK

The objective of this study was to evaluate the TH1 and TH2 cytokine profile in patients with brain tumors. IL-12 is a key cytokine mediating a TH1-type response, and IL-10 is a TH2-type pleiotrophic cytokine. These two cytokines act in an antagonistic fashion, with a balance point that varies between the physiological and pathological state (O'Hara et al., Clin. Cancer Res. 4, 1943, 1998). The study was designed as a prospective analysis to compare changes in serum IL-10 and IL-12 levels in patients with newly diagnosed brain tumors. An initial cohort of 65 patients aged 24 to 78 years were recruited between December 2002 and December 2003 following radiological diagnosis of a space-occupying lesion. Serum samples were obtained prior to surgery. Age and sex matched control patients (n = 36) were also recruited. Serum IL-10 and IL-12 were measured by quantitative ELISA (BioSource International.). There were 12 benign, 5 low-grade, 30 high-grade, and 18 metastatic tumors. Serum IL-10 was detected in only 47% of the controls (median [m] 0.0 pg/ml). IL-10 was detected in 92% of patients with benign tumors (m = 2.4 pg/ml), 60% of low-grade tumors (m = 0.0 pg/ml), 67% of high-grade tumors (m = 0.1 pg/ml), and 78% of metastatic tumors (m = 0.3pg/ml). Conversely, serum IL-12 was detected in all patients in the control group (m = 52.2 pg/ml). However, IL-12 was only detected in 67% of benign tumors (m = 12.1 pg/ml), 80% of low-grade tumors (m = 41.1 pg/ml), 27% of high-grade tumors (m = 0.0 pg/ml), and 33% of metastatic tumors at 0.0 pg/ml. The only factor that showed statistical significance was serum IL-12 levels where there was reduction in the high grade and metastatic group compared with controls ($P < 0.001$). The TH1-like cytokine IL-12 decreased significantly as tumor stage progressed; however no increase in the antagonistic TH2-like cytokine IL-10 was observed.

359. MODIFICATION OF TUMOR MICROENVIRONMENT BY DELIVERY OF CYTOKINE-GENE TRANSFECTED DENDRITIC CELLS ENHANCES THE EFFICACY OF TYPE-1 ANTIGEN-SPECIFIC T CELL ADOPTIVE TRANSFER

F.N. Nishimura,[1] J.E.D. Dusak,[1] M.H. Hatano,[1] J.E. Eguchi,[1] W.G. Gao,[2] A.G. Gambotto,[2] W.J.S Storkus,[3] F.M. Monzon-Bordonaba,[4] J.L. Lyons-Weiler,[4] and H.O. Okada[1]; [1]Neurological Surgery, [2]Surgery, [3]Dermatology, and [4]Microarray and Analysis Shared Services and Bioinformatics Shared Services, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA

We have previously demonstrated that injection of murine central nervous system (CNS) tumors with dendritic cells (DC) secreting interferon (IFN)-a (DC-IFNa) remarkably enhanced the therapeutic effect of peripheral vaccinations with ovalbumin (OVA)-specific T cell epitopes. The injected DC-IFNa migrated from the CNS tumor site to the draining cervical lymph nodes (CLNs), where they cross-primed tumor antigen-specific CTLs (Okada et al., 2004). To further determine the effect of this "prime and boost" vaccine-strategy on the effector cells, we employed adoptive transfer of OVA-specific type I CD8 effector T (Tc1) cells derived from OVA-specific T cell receptor transgenic OT-1 mice. In addition, to further understand molecular events induced by the IFN-a delivery in the CNS tumors, we analyzed the gene-expression profiles within the tumor microenvironment using Codelink Mouse 20K microarray system. Syngeneic C57BL/6 mice bearing day 5 OVA-transfected B16 melanoma (M05) in the CNS received intratumoral (i.t.) delivery of DCs ex vivo transfected with adenoviral murine IFN-a or control vector y5. On day 6, these mice also received intravenous injections with 5×10^6 Tc1 cells. DC-IFNa induced expression of IFN-inducible protein (IP)-10, which is a potent chemokine for type I immune effector cells, from M05 cells. Tc1 cells also expressed CXCR3, which is a primary receptor for IP-10 and a high level of CD44 activation marker. As expected from these observations, mice treated with i.t. DC-IFN-a and i.v. Tc1 injections resulted in prolonged survival of the host animals and accumulation of higher numbers of OVA-tetramer+/CD8+ cells in the cervical lymph nodes in comparison to the control treatment mice that received i.t. DC-y5 and i.v. Tc1. Microarray analyses of the CNS tumors indicated that local IFN-a expression upregulated a variety of immuno-regulatory molecules such as major histocompatibility complex (MHC) class I chains, a DC maturation factor 2',5' oligoadenylate synthetase and T cell activation marker CD69, suggesting that local IFN-a expression enhances antigen presentation processes and activation of effector T cells within the tumor microenvironment. In addition, as interleukin (IL)-23 has been recognized as a critical mediator of chronic inflammatory responses in the CNS, we evaluated the effect DC-mediated delivery of IL-23 to the CNS tumors. Although IL-23 did not induce IP-10 from the tumor cells or IFN-g from the Tc1 cells, tumor infiltrating lymphocytes from IL-23 and Tc1 treated mice demonstrated a remarkably increased number of OVA-tetramer+/CD44+ cells in comparison to the control and IFN-a treated animals, suggesting that IL-23 has a unique ability to attract antigen-specific effector cells to the tumor via distinct mechanisms than IFN-a. These results warrant further evaluation of a combined approach with IFN-a and IL-23 delivery to CNS tumors.

360. PILOMYXOID ASTROCYTOMA: A RARE BUT CLINICALLY SIGNIFICANT SUBGROUP OF OPTIC PATHWAY GLIOMAS

G. Kammler,[1] C. Hagel,[2] K. Bentele,[3] K. Ittner,[3] U. Grzyska,[4] *K. Lamszus*,[1] M. Westphal[1]; Departments of [1]Neurosurgery, [2]Neuropathology, [3]Pediatrics, and [4]Neuroradiology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Optic pathway/hypothalamic gliomas occur mostly in children, belong to the histological subgroup of pilocytic astrocytomas (PA), and take highly variable clinical courses during adolescence and later in adulthood. It has been stated that the pilomyxoid variant (pmPA) is more aggressive and makes up a high percentage of PAs. We review a series of 59 cases of recent pediatric and adult cases including three pmPAs and specifically focus on one particular case of pmPA and its remarkable clinical and neuroradiological course. Between 2000 and 2004, 37 children and 22 adults were operated with intracranial PAs of which 12 were optic pathway PAs (20.1%). Of the 8 pediatric cases, 3 were limited to the optic nerves, chiasm, and retrochiasmatic "extracerebral" aspect of the optic tract. Two cases expanded into the hypothalamus/third ventricle, and three were spread through the hemisphere. In the adult population, only two cases were related to the optic system, one being optochiasmatic with extension into the third ven-

tricle and one originating from the optic tract with mainly temporal extension. As for the neuroradiological growth pattern, three groups of tumors can be distinguished in our group: (1) solid, non-cystic, mostly extracerebral tumors, (2) cystic intraparenchymal/ intraventricular tumors, and (3) solid, non-cystic inhomogenously enhancing tumors. A case of pmPA was found among the latter group, diffusely spreading from throughout the hemisphere and the brainstem. Within this tumor, strongly enhancing nodules with rapid growth appeared at different times and places requiring transylvian and transventricular decompression, respectively, for neurological symptoms. These nodules both turned out to be of the pilomyxoid subtype and also during surgery were clearly distinct, being of a greenish jelly-like texture. Complete removal of these nodules resulted in stable local control while new nodules rapidly occurred. The pilomyxoid variant of PA seems to harbor cell clones that rapidly expand despite absence of any signs of anaplasia. They appear to have limited invasive capacity because even within a very diffuse tumor, they can be locally controlled by surgery. The highly diverse behavior of the different compartments of this tumor allow recommendation of aggressive treatment either by microsurgery or interstitial radiosurgery for these spherical nodules which determine the poorer outcome of these cases.

361. DUMBBELL TRIGEMINAL SCHWANNOMA IN A CHILD: COMPLETE REMOVAL BY A ONE-STAGE PTERIONAL SURGICAL APPROACH

C.P. Verstappen, T. Beems, and E.J. van Lindert; Neurosurgery, University Medical Centre Nijmegen, Nijmegen, The Netherlands

The objective of our study was to describe a rare case of a trigeminal schwannoma in a child and the surgical procedure performed for therapy. A six-year-old girl presented with tiredness, pain in the neck region, gait disturbances, and dysarthric speech. She did not have any skin manifestations like café au lait spots or subcutaneous tumors. Family history did not suggest familial neurofibromatosis. Imaging studies revealed a unilateral dumbbell-shaped tumor, both extending in the middle and posterior fossa, centered over Meckel's cave. A one-stage surgery was performed by pterional craniotomy. The tumor was first debulked in the middle fossa, then peeled from the wall of the cavernous sinus, followed by extirpation of the tumor from the posterior fossa. The tumor extended to the caudal cranial nerves, and was completely removed. Trigeminal fascicles were distributed throughout the tumor. Histopathological examination revealed a schwannoma. Trigeminal schwannoma is a rarely occurring tumor in childhood, only nine cases having been reported in the literature thus far. Furthermore, although several often multi-staged surgical strategies for dumbbell tumors are described, in this patient the tumor was eradicated by a one-stage pterional approach.

362. ATYPICAL TERATOID/RHABDOID TUMORS: A NEW MALIGNANT PEDIATRIC BRAIN TUMOR TO BE DISTINGUISHED FROM MEDULLOBLASTOMA/PNET

H. Hidehiro and F. Kiyotaka; Neurosurgery, Kitasato University, Sagamihara, Japan

The clinicopathological features of atypical teratoid/rhabdoid tumor (AT/RT), a new entity among malignant pediatric brain tumors, and the differential diagnosis from primitive neuroectodermal tumor (PNET)/ medulloblastoma are described. The clinicopathological features of 140 AT/RTs including 7 Japanese cases, 70 cases of Mayo Clinic and American Pediatric Oncology Group, and 63 published cases subject to analysis included patient age and sex at presentation, symptoms, neurological signs, tumor location, histopathological features and outcome. The patients with AT/RT aged from 22 days to 14.9 years. There was a 1.5:1 male predominance. The clinical symptoms were related to tumor location and usually nonspecific in nature. Of the 140 AT/RTs, 61% were located in the posterior fossa, 20% in the cerebral hemispheres, 5% in the suprasellar and/or third ventricular regions, 5% in the pineal region. Histologically, AT/RT is defined as a polymorphous neoplasm often featuring rhabdoid, PNET, epithelial, and mesenchymal components. AT/RTs usually include PNET components and occur mainly in the posterior fossa, so mimic medulloblastoma. AT/RT is characterized by the cytogenetic finding of monosomy 22 rather than i(17q). The tumor is similarly mistaken for PNET at supratentorial sites. Germ cell tumors also enter into the differential diagnosis due to their histological immunophenotypic diversity, particularly features indicative of epithelial and mesenchymal differentiation. Nonetheless, the remarkable spectrum of tissues that typify teratoma is absent in AT/RT. The prognosis of AT/RT is far less favorable than that of PNET/medulloblastoma of malignant or germ cell tumor. Meta-analysis of 140 cases confirms that AT/RT is as a clinicopathological entity and emphasizes the necessity for distinguishing this unique tumor from other pediatric central nervous system neoplasms.

363. THERAPEUTIC EFFICACY AND PROGNOSTIC FACTORS IN MEDULLOBLASTOMAS

T. Ota,[1] T. Watanabe,[1] J. Kurihara,[2] A. Yoshino,[1] Y. Katayama,[1] H. Nishimoto,[2] and H. Kishimoto[3]; [1]Neurological Surgery, Nihon University School of Medicine, Tokyo; [2]Neurological Surgery and [3]Pathology, Saitama Children's Medical Center, Saitama; Japan

Medulloblastomas are the most common central nervous system malignancies in children. Combined modality treatment with radiation and chemotherapy has substantially improved disease control and extended patient survival. Nevertheless, medulloblastoma remains a treatment challenge, and considerable controversy exists as to the best therapeutic management for patients with such tumors. In the present study, we retrospectively analyzed a series of 21 patients with newly diagnosed medulloblastomas treated by surgery, radiation, and adjuvant chemotherapy. For the entire study population, the median overall survival (OS) was 56 months, with 5-year OS rate of 47%, and the median disease-free survival (DFS) was 41 months, with 5-year DFS rate of 44%. Radical surgery, low-stage according to the Chang Staging System, and platinum chemotherapy were significantly associated with longer DFS and/or OS. Patients who were less than 3 years of age exhibited a tendency toward a shorter survival. None of the immunohistochemical markers including GFAP, S-100, NSE, synaptophysin, TrkA, TrkC, and MIB-1 revealed a correlation with the survival. Our data indicate that despite optimal treatment with surgery, radiation therapy, and adjuvant chemotherapy, the prognosis for patients with medulloblastoma remains dismal. Further clinical studies will need to address the exploration of more aggressive therapeutic strategies in combination with conventional craniospinal irradiation and intensive platinum-based chemotherapy.

364. PEDIATRIC OLIGODENDROGLIOMA IN THE MOTOR STRIP

J.V. Anton,[1] M. Prelog,[2] H. Zunterer,[3] H. Maier,[4] E. Grasbon-Frodl,[5] W. Eisner,[1] T. Fiegele,[1] L.B. Zimmerhackl,[2] J. Burtscher,[1] and K. Twerdy[1]; [1]Neurosurgery, [2]Pediatrics, [3]Radiology II, and [4]Pathology, Medical University Innsbruck, Innsbruck, Austria; [5]Centre for Neuropathology and Prion Disease, Ludwig-Maximilians-University Munich, Klinikum Großhadern, Munich, Germany

We present the case of an eight-year-old boy who presented with focal seizures. MRI showed a mass (diameter 4 cm) in the left motor cortical area with slight contrast enhancement on the floor of left sulcus centralis and no contrast enhancement in the motor strip. Subtotal surgical resection was guided by intraoperative ultrasound and intraoperative CT. Direct bipolar cortical electrophysiological mapping informed the neurosurgeon about the motor functionality of the exposed structures, which were infiltrated by tumor tissue. Finally, the contrast medium enhanced tumor part could be resected due to combined use of intraoperative imaging and intraoperative electrophysiology. Tumor within the motor strip was spared to avoid functional impairment. Although Ki-67 staining showed elevated proliferative activity, a low-grade oligodendroglioma (WHO grade II) was diagnosed after histomorphological examination. Loss of heterozygosity (LOH) analysis was carried out using microsatellite markers and PCR based assay. No allelic losses on chromosome arms 1p and 19q were shown. Post-surgery the boy presented with paresthesia and mild motoric function disability of the right hand, which diminished after a few days. The focal seizures of the right arm persisted after surgery. Postoperative MRI (after 3 and 11 months) demonstrated a stable, remaining T2 extension in the motor strip. There were no signs of tumor progression. As a consequence, the patient received no further adjuvant chemotherapy.

365. PEDIATRIC NEURO-ONCOLOGY FROM 2500 BC TO NOW: A HISTORICAL PERSPECTIVE

D. Walker,[1] C. Bailey,[2] R. Taylor,[2] G. Perilongo,[3] and J. Punt[1]; [1]Children's Brain Tumor Research Centre, University of Nottingham, Nottingham, UK; [2]Department of Paediatric Oncology, St James' Hospital, Leeds, UK; [3]Department of Paediatric Oncology, University of Padova, Padova, Italy

Pediatric neuro-oncology has evolved as an international subspecialty since the establishment of the International Symposium of Paediatric Neuro-Oncology in 1985. Historically, the scientific body knowledge which forms the basis for modern practice can be traced to 2500 BC. Studying the evolution of this specialty identifies development of scientific discoveries, technical inventions, and combined modality working from the late nineteenth century to the present day. Consultation of a wide variety of texts, Web sites, experienced colleagues, and other historical sources helped create a historical timeline, depicting events, people, institutions, journals, scientific advances, awards and political events that have shaped the current state of knowledge and practice. This information will be used to stimulate

further contributions of important historical events, nationally and internationally, by a request to delegates to identify additional events as part of an ongoing chronology of events that will evolve. The first draft has already been incorporated in Brain & Spinal Tumors of Childhood, Editors, D.A. Walker, J.A.G. Punt, R. Taylor, and G. Perilongo (Arnold). A summary of the timeline will be presented of the evolution of neuroscience, neurosurgical practice, institutions, diagnostic imaging, radiotherapy, chemotherapy, cancer science and epidemiology that have been identified so far as making major contributions to the clinical and scientific practice of neuro-oncology. Further collection and subsequent dissemination of information from this timeline will stimulate international debate of the national and international scientific and clinical developments critical to establishment of effective clinical services for children with brain and spinal tumors in our respective national health systems.

366. MALIGNANT CENTRAL NERVOUS SYSTEM TUMORS IN CHILDREN UNDER 3 YEARS OF AGE: SINGLE INSTITUTIONAL EXPERIENCE

C. Wynne, S. Mackie, and J. Sastry; Paediatric Oncology, Southampton General Hospital, Southampton, UK

It is perceived that the prognosis is poor in central nervous system (CNS) tumors diagnosed in children less than 3 years of age. Surgery and chemotherapy are the mainstays of treatment in this group of patients. Radiotherapy is rarely used as a modality of treatment, primarily due to concerns about its effects on brain growth and neurodevelopmental outcome. The purpose of this study was to review the types of tumors, mode of presentation, and management strategies used, as well as to assess outcome. Patients under the age of 3 years diagnosed with CNS tumors between 1 September 1994 and 31 August 2004 were identified retrospectively from the database in a UKCCSG pediatric oncology centre. Analysis of management and outcome was performed using patient records. Thirty-four patients were identified, of which 18 were male and 16 female. The median age at diagnosis was 20.8 months (interquartile range, 12.4–28.5 months). The pathological diagnosis was available in 32 patients: primitive neuroectodermal tumor (PNET) (12 patients), astrocytoma (8 patients), optic glioma (2 patients), tectal glioma (2 patients), brain stem glioma (1 patient), oligodendroglioma (1 patient), choroid plexus papilloma (2 patients), ependymoma (2 patients), gangliocytoma (1 patient), and pineoblastoma (1 patient). Two additional patients with CNS tumors had no pathological diagnosis. Six patients were treated with surgery alone, 5 patients with chemotherapy alone, and 7 patients with both modalities. Surgery, chemotherapy, and radiotherapy were used to treat 1 patient with optic glioma and 1 patient with ependymoma. The patient with brain stem glioma was treated with radiotherapy alone. Five patients, including 1 with PNET, received no active treatment and remain alive. Six patients with PNET and the 2 patients without pathological diagnoses died, having not received active treatment. At the end of the 10-year period, 19 patients overall were still alive. Of the children who had not survived, 11 had PNET, 1 had astrocytoma, and 2 did not have a pathological diagnosis. There was no apparent trend of improving survival in the last 5 years. In this local group of patients diagnosed with a CNS tumor below the age of 3 years, 19 out of 34 patients (55%) are alive at the time of the study. The prognosis remains poor, but this appears to be largely attributable to the high mortality associated with PNET, the most common CNS tumor in this series.

367. CHRONIC LOW-DOSE CHEMOTHERAPY FOR REFRACTORY OR RECURRENT BRAIN TUMORS OF CHILDHOOD

T. Yanagisawa,[1] M. Akiyama,[1] K. Yokoi,[1] Y. Yuza,[1] Y. Kato,[1] K. Fujisawa,[1] T. Joki,[2] S. Ohi,[2] and Y. Eto[1]; [1]Paediatric Oncology; [2]Neurosurgery, Jikei University School of Medicine, Tokyo, Japan

Prolonged low-dose chemotherapy, which exploits the schedule dependency of the agents, has sometimes demonstrated increased activity in both hematological and solid refractory malignancies of childhood compared with bolus administration. This makes it a reasonable choice for palliative treatment in children who are otherwise incurable. A prospective phase 2 study has been conducted in our institute to evaluate the efficacy and toxicity of prolonged schedule oral etoposide in children with refractory or relapsed malignancies in a palliative treatment setting. Patients were treated with oral etoposide (50 mg/m^2 per day given daily for 21 consecutive days every 4 to 5 weeks) after failing in medical, radiological, and/or surgical treatments. End point for follow-up was disease progression. Four patients with brain tumors were entered. Patient 1 was a 6-year-old boy with suprasellar mature teratoma with malignant component. Two years after the initial treatment with repeated surgeries, radiotherapy, and chemotherapy, he had local relapse. Failing in ICE and BEP regimen chemotherapy and gamma-knife treatment, oral etoposide was initiated for progressive tumor. Stable disease duration was 28 months with oral etoposide. Patient 2 was a 4-year-old girl with suprasellar immature teratoma. Six months after high-dose chemotherapy with thiotepa and toposide regimen and PBSCT for the first local relapse, tumor progression was noticed. Oral etoposide was initiated for the recurrent tumor after failing in ICE regimen chemotherapy. Progression-free survival was 11 months. Patient 3 was a 1-year-old girl with pontine low-grade astrocytoma. Oral etoposide was begun after failing in initial chemotherapy with vincristine and carboplatin. She has been alive at home for 31 months without any signs of tumor progression. Patient 4 was a 13-year-old boy with brain stem tumor. Failing in initial radiotherapy (54 Gy), oral etoposide was begun. Four months after the treatment, MRI revealed tumor progression and weekly vinblastine (6 mg/m^2) was initiated. He has been at home without tumor progression for 8 months. Chronic oral etoposide and weekly vinblastine appeared to be well tolerated, had modest toxicity, and showed excellent palliative effect in the children with refractory and progressive brain tumors. Further large-scale investigation seems justified to determine the indication and the optimal use of these treatments.

368. THERAPEUTIC STRATEGY FOR ATYPICAL TERATOID/RHABDOID TUMOR: A REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

A. Gomi[1], Y. Koizumi[1], H. Suzuki[1], T. Miyata[1], E. Matsumoto[1], H. Fujii[1], S. Sakurai[2], K. Fuse[1], and E. Watanabe[1]; [1]Surgical Neurology; [2]Pathology, Jichi Medical School, Tochigi, Japan

Atypical teratoid/rhabdoid tumor (AT/RT) of the central nervous system is a highly malignant neoplasm that primarily affects children less than 5 years of age. Despite aggressive multimodality therapy including surgical resection, chemotherapy, and irradiation, prognosis is dismal. Here we report two young AT/RT patients with extended survival. The first patient was a male, 11 months of age at presentation. The tumor was located in the right cerebellar hemisphere without radiographic metastatic disease. Initial therapy consisted of gross total surgical resection followed by 7 cycles of mild chemotherapy (cisplatin and etoposide). No relapse occurred within 33 months of initial treatment. The second patient was a female, 24 months of age at presentation. The tumor was located in the fourth ventricle without radiographic metastatic disease. Initial therapy consisted of gross total surgical resection followed by 3 cycles of mild chemotherapy (cisplatin and etoposide). Relapse occurred within 3 months of initial treatment. Postrecurrence treatment consisted of surgical resection and extended local irradiation to a dose of 5600 cGy. After 17 months of continued remission, cerebrospinal dissemination occurred. Following extended chemotherapy (cisplatin/etoposide and ifosfamide), the size of the tumor was reduced. After 4 months of continued remission, dissemination around the brainstem recurred, and the patient died at 27 months of initial surgery. It is important to consider the therapeutic strategy best suited to each individual AT/RT patient. For children less than 3 years of age, we hesitate to use radiotherapy because it impairs physical and mental development; therefore, extended surgical resection and chemotherapy should be selected as initial therapies. Some cases of AT/RT may not be sensitive to chemotherapy, and once we have determined that chemotherapy is ineffective, we should not hesitate to induce radiotherapy even if the child is less than 3 years old. Previous reports have indicated that most AT/RT are radiosensitive. In the event of recurrence, we recommend surgical resection and intensive chemotherapy.

369. RESIDUAL DISEASE IN INTRACRANIAL MALIGNANT GERM CELL TUMORS (GCTS): FINDINGS GENERATED IN SIOP CNS GCT 96 AND THEIR PROGNOSTIC IMPLICATION

G. Calaminus,[1] J. Nicholson,[2] D. Frappaz,[3] L. Garré,[4] C. Alapetite,[5] U. Riccardi,[6] F. Saran,[7] T. Czech,[8] R. Kortmann,[9] and U. Göbel[1]; [1]Pediatric Oncology, Hematology and Immunology, Heinrich-Heine-University, Duesseldorf, Germany; [2]Pediatric Oncology, Addenbrookes Hospital, Cambridge, UK; [3]Centre Leon Berard, Lyon, France; [4]Hematology/Oncology, G. Gaslini Children's Hospital, Genova, Italy; [5]Institut Curie, Paris, France; [6]Radiation Oncology, Ospedale Infantile Regina Margherita, Torino, Italy; [7]Radiotherapy, Royal Marsden Hospital NHS Trust, Sutton, UK; [8]Neurochirurgie, Allgemeines Krankenhaus Wien, Wien, Austria; [9]Radioonkologie, Universität Leipzig, Leipzig, Germany

Residual disease is a predominant risk factor in most of the malignant brain tumors. Therefore, the impact of residual tumor at the end of treatment has been analyzed in patients with intracranial malignant GCTs who are registered in the SIOP CNS GCT 96 trial. Residual disease is defined as any abnormal contrast enhancement persisting in the former tumor region. The patients under study are considered to be either germinoma patients or non-germinoma patients. For the germinoma patients, after diagnosis, treatment consists either of craniospinal irradiation 24 Gy/16 Gy tumor boost (option A) or a chemotherapy with carboplatin/VP16/ifosfamide fol-

lowed by 40 Gy focal radiotherapy (RT) (option B). One hundred seven patients are evaluated. MRI is assessed after operation, 3 months after RT, and 1/2 year after diagnosis. Thirteen children have a complete resection and are not analyzed for response. Fifty-four patients have a subtotal/partial resection, of whom 39 receive a craniospinal RT (option A), and 15 children have a combined treatment (option B). A stereotactic biopsy has been performed in 40 patients, of whom 31 receive option A and 9 children option B. In summary, 30 patients have a residual lesion after RT between 0.2 and 2.0 cm in diameter. No patient undergoes a second operation or other additional therapy. During follow-up, 13 tumors resolve, and only one of 30 patients develops a progression. In comparison to the group without residual lesion at the end of therapy (n = 64, five events) no difference in event-free survival (EFS) can be detected (no residual 0.88 + 0.06 vs. residual 0.97 + 0.03). For the non-germinoma patients, treatment after diagnosis by markers and/or histology consists of either 4 courses PEI and focal RT with 54 Gy or 30 Gy craniospinal RT/24 Gy tumor boost in case of metastatic disease. One hundred fourteen children are evaluated. MRI is assessed after operation, after chemo, and 3 months after RT. Thirty-six patients show residual tumor after completion of therapy. In 26 patients, no information about the status at the end of treatment is available. Of the 52 patients without residual tumor, 43 are in CR in contrast to 20 of the 36 children with residual disease. (EFS no residual 0.80 + 0.07 vs. residual 0.42 + 0.04). The impact of persisting tumor on prognosis in intracranial malignant GCTs highlights the biological divergence of germinoma and non-germinoma. Whereas in germinoma in the case of a residue an observant position is justified, in non-germinoma a surgical excision should be performed whenever possible to decrease the high risk of recurrence. This work was supported in part by Deutsche Krebshilfe.

370. MGMT AND p53 STATUS PREDICT TEMOZOLOMIDE SENSITIVITY IN HUMAN MALIGNANT GLIOMA CELLS

M. Hermisson,[1] A. Klumpp,[1] W. Wick,[1] J. Wischhusen,[1] G. Nagel,[2] W. Roos,[2] B. Kaina,[2] and M. Weller[1]; [1]General Neurology, University of Tuebingen, Tuebingen; [2]Toxicology, University of Mainz, Mainz; Germany

Temozolomide (TMZ) is an alkylating agent which prolongs survival when administered during and after radiotherapy in the first-line treatment of glioblastoma and which also has significant activity in recurrent disease. O^6-methyl-guanine-DNA methyltransferase (MGMT) is a DNA repair enzyme attributed a role in cancer cell resistance to O^6-alkylating agent-based chemotherapy. Using a panel of human glioma cell lines, we demonstrate here that the levels of MGMT expression are a major predictor of TMZ sensitivity in human glioma cells. The MGMT inhibitor, O^6-benzylguanine (O^6-BG), sensitizes MGMT-positive glioma cells to TMZ, whereas MGMT gene transfer into MGMT-negative cells confers protection. The anti-apoptotic BCL-X_L protein attenuates TMZ cytotoxicity in MGMT-negative LNT-229, but not in MGMT-positive LN-18 cells. Neither irradiation nor dexamethasone modulates MGMT activity or TMZ sensitivity. Abrogation of p53 wild-type function by RNA interference or a dominant-negative p53 mutant strongly attenuates TMZ cytotoxicity. Conversely, p53 mimetic agents designed to stabilize the wild-type conformation of p53 sensitize glioma cells for TMZ cytotoxicity. Collectively, these results suggest that the determination of MGMT expression and p53 status will help to identify glioma patients who will or will not respond to TMZ.

371. THE COMPARABLE EFFECT OF EXO- AND ENDOGENOUS CANNABINOIDS ON APOPTOSIS IN HUMAN MEDULLOBLASTOMA AND RAT GLIOMA CELLS

L. Storer,[1] A. Loku Kalutotage,[2] D. Walker,[1] and T. Parker[2]; [1]Department of Child Health; [2]School of Biomedical Sciences, University of Nottingham, Nottingham, UK

Synthetic cannabinoids act by mimicking endogenous substances (endocannabinoids) which activate specific cannabinoid receptors (Guzman, 2003). Cannabinoids have been shown to induce apoptosis, decrease tumor growth, and inhibit tumor angiogenesis in a number of different transformed cell lines and primary cells (Blazquez et al., FASEB J. 17, 529, 2003; Guzman, Nature Rev. 3, 745, 2003; Sanchez et al., FEBS Lett. 472, 39, 1998). In this study the effect of cannabinoids and endocannabinoids was investigated on human medulloblastoma (Daoy) and rat glioma (C6) cells. Cells were seeded in 6-well plates at a density of 50,000 cells/ml. The apoptotic effect of cannabinoids and endocannabinoids was studied by replacing the media after 24 h with either anandamide, 2-arachidonylglycerol (endogenous cannabinoid agonists), WIN-55,212-2 (CB_1 and CB_2 agonist), Methandamide (CB_1 agonist) or AM281 (CB_1 antagonist). Drug concentrations used were 10^{-4} M, 5×10^{-5} M, and 10^{-5} M. Positive controls were incubated with 10 ÏM MG132, a known apoptotic inducer. Cells incubated in the presence of DMSO or ethanol (carriers) provided the baseline level of apoptosis. Cells were incubated for 3 and 7 days. Cells were harvested and incubated with Annexin V and propidium iodide. The number of live and the number of apoptotic cells were counted by using flow cytometry. The results showed drug-dependent apoptosis after 3 days in 4 Daoy cells incubated with WIN-55,212-2 at 5×10^{-5} M. However, there is little drug-dependent apoptosis following 3 days incubation of Daoy and C6 cells with all other drugs tested. Trends show that after 7 days of incubation the levels of apoptosis increased dramatically with all agonists. Treatment with AM281 alone did not result in apoptosis at all concentrations. Control experiments showed the carriers had no significant effect on apoptosis at concentrations lower than 5×10^{-4} M on both cell lines. These results provide strong evidence to justify further investigation of exo- and endogenous cannabinoids as therapeutic agents in the treatment of medulloblastoma and glioma brain tumors.

372. LEVETIRACETAM (LEV) MONOTHERAPY IN PATIENTS WITH PRIMARY BRAIN TUMORS (PBTS): EFFICACY, SIDE EFFECTS, AND CSF AND PLASMA PHARMACOKINETICS

M. Previti,[1] M. Chamberlain,[3] S. Kesari,[4] P. Wen,[4] K. Edwards,[1] A. Van Horn,[1] and *M. Glantz*[2]; [1]Neuro-oncology, University of Massachusetts, Worcester, Massachusetts; [2]Neurology, University of Massachusetts Memorial Medical Center, Worcester, Massachusetts; [3]Neuro-oncology, University of Southern California, Los Angeles, California, [4]Neuro-oncology, Massachusetts General Hospital, Boston, Massachusetts; USA

LEV is a broad-spectrum antiepileptic drug (AED) with a novel mechanism of action, excellent tolerability, no induction of the hepatic P-450 system, and a half-life permitting twice daily dosing. These features suggest that LEV would be an attractive AED for patients with PBTs, in whom seizures are common and often poorly controlled, and in whom P-450 interactions are a critical issue. Fourteen patients with PBTs and intraventricular reservoirs for administration of intra-CSF chemotherapy received a single oral loading dose of LEV at doses between 500 and 3000 mg. Plasma and CSF LEV concentrations were measured serially between 0 and 48 h after LEV administration. Data including epidemiologic features, seizure characteristics, tumor type and therapy, concurrent medications, prior AEDs, and dose, side-effects, and efficacy of LEV and any other AEDs was also prospectively collected on 150 consecutive PBT patients receiving LEV either as initial or replacement monotherapy for tumor-related seizures. Loading doses of LEV of up to 3000 mg were well tolerated. The mean T_{max} of LEV in plasma was 5.2 h (range, 1–7), and in CSF 7.3 h (range, 3–15). The mean t½of LEV in serum was 13.3 h (14–20), and in CSF 24 h (13–40). No patients discontinued LEV secondary to adverse events. The most common adverse events were mood disturbance (8%) and sedation/fatigue (7%). Seventy-five percent of patients had complete or good ($\leqslant$1 seizure/month) seizure control with LEV, 83% of patients were receiving at least one p-450 metabolized drug, and 76% of patients were treated with at least one p-450 metabolized chemotherapeutic agent. Peak levels of LEV are achieved rapidly in serum and CSF after a single oral loading dose. The long t½ LEV in the CSF suggests a long duration of action and permits twice daily dosing. Efficacy and tolerability are excellent, and P-450 interactions are absent. LEV should be considered as first- and second-line therapy in patients with PBTs.

373. CHIMERIC PEPTIDES AS TUMOR-SELECTIVE DELIVERY SYSTEMS

S. Jones and J. Howl; Molecular Pharmacology Group, School of Applied Sciences, University of Wolverhampton, Wolverhampton, UK

The cell-type-specific targeting of cytotoxic agents and other functional moieties can be achieved by using peptidyl *address* motifs that selectively bind protein targets expressed at high density at the cell membrane. Indeed, numerous studies have confirmed the utility of ligands for G protein–coupled receptors as components of heterofunctional peptide chimeras that are selective biological probes. Our current efforts are directed toward the further development of chimeric peptidyl constructs that employ sequences derived from GPCR ligands or cell penetrant motifs to affect the selective delivery of cytotoxins and signal transduction modulators to tumor cells. We have designed and synthesized a range of hybrid constructs consisting of cytotoxins (peptide and non-peptide) covalently linked to an address peptide derived from the C-terminal of gastrin (G7; H-AYGWMDF-NH_2). The G7 *homing* motif targets a novel binding site expressed by U373MG astrocytic tumor cells that is distinct from classical CCK_1/CCK_2 receptors. Moreover, biological responses following activation of this novel membrane-bound protein may offer additional therapeutic advantages. For example, G7 receptor activation is reported to inhibit the motility of malignant astrocytoma in vivo while avoiding the growth-promoting effects of gastrin (Pannequin et al., J. Pharmacol. Exp. Ther. 302, 274, 2002). We

evaluated the cytotoxicity of our chimeric peptides by comparing changes in cellular viability using MTT conversion assays. Our data indicate that chimeric peptides dose-dependently and rapidly (<8 h) reduced the viability of U373MG cells. Moreover, as a chimeric amino-terminal extension, the G7 *address* motif enhanced the cytotoxicity of both mastoparan (H-INLKALAALAKKIL-NH_2) and $_D$(KLAKLAK)$_2$ peptides reported to stimulate necrosis and/or apoptosis of eukarytoic cells. In conclusion, hybrid G7 chimeras enhance the efficacy of cytotoxic agents and may be valuable probes to investigate and manipulate additional aspects of astrocytoma cell biology. This work was supported by The Wellcome Trust.

374. INTRATUMORAL PHARMACOKINETICS DETERMINED WITH MICRODIALYSIS IN A PATIENT WITH GLIOBLASTOMA MULTIFORME FOLLOWING SYSTEMIC ADMINISTRATION OF HIGH DOSE METHOTREXATE

J. Olson, J. Supko, S. Phuphanich, E. McKenzie, and *S. Grossman*; NABTT CNS Consortium, Johns Hopkins Hospital, Baltimore, Maryland, USA

Delivering potentially therapeutic concentrations of chemotherapeutic agents to brain tumors remains a major concern in the design and interpretation of clinical trials in neuro-oncology. This is the first report of the use of microdialysis in a human to monitor local concentrations of a systemically administered anticancer drug within a brain tumor. This study was conducted under a research protocol approved by the National Cancer Institute and Institutional Review Board at each participating site. Patients with recurrent and refractory glioblastoma multiforme (GBM) where a surgical biopsy or partial resection followed by high-dose methotrexate (MTX) was planned were eligible. A microdialysis catheter was placed within residual contrast-enhancing tumor at the time of surgery. Modified Ringer's solution was delivered to the catheter at 1 ml/min. On the following day, 12 g/m^2 of MTX (adjusted for renal function) were administered as a 4-h intravenous infusion. Plasma and microdialysate were collected at 30-min intervals from one hour before dosing to 24 h after completing the infusion. MTX was assayed using high-performance liquid chromatography with mass spectrometric detection, with a 0.5 ng/ml lower limit of quantitation. The first patient to be evaluated was a 51 year old who had a biopsy to confirm tumor progression after prior surgery, radiation, Gliadel, and temozolomide. The plasma pharmacokinetics of MTX behaved as expected. Renal function remained unchanged, and the leukovorin rescue was administered as per protocol. MTX concentrations in extracellular fluid within the GBM rose rapidly to a maximum of 57 mM and subsequently decayed in a monoexponential manner with a half-life of 12.3 h. As this was considerably slower than the loss of MTX from plasma, MTX concentrations in the dialysate actually exceeded the plasma levels beginning at 16 h after the MTX infusion ended. The patient subsequently developed urosepsis with neutropenia, but no mucositis or diarrhea, and died 3 weeks later after care was withdrawn. Detailed review by NABTT, CTEP, and the study site found no association with the microdialysis catheter and an uncertain relationship to MTX. This initial experience has demonstrated the ability to measure intratumoral concentrations of a chemotherapeutic agent in a patient with a brain tumor using microdialysis techniques. This approach could significantly impact the selection of drugs, dosing schedules, and routes of administration in clinical neuro-oncology trials.

375. A NOVEL MECHANISM OF PKA- AND PKC-DEPENDENT DRUG RESISTANCE IN HUMAN GLIOMAS INVOLVING PHOSPHORYLATION AND METABOLIC ACTIVATION OF GLUTATHIONE S-TRANSFERASE P1 (GSTP1)

F. Ali-Osman,[1] H. Lo,[2] G. Antoun,[1] A. Friedman,[1] H. Friedman,[1] and D. Bigner[1]; [1]Surgery, Duke University Medical Center, Durham, North Carolina; [2]Molecular Oncology, M.D. Anderson Cancer Center, Houston, Texas; USA

The human GSTP1 protein, involved in phase II metabolism of many anticancer agents used in brain tumor therapy and in the regulation of cell signaling in response to stress, is highly expressed in a significant proportion of human malignant gliomas. The overexpression and nuclear localization of GSTP1 has been associated with drug resistance and poor outcome in gliomas and medulloblastomas. We report here a novel mechanism of glioma resistance to chemotherapy that involves crosstalk between the GSTP1 protein and two Ser/Thr protein kinases, PKA and PKC, frequently activated in human gliomas, resulting in phosphorylation and significant enhancement of GSTP1 metabolic activity. Using cell-free systems and MGR3 human glioblastoma cells, we showed both PKA and PKC to phosphorylate GSTP1 GSH-dependently, with a stoichiometry of 0.4 + 0.03 and 0.53 + 0.02 mole phosphate per mole GSTP1 protein. In the presence of GSH, eight different PKC isoforms (a, bI, bII, d, e, g, h, and z), belonging to the three major PKC subclasses phosphorylated the GSTP1 protein, albeit with varying efficiencies. Enzyme kinetic studies with GSTP1 proteins mutated at candidate amino acid residues established Ser42 and Ser184 as putative phospho-acceptor residues for both kinases in the GSTP1 protein. The catalytic efficiency, kcat/Km, of the phosphorylated GSTP1 was more than double that of the unphosphorylated protein. In glioblastoma cells, activation of PKA and PKC signaling resulted in almost a 3-fold increase in specific GSTP1 activity and a significant increase in the resistance of the glioblastoma cells to BCNU, cisplatin, and 4-hydroxyifosphamide. These findings demonstrate PKA- and PKC-dependent phosphorylation of GSTP1 as a significant post-translational mechanism of regulation of GSTP1 function. The GSH-dependence of the phosphorylation suggests that under high intracellular GSH conditions, such as is present in most drug-resistant tumors, the GSTP1 protein will exist in a hyperphosphorylated and metabolically more active state. The increased resistance of glioblastoma cells following activation of PKC and PKA and the associated increase in GSTP1 metabolic activity indicate the crosstalk between the Ser/Thr kinases and GSTP1 as a novel mechanism of drug resistance in glioma cells. The findings also explain, in part, the high resistance of PKC-overexpressing gliomas to chemotherapy and suggest that PKC inhibitors have a potential to overcome GSTP1-associated drug resistance in gliomas. This work was supported by grants RO1 CA91438, RO1 CA79644, and P50-CA108786 from the NIH (USA).

376. ICOVIR-2, AN OLD FRIEND WITH A NEW FACE: E2F-1 MEDIATED GLIOMA SELECTIVITY FOR DELTA24-RGD

M. Alonso,[1] R. Alemany,[2] C. Gomez-Manzano,[1] W.K.A. Yung,[1] H. Jiang,[1] and J. Fueyo[1]; [1]Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [2]Terapia Genica, Instituto Catalan de Oncologia, Barcelona, Spain

Oncolytic adenoviruses are promising therapies for the treatment of gliomas. However, the major hurdles that these treatments encounter are selectivity and efficacy. Efficacy can be enhanced by broadening the tropism of the adenovirus. In this regard, we increased the infectivity of the D24 adenovirus inserting and integrin-binding motif (RGD) into the adenoviral fiber. Specificity can be achieved by means of regulatory elements that provide both cell-specific gene expression and viral replication in cancer cells. In this study, we developed a system in which E2F-1 promoter drives the expression of the mutant E1A, in the context of D24-RGD construct (Icovir-2). We hypothesized that E2F1 promoter will increase the selectivity of Icovir-2 in gliomas due to the absence of a functional RB pathway in these tumors and the low activity of E2F1 in normal brain. In order to characterize the therapeutic potential of Icovir-2 we carried out in vitro studies using two different gliomas cell lines, U-251MG and U-87MG, expressing high and low CAR, respectively. Icovir-2 showed cytopathic effect and replication capacity in both cell lines. Interestingly, restoration of the Rb-pathway by means of transfer of pRB or p21 completely abrogated the oncolotyc effect of Icovir-2. Importantly, restoration of the RB-pathway in cells infected with D24-RGD showed partial rescue (50%) from the cytotoxic effect. These data suggested an increased specificity of Icovir-2 for glioma cells in comparison to D24-RGD. Assessment of E1A levels by Western blot revealed also reduced expression levels of the protein in cells infected with Icovir-2 versus D24-RGD. Moreover, fiber expression was detected in D24-RGD but not in Icovir-2 infected cells. Importantly, Icovir-2 was unable to replicate in arrested NHA, and the MTT analysis showed a significantly reduced cytotoxicity in comparison to D24-RGD. Expression levels of E1A in NHA were also substantially reduced in comparison to NHA infected with D24-RGD. ChIP analysis showed that the cellular E2F1 was able to bind and activate the Icovir-2 promoter and that transfer of pRB resulted in transcriptional repression of E2F1 at the promoter location by pRb. Notably, in vivo studies involving tail vein injection (5^{10} vp) followed by monitoring the levels of GPT (IU/L) showed that liver toxicity induced by Icovir-2 was significantly lower than toxicity induced by D24-RGD. Our data showed that Icovir-2 presents improved therapeutic index in glioma cell lines versus D24-RGD. We concluded that cancer-specific promoters could complement tropism-enhancer strategies in the fight of oncolytic adenovirus against cancer cells.

377. EFFECT OF DEXAMETHASONE ON THE CYTOTOXIC EFFECT OF CLOMIPRAMINE IN HUMAN ASTROCYTIC CELLS IN VITRO

S. Amar, K. Parker, R. Lisle, and G.J. Pilkington; Cellular & Molecular Neuro-Oncology Group, School of Pharmacy & Biomedical Sciences, University of Portsmouth, White Swan Road, Portsmouth, UK

Dexamethasone is a steroid frequently prescribed to patients with primary high-grade brain tumors in order to control cerebral edema. Our laboratories have already demonstrated that a tricyclic antidepressant, clo-

mipramine, selectively induces apoptosis in cultured brain tumor cells by compromising the mitochondrial respiratory chain via complex III and consequently activating a caspase pathway to cell death. It has previously been also shown that dexamethasone inhibits and promotes apoptosis in vitro. Clomipramine and dexamethasone are known to be interacting medications. Additive, synergistic, or inhibitory effects of dexamethasone on the apoptotic effect of chlomipramine need therefore to be considered. In a series of experiments IPSB-18 (passage 46), cultured anaplastic astrocytoma cells, and UPESC (passage 11), a non-neoplastic astrocyte-rich population of cells derived from the temporal lobe of an epileptic patient were exposed to clomipramine (10 μM) or dexamethasone (0–50 μM) for 24 h. They were then exposed to clomipramine (0–30 μM) or dexamethasone (0–50 μM) for 72 h (i.e., alternative clomipramine/dexamethasone and dexamethasone/clomipramine treatment schedules). MTT assays were performed to assess cell viability after drug exposure. We have also shown that dexamethasone pretreatment of neoplastic astrocytes modulates oxygen utilization using a Clarke Oxygen electrode assay and promotes apoptosis (Annexin V assay). Pretreatment with clomipramine and subsequently with dexamethasone showed no treatment-related effect in both cell lines. However, in tumor cells, pretreatment with dexamethasone followed by clomipramine treatment resulted in shift from cell death at < 10 μM (dexamethasone followed by dexamethasone) to cell death at concentration <2 μM. This effect was not observed in non-neoplastic cells. Studies are currently in progress in order to elucidate the molecular mechanisms underlying these effects. This research is supported by the Samantha Dickson Research Trust.

378. CYTOKINES REGULATE INTERLEUKIN 13 RECEPTOR ALPHA2 EXPRESSION IN GLIOMA CELLS

W. Debinski, N. Hu, and D.M. Gibo; Brain Tumor Center of Excellence, Wake Forest University, Winston-Salem, North Carolina, USA

We documented that a vast majority of patients with high-grade gliomas (HGG) overexpress IL13Ra2, a restricted receptor for interleukin13 (IL13) and that this receptor is a very attractive target for anti-HGG molecularly targeted therapies. We had suggested that epigenetic mechanisms involving activating protein 1 (AP-1) activity are involved in the regulation of expression of IL13Ra2 in HGG, and recent studies revealed that the promoter region of the receptor's gene possesses AP-1 and also STAT-6 binding sites. However, little is known how the expression of this receptor is biologically regulated, and thus we conducted series of experiments in which HGG cells were treated with either AP-1 or STAT-6 stimulatory cytokines, individually or in combination, and the levels of the receptor were monitored. The IL13Ra2 protein levels were examined by Western blot and immunohistochemistry (IH) in a variety of HGG cells and normal cells. Thus, the cells were treated with epidermal growth factor (EGF) or IL4 or tumor necrosis factor alpha (TNFa), AP-1, and STAT-6 stimulants, respectively. We have found that serum-starved HGG cells, such as A-172 MG, U-251 MG, G48a, SNB-19, and U-87 MG, had the levels of immunoreactive IL13Ra2 decreased significantly as detected by Western blotting and IH. This is suggestive that IL13Ra2 is overexpressed in HGG in a nonconstitutive manner. The addition of EGF or TNFa or IL4 to cells increased prominently the levels of IL13Ra2 protein, usually by three- to tenfold, the extent of which was cytokine-, cell line- and time-dependent. For example, EGF upregulated the receptor most potently after 24-h treatment in the U-251 MG cells and after 36-h treatment in G48a cells, while IL4 alone had little effect. We next examined whether an already elevated IL13Ra2 can be further upregulated by the studied cytokines in the presence of serum in HGG cells. We found that supra-physiologic concentrations of EGF and TNFa, and less that of IL4, could further increase the levels of the receptor in HGG cells. The same experiments were performed on transformed normal glial cells (SVGp12), human endothelial cells (HUVEC), and keratinocytes (HaCat). In general, the background levels of immunoreactive IL13Ra2 were very low in those cells when compared with HGG cells. EGF, TNFa, or IL4 produced an increase in the receptor levels, but the levels of IL13Ra2 were still much lower than in HGG cells. IL13Ra2 is currently utilized preclinically and clinically as a target for a variety of therapeutic approaches, such as targeted recombinant cytotoxins, viruses, cytolytic T cells, and vaccines. In this work, we demonstrate that a short-term pretreatment of HGG cells with AP-1 and/or STAT-6 stimulatory cytokines should provide further therapeutic advantage by upregulating the levels of IL13Ra2.

379. NATURAL SESQUITERPEN ALCOHOL ALPHA-BISABOLOL, A NONTOXIC COMPOUND, STRONGLY INDUCES APOPTOSIS IN GLIOMA CELL LINES

E. Cavalieri,[1] S. Mariotto,[1] *L. Berra*,[2] A. Carcereri de Prati,[1] A. Ciampa,[1] C. Fabrizi,[3] G. Lauro,[3] S. Leone,[3] R. Gottardo,[4] and H. Suzuki[1]; Departments of [1]Neuroscience and Vision, Section of Biochemistry, [2]Neuroscience and Vision, Section of Neurosurgery, and [4]Medicine and Public Health, Unit of Forensic Medicine, University of Verona, Verona; [3]Department of Biology, University of Roma Tre, Rome; Italy

Among human tumors, glioblastoma is one of the most malignant ones, and despite aggressive surgical resection and radiotherapy, the median survival in these patients does not normally exceed 1 year (De Angelis, N. Engl. J. Med. 344, 114, 2001; Surawicz et al., J. Neurooncol. 40, 151, 1998). The use of systemic chemotherapy may improve the efficacy of treatment, but its use is associated with significant toxicity, and the long-term prognosis remains poor (Parker et al., CA Cancer J. Clin. 46, 5, 1996). Carmustine (BCNU) is, at concentration corresponding to LD10 (13 mg/kg), not able to completely kill glioma cells *in vivo* (Rosenblum et al., J. Neurosurg. 58, 177, 1983). Numerous natural compounds have been reported to be potential anti-glioma agents, although major parts of these sank into oblivion. a-bisabolol is a small, oily sesquiterpene alcohol with molecular mass of 222.37 Da, isolated from the essential oil of a variety of plants, shrubs, and trees. Its toxicity in animals is very low (LD50 = 13–14 g/kg, Merck Index). In the present study, we report the cytotoxic effect and the type of death induced by a-bisabolol in glioma cells. We examined, as a human glioma cell model, T67 and U87 cell lines and, as an animal model, the rat glioma cell line C6. At 2.5–3.5 mM, the viability of these cells was reduced to 50% with respect to untreated cells in 24 h. Furthermore, the same concentrations failed to affect the viability of normal rat astroglial cells, in line with its reported non-toxicity in rats (Hernandez-Ceruelos et al., Toxicol Lett. 135, 103, 2002; Villegas et al., J. Nat. Prod. 64, 1357, 2001). At higher concentrations (10 mM) a-bisabolol killed completely the cells. Judging from caspase 3 activation, poly(ADP-ribose) polymerase cleavage, DNA ladder formation, and hypo-G1 accumulation, the cytotoxicity triggered by a-bisabolol results from the induction of apoptosis. Two major routes, extrinsic and intrinsic, have been identified through which cytotoxic drugs induce apoptosis. The first one is mediated by death receptors. In the second pathway, mitochondria play essential roles. The dissipation of mitochondrial-inner transmembrane potential and the release of cytochrome c from mitochondria indicate that apoptosis occurs, in our experiments, through the intrinsic pathway. Taken together, these results point out that a-bisabolol may be considered a novel compound able to inhibit glioma cell growth and survival.

380. ENHANCED EFFICACY AND MR IMAGING OF CONVECTION-ENHANCED DRUG DELIVERY

Y. Mardor,[1] O. Rahav,[2] Z. Lidar,[2] A. Ocherashvilli,[1] D. Daniels,[1] Y. Roth,[1] S. Maier,[3] J. Zauberman,[4] and Z. Ram[2]; [1]The Advanced Technology Center, Sheba Medical Center, Ramat-Gan, Israel; [2]Neurosurgery Department, Tel-Aviv Medical Center, Tel-Aviv, Israel; [3]Radiology, Brigham and Women's Hospital, Boston, Massachusetts, USA; [4]Neurosurgery Department, Sheba Medical Center, Ramat-Gan, Israel

Convection-enhanced delivery (CED) is a promising technique for distribution of drugs into brain and tumor tissue, currently being tested in several phase 2/3 clinical trials. We have previously presented the clinical application of diffusion-weighted MRI (DWMRI) for monitoring CED of Taxol in recurrent GBM patients. CED is known to depend on several physical and physiological parameters. After accounting for these variables, initial clinical experience shows significant variability in the extent of convection among patients and among drugs. Therefore, increasing tumor response to CED is essential, as well as real-time monitoring of the extent of convection and its early effects on the tissue. Solutions containing combinations of Cremaphore, Taxol, carboplatin, ethanol, sucrose, and human serum albumin in different concentrations were mixed with Gd-DTPA and infused into normal rat striatum or intratumorally in rats bearing large 9L tumors. T1 MRIs were acquired immediately post-treatment to assess the extent of convection, and DWMRIs were acquired 24 h later to assess tissue response. Some rats were monitored by MRI for an additional 4 days to demonstrate subsequent formation of necrosis. The extent of convection was reflected by the T1 MRIs. Limited convection was characterized by significant backflow along the catheter and into the ventricles, while efficient convection presented significant spread into the striatum. CED with cytotoxic infusates was followed by significant changes in subsequent DWMRIs. The extent of these changes correlated significantly (Taxol, $r^2 = 0.75$, $P < 0.004$) with the extent of convection as depicted by T1 MRIs. When convection was not achieved, including with toxic infusates, no changes were detected on DWMRIs. The efficacy and extent of convection were found to correlate significantly with infusate viscosity ($r^2 = 0.73$, $P < 0.003$). While low-viscosity infusates tend to backflow, high-viscosity infusates tend to

form efficient convection. Increasing the viscosity of a carboplatin solution with sucrose led to a significant increase of infusate distribution within the striatum, depicted by the immediate T1 MRIs ($P < 0.008$). This effect was accompanied by a corresponding increase in toxic tissue changes, depicted by the later DWMRIs ($P < 0.007$). In all 9L tumor–bearing rats, DWMRI revealed no tumor response, consistent with the immediate T1 MRIs, which showed that the Taxol leaked out of the tumor and accumulated in normal surrounding brain tissue. Our data suggest that Gd MRI and DWMRI can be used for real-time assessment of CED efficiency and early assessment of tissue response. In addition, increasing the viscosity of solvents may be a simple way to significantly enhance the efficacy of CED treatments, thus increasing their antitumor effect.

381. TARGETING MALIGNANT GLIOMAS WITH A LOW-MOLECULAR-WEIGHT RAF/VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR INHIBITOR

S. Sathornsumetee,[1] D. Batt,[3] M. Kieran,[2] S. Kier,[1] T. Ramsey,[3] N. Yusuff,[3] D. Reardon,[1] D. Bigner,[4] H. Friedman,[1] and *J. Rich*[1]; Departments of [1]Surgery, [4]Pathology, and [5]Medicine, Duke University Medical Center, Durham, North Carolina; [2]Pediatrics, Dana-Farber Cancer Institute, Boston, Massachusetts; [3]Oncology, Novartis Institutes for Biomedical Research, Cambridge, Massachusetts; USA

Low-molecular-weight tyrosine kinase inhibitors against several growth factor receptors have shown promise in preclinical malignant glioma studies, but more modest activity in clinical trials. We are currently investigating novel intracellular kinases that may be frequently active in gliomas. Raf is a critical intracellular mitogenic signaling mediator downstream from the G-protein Ras family members that is occasionally mutated in gliomas. Additionally, RAS and Raf activities are increased in most malignant gliomas through activation of growth factor receptor pathways. As multiple growth factor receptors converge onto Raf, disrupting Raf function may offer broad activity against tumors dependent on this pathway. AAL881 is a novel, orally administered, small-molecule inhibitor of kinase activity associated with Raf and vascular endothelial growth factor (VEGF) receptors. We have now shown that AAL881 treatment of a highly resistant human glioma cell line, D54MG, inhibits phosphorylation of downstream signaling effectors, colony formation, VEGF secretion, and invasion through an artificial matrix. In addition, AAL881 inhibits cellular proliferation by producing cell-cycle G_1 arrest without inducing significant apoptosis. In in vivo studies of athymic mice, AAL881 treatment was well tolerated without significant weight loss. A short-term (two-week) course of AAL881 treatment in athymic nude mice with established subcutaneous D54MG xenografts not only significantly delayed tumor growth in 5/10 mice, but also cured 5/10 mice in repeated trials. Tumors less responsive to AAL881 treatment displayed decreased proliferation relative to control tumors. AAL881 therapy in athymic mice with intracranial D54MG xenografts more than doubled median survival compared to the control group. Of note, D54MG xenografts display minimal sensitivity to VEGFR tyrosine kinase inhibitors suggesting that either Raf plays an important role in tumor growth or targeting combined Raf/VEGFR activity is highly active against this tumor. Based on these results, Raf may represent a useful therapeutic target in gliomas. AAL881, a novel Raf/VEGFR kinase inhibitor, may offer a promising therapy against malignant gliomas. This work was supported by grants from Accelerate Brain Cancer Cure and the Pediatric Brain Tumor Foundation of the U.S. J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

382. COMBINATION THERAPY WITH PERIFOSINE AND TEMOZOLOMIDE FOR GLIOMA TREATMENT: PRECLINICAL TRIAL USING A MOUSE GLIOMA MODEL

H. Momota,[1] E. Nerio,[1] J. Finney,[1] Y. Lyustikman,[1] and E. Holland[1,2,3]; [1]Cancer Biology and Genetics, [2]Surgery (Neurosurgery), and [3]Neurology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

Glioblastoma multiforme (GBM) is an incurable glial tumor even with intensive therapy consisting of surgery followed by radio-chemotherapy. Many newer forms of treatment have been tried for GBM, but these have not been efficacious. Presenting, the best chemotherapeutic drugs for GBM are alkylating agents such as carmustine (BCNU) and temozolomide. Alkylphospholipid is in a novel class of antitumor agents, and perifosine is a first oral alkylphospholipid with a marked cytotoxic effect and fewer side effects. Unlike most chemotherapeutic drugs that target the nuclear DNA, perifosine interacts with the cell membrane and blocks signal transduction pathways. Recent studies have suggested the molecular mechanism of perifosine action has the capacity to enhance radiation or other anticancer drugs. However, the exact mechanisms of perifosine's effect on gliomas are still unclear. To develop a new paradigm for glioma therapy, we used mouse glioma cell lines to investigate the mechanism of action of perifosine alone and in combination with temozolomide. We also addressed this in vivo by using a mouse glioma model. Mouse glial cells transformed with PDGF-B, Kras, Akt, Kras+Akt, and LacZ were used as an in vivo model. These cells were treated with 300 μM temozolomide and 45 μM perifosine and analyzed with cell proliferation assay, Western blot, and cell cycle analysis. Gliomas ware induced in mice by PDGF gene transfer to the nestin-expressing neural progenitor cells. Tumor-bearing mice were detected by bioluminescence imaging, and image-positive mice were treated with daily intraperitoneal administration of 100 mg/kg temozolomide and oral administration of 30 mg/kg perifosine. Mice were then sacrificed and tumor histology was examined. Perifosine and temozolomide inhibited mouse glioma cell growth in a dose-dependent manner, and these drugs had a synergistic effect in cell culture. Cell cycle analysis showed a stronger G1 arrest with this combination rather than with each drug alone. In vivo, the mouse glioma model also demonstrated reduction in tumor size by imaging and minimal staining for proliferation markers by immunohistochemistry. Combination therapy of perifosine and temozolomide is effective in a mouse glioma model and could be a new candidate in treatment of human gliomas.

383. THE LUMINESCENT ATP ASSAY OR THE COLORIMETRIC MTS ASSAY—WHICH IS BETTER FOR CHEMOSENSITIVITY TESTING IN MALIGNANT GLIOMAS?

R.V. Iyer,[1] T. Dawson,[1] K. Ashton,[1] C.H.G. Davis,[1] A. Golash,[1] P. Roberts,[2] and R.W. Lea[2]; [1]Neurosurgery, Lancashire Teaching Hospitals NHS Trust, Preston; [2]Biomedical Research unit, University of Central Lancashire, Preston; UK

Chemosensitivity testing has been used clinically most successfully in leukemia. Non-neurological malignancies provide large amounts of tumor tissue for drug testing, while very little tissue is available in malignant astrocytomas due to limited surgery. There is a need for an assay methodology sensitive enough to deal with small numbers of glial cells. Toward this end we have developed a cell death assay by measuring ATP levels following exposure of primary glial cells to different chemotherapeutic agents which were compared with a tetrazolium dye reduction assay (MTS). Also, we have investigated the role of apoptotic cell death by determining the level of caspase enzyme activation within the cells. Astrocytic tumor tissue obtained at surgery was disaggregated and plated in culture flasks. At confluence the cells were trypsinized and transferred to 96-well microtiter plates. At 70% confluence, five concentrations of each drug (cisplatin, BCNU, paclitaxel, and etoposide) were added to the wells. After a period of 72 h, cell proliferation was measured by using the one-step MTS and ATP assays (Promega). A separate luminescent microtiter plate was used to measure apoptotic activity by measuring caspase levels by using the one-step caspase assay (Promega). All tumors were either WHO grade III or IV astrocytomas. Assays were performed at the first passage, although the rate of cell growth was low with some cultures limiting the plating density on the microtiter plates. A high correlation ($R^2 > 0.9$) was observed between the ATP and MTS assays when plating density per well was high (>1000). However, the correlation was poor ($R^2 < 0.5$) with low plating densities. There was variability in the sensitivity of different primary cultures to the drugs used, with only cisplatin showing a consistently toxic effect in the assays used. High levels of caspase activation were only observed with paclitaxel and cisplatin at the highest concentrations used, despite cell death being seen at lower concentrations. The luminescent ATP assay has a greater sensitivity to predict drug response in glial cells, with lower cell numbers than the colorimetric MTS assay, making it a potential tool for testing small biopsies. Estimation of caspase levels show that at lower concentrations cisplatin and paclitaxel may cause cell death through non-apoptotic mechanisms.

384. TISSUE INHIBITOR OF METALLOPROTEINASES-3 EXPRESSION IN THE CONTEXT OF AN ONCOLYTIC ADENOVIRUS INHIBITS MATRIX METALLOPROTEINASE ACTIVITY IN VIVO BUT DOES NOT ENHANCE ANTI-TUMOR EFFICACY IN MALIGNANT GLIOMA

M. Lamfers,[1] D. Gianni,[2] C.H. Tung,[3] S. Idema,[1] P.H. Quax,[4] V.W. van Beusechem,[5] W.P. Vandertop,[1] C.M.F. Dirven,[1] E.A. Chiocca,[2] and W.R. Gerritsen[5]; [1]Department of Neurosurgery, VU University Medical Center, Amsterdam, The Netherlands; [2]Molecular Neuro-Oncology Laboratories, Neurosurgery Service and [3]Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts, USA; [4]Gaubius Laboratory TNO-PG, Leiden, The Netherlands; [5]Department of Medical Oncology, Division of Gene Therapy, VU University medical center, Amsterdam, The Netherlands

A promising new approach to treatment of solid tumors is the use of oncolytic viruses designed to replicate specifically in malignant cells. We hypothesized that insertion of a transgene encoding a secreted protein

which exerts effects on angiogenesis, apoptosis, and tumor cell infiltration would improve the anti-tumor activity of these agents. Tissue inhibitor of metalloproteinases 3 (TIMP-3) constitutes an interesting transgene candidate for such an approach considering its reported inhibitory effects on these processes. To assess the effects of TIMP-3 gene transfer to glioma cells, we first employed a replication-defective adenovirus encoding TIMP-3 (Ad.TIMP-3). Infection of U-87MG, U-87dEGFR, and U-251MG glioma cells with Ad.TIMP-3 inhibited in vivo invasion up to 86%. In addition, Ad.TIMP-3 infection of a panel of primary glioma cell cultures obtained from patients material decreased the viability of these cells and induced morphological changes characteristic for apoptosis. On the basis of these findings, we proceeded to construct a conditionally replicating adenovirus encoding TIMP-3. The TIMP-3 expression cassette was inserted into the E3 region of the adenoviral backbone containing a 24 bp deletion in E1A. This novel oncolytic adenovirus, AdD24TIMP-3, demonstrated enhanced oncolytic activity on a panel of glioma cell lines and primary cell cultures compared to the control oncolytic virus AdD24Luc. To confirm inhibition of in vivo MMP activity by AdD24TIMP-3, nude mice bearing subcutaneous glioma xenografts received intratumoral injections of AdD24TIMP-3 or AdD24Luc. The functional activity of TIMP-3 was imaged noninvasively by using a near-infrared fluorescent MMP-2-activated probe. Tumoral MMP-2 activity was significantly reduced by 58.3% in the AdD24TIMP-3 treated tumors 24 h after infection. A study into the therapeutic effects of combined oncolytic and antiproteolytic therapy was performed in both a subcutaneous and an intracranial model for malignant glioma comparing AdD24TIMP-3 to AdD24Luc. Treatment of subcutaneous (U-87MG) or intracranial (U-87dEGFR) tumors with AdD24TIMP-3 and AdD24Luc both significantly inhibited tumor growth and survival compared to PBS-treated controls. However, no significant difference in anti-tumor activity between these oncolytic viruses was found. TIMP-3 was produced by glioma cells infected with AdD24TIMP-3 in vivo and in vivo. The biological effect resulting from an oncolytic adenovirus expressing TIMP-3 can be imaged in vivo non-invasively demonstrating the functional expression of the gene of interest. The anti-tumor effects of AdD24 could not be improved by insertion of the TIMP-3 gene when injected during tumor development in two murine models for malignant glioma.

385. OPTIMAL BIOLOGICAL DOSE AND SCHEDULE OF INTERFERON ALPHA TO REDUCE THE GROWTH OF HUMAN GLIOBLASTOMA IMPLANTED ORTHOTOPICALLY IN NUDE MICE

D. Nam, M. Kim, and K. Park; Department of Neurosurgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

The purpose of this study was to optimize the antitumor activities of pegylated IFN-alpha (PEG-IFN-alpha) against U87MG human glioblastoma cells growing orthotopically in nude mice. Twenty days after the intracranial inoculation of tumor cells, groups of mice (n = 5) were injected with different doses of PEG-IFN-alpha (1,000, 5,000, 10,000, 25,000, and 125,000 units) twice per week subcutaneously. PEG-IFN-alpha at 10,000 units decreased the expression of basic fibroblast growth factor and matrix metalloproteinase-2 most effectively. More than 25,000 unit injection did not show the reduced expression of proangiogenic molecules by interferon. Administration at the optimal biological dose (10,000 units, twice a week) decreased tumor uptake (control: 6/6; PEG-IFN-alpha: 2/6) and progressive growth of human glioblastoma cells. Mice that received chronic treatment (4 weeks) reduced in tumor uptake and size compared with the short-term treatment (2 weeks). With the immunohistochemical study, there was significant inhibition in the expression of proangiogenic molecules with decreased microvessel density by PEG-IFN-alpha. The data suggest that determination of optimal biological dose for PEG-IFN-alpha is important in the clinical trial for glioblastoma patients.

386. PRECLINICAL FEASIBILITY AND SAFETY STUDY OF A NOVEL ULTRASONIC DISPERSION SYSTEM FOR INTRAPARENCHYMAL DRUG DELIVERY IN BRAIN TUMORS

A.A. Kanner,[1] Y. Mardor,[2] H. Ezer,[1] L. Trejo,[1] Y. Wollman,[1] D. Massad,[3] M. Goldman,[3] and Z. Ram[1]; [1]Neurosurgery, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

We have developed a novel ultrasonic dispersion device (UDD) to treat infiltrative primary brain tumors by intra-tumoral delivery of therapeutic molecules. The UDD consists of a dispersion wire within a guide tube. The transmitter tip can be coated with a variety of particles of different size and character. Ultrasonic pulses of different profiles induce a high frequency vibration (sonification) of the wire tip, which disperse particles from the coated tip into the adjacent tissue. We conducted initial studies in a rat brain model to investigate the local and remote effects of UDD on normal brain tissue, and subsequently the feasibility and characteristics of in situ compound dispersion of various compounds. The tested microparticles were purple DS02/5290 (30 nm, Bangs Lab.), blue Fe_3SO_4 (100 nm), and blue Polystyrene (180 nm). The UDD probe was introduced stereotactically into the right frontal lobe of male Fischer rats. The sonication was performed with two different power profiles as a single treatment cycle. Profile I lasted for 60 s, source power 1 Watt with pulses of 1 s. Profile II lasted for 120 s, source power 2 Watt with pulses of 2 s. After the procedure the probe was removed and the brains were harvested after 30 min, 24 h, 96 h, and 10 days. Macroscopic and microscopic evaluation was performed on H&E stained histological brain sections by a neuropathologist. Summary of the research results: A total of 24 rats received a single cycle of treatment. The stratification of the study groups was performed according to the two different sonification treatment profiles with 3 rats each per profile (2) and time point (4). A significant dispersion of the coating material (both solid and suspension) within brain tissue was observed in all rats. Longer sonification periods resulted in more extended distribution. During the post-treatment observation period, no abnormal behavior of the study animals was evident. Macroscopic and microscopic examinations of the brain tissue specimens were negative for any evidence of tissue damage, cyst formation, or necrosis. We have demonstrated the feasibility and safety of the UDD in a rat brain model. Further efficacy studies in tumor-bearing rats are ongoing.

387. INCREASED ONCOLYTIC POTENCY OF THE CONDITIONALLY REPLICATIVE ADENOVIRUS ADD24-P53 WHEN COMBINED WITH RADIOTHERAPY IN VIVO

S. Idema[1], M. Lamfers,[2] S. Heukelom,[3] V. van Beusechem,[2] W. Gerritsen,[2] W. Vandertop,[1] and C. Dirven[1]; Departments of [1]Neurosurgery, [2]Medical Oncology, and [3]Radiotherapy, VU University Medical Center, Amsterdam, The Netherlands

Conditionally replicative adenoviruses (CRAds) represent a promising class of agents used against therapy-resistant brain tumors such as glioblastoma multiforme (GBM). The adenovirus Ad*f*24-p53 has a 24 bp deletion in the E1a region limiting replication to Rb mutant cells and encodes the p53 tumor suppressor protein. It has shown superior oncolytic activity in glioma compared to the parental control Ad*f*24. As both replication competent adenoviruses and exogenous p53 expression have been shown to enhance radiosensitivity, the current experiments were performed to assess the effect of Ad*f*24-p53 and Ad*f*24 in combination with radiotherapy in GBM monolayer cultures, multicellular spheroids, and mouse xenografts. U-87 and U-251 glioma cells growing in monolayers were irradiated with 3, 6, and 9 Gy. After 24 h, cells were infected at a multiplicity of infection (MOI) of 0.1 or 0.01 with Ad*f*24-p53 or the control Ad*f*24. Eleven days after treatment, viability was assessed by using a quantitative crystal violet assay. U-87 spheroids were irradiated at 4 and 9 Gy and infected with 5×10^4 plaque-forming units (PFU) of Ad*f*24-p53 or Ad*f*24. Viability was measured 12 days after infection using the tetrazolium salt–based WST-1 assay. U-87 subcutaneous mouse xenografts of approximately 170 mm^3 in size were locally irradiated with 2×10 Gy on days 0 and 4 and received 4×10^8 PFU of Ad*f*24-p53 on days 0, 2, and 4. Tumor size was measured 3 times weekly. The combination of Ad*f*24-p53 and irradiation in U-87 or U-251 cells synergistically increased cell kill with maximally 72% compared to single treatments (ad*f*24: *f*24-p53 and 9 Gy significantly reduced viability from 76% (infection alone) to 50% (ad*f*24: 80% vs. 75%, respectively). Combination treatment in U-87 mouse xenografts increased tumor growth delay from 2 days (Ad*f*24-p53) and 23 days (irradiation) to 32 days, with 80% complete regression and 5/10 long-term survivors compared to 0/10 (irradiation) and 2/9 (Ad*f*24-p53). Combining the radiosensitizing effects of a conditionally replicative adenovirus and p53 expression has the potential to greatly enhance the effect of radiotherapy in GBM. Here we show that combining the CRAd Ad*f*24-p53 with irradiation improves the potency of both single treatments in vivo and in vivo. These results show great promise for a future clinical trial combining radiotherapy with Ad*f*24-p53.

388. NEWCASTLE DISEASE VIRUS INDUCES APOPTOSIS IN GLIOBLASTOMA CELLS

M. Remmers,[1] B. König,[2] R. Firsching,[1] and T.S. Schneider[1]; [1]Department of Neurosurgery and [2]Institute of Microbiology, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany

Malignant gliomas remain incurable brain tumors despite aggressive treatment with surgery, irradiation, and chemotherapy. As a new approach the therapy with different kind of virus, for instance genetically engineered, achieved more and more interest. Some naturally occurring strains of Newcastle disease virus (NDV) may have an oncolytic potential against tumor cells. Therefore we asked whether NDV may induce cell death in glioblas-

toma cells. We analyzed the effects of lytic and non-lytic strains of NDV on two established glioblastoma cell lines (U87, U138) with regard to parameters of cell death. For this purpose the tumor cells were treated with NDV at concentrations from 0.0001 to 100 HU (hemagglutination units) for up to 96 h. At different time points, cell viability was measured by using the tetrazolium salt WST-1. Additionally, the induction of apoptotic parameters was investigated, as well as DNA-fragmentation with Tunnel-assay. Normal rat astrocytes were treated as a control group. All three NDV strains induced cell death in both glioblastoma cell lines in a dose-dependent manner. However, the most significant effects were observed with the lytic NDV strain "73 T". In parallel we observed modulation of distinct key parameters in the apoptotic signaling pathway, and especially DNA-fragmentation could be detected as NDV-dose dependent. The same effects were not seen in normal rat astrocytes. Oncolytic strains of NDV, described as apathogenic for human, seem to be a very promising option for therapy of glioblastoma. The in vitro results require an in vivo testing using an animal model as the next step to therapeutical application.

389. GEMCITABINE SENSITIZES ASTROCYTOMA CELL LINE TO RADIATION EXPOSURE INCREASING APOPTOSIS

A. Di Palma,[3] B. Bucci,[2] A. Citti,[2] E. Brunetti,[2] *C. Carapella*,[1] G. Raza,[3] R. Miceli,[3] and U. De Paula[3]; [1]Department of Neurosurgery, Regina Elena Institute, Rome; [2]Research Center, Ospedale San Pietro AFaR-FBF and [3]Oncological Radiotherapy Unit, Ospedale San Pietro FBF, Rome; Italy

Gemcitabine (dFdCyd, Gem) is a deoxycytidine analogue, recently indicated as a radiosensitizer agent in different tumor cell lines. In this study, we investigated the ability of Gem to enhance the radiosensitivity of human astrocytoma cell line (ADF). The effects of ionizing radiation (IR) and/or Gem, on cell growth, cell cycle distribution, and apoptosis were assessed. Cell counts and viability were determined by trypan blue exclusion test. Cell cycle perturbation and apoptosis were analyzed by flow cytometry. Escalating doses of 5, 10, and 15 Gy IR exposure caused a dose-dependent inhibition on the cell proliferation. A 5-Gy IR exposure produced a moderate antiproliferative effect, within 120 h from treatment, with an inhibitory effect less than 30%. Conversely, 10-Gy IR treatment determined significant inhibition in cell growth (67%), already at 24 h after treatment associated with an elevated amount of apoptosis (29%) at 48 h. Instead, in cells treated with 15 Gy, the inhibition of cell growth was mainly due to necrosis. For these experiments, we have chosen 10 Gy in combination with Gem treatment (Gem/IR) at the IC_{50} dose (0.0175 mM). The analysis of cell growth showed that Gem/IR combined treatment enhances the lethal effect induced by IR alone. Gem exposure, determining an accumulation in G_1 phase (about 64%) influences cell sensitivity to IR-induced apoptosis. The IR alone treatment induced a remarkable apoptotic cell death (40%) that was increased by the Gem/IR exposure (68%). Gem sensitizes astrocytoma cell line to the radiation exposure rendering these cells more prone to radiation-induced apoptosis.

390. PRO-DRUG CONVERTING NEURAL STEM CELLS FOR THE LOCAL INTRACEREBRAL CHEMOTHERAPY OF HUMAN GLIOBLASTOMA XENOGRAFTS

N.O. Schmidt,[1] M. Ziu,[2] W. Przylecki,[2] T. Cargioli,[2] P. Black,[2] K. Aboody,[3] and R. Carroll[2]; [1]Department of Neurosurgery, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]Neurosurgical Oncology Laboratory, Neurosurgery/Brigham & Women's Hospital, Boston, Massachusetts, USA; [3]Divisions of Hematology/HCT, City of Hope National Medical Center and Beckman Research Institute, Duarte, USA

Previous reports have demonstrated that neural stem cells (NSCs) distribute throughout experimental intracranial gliomas and are able to "track" invading tumor cells when implanted in the adult rodent brain. Based on this extensive tumor tropism NSCs are attractive candidates as a potential delivery system for therapeutic gene products in the treatment of invasive gliomas. Here, we investigated the therapeutic effectiveness of glioma-targeting NSCs expressing cytosine deaminase (CD) to convert systemically administered nontoxic pro-drug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU), which diffuses out of the NSCs and selectively kills dividing tumor cells. Murine NSCs C17.2 (lacZ/cytosine deaminase positive or negative) were stereotactically implanted distant (occipital) to well-established intracerebral U87 human glioblastoma xenografts in adult nude mice. Systemic treatment with 5-FC at 500 mg/kg/d started three days after NSCs injection. Tumor growth was assessed by T1-Gd-enhanced magnetic resonance imaging and NSCs distribution by X-gal immunohistochemistry. Intracerebral implantation of 1.5×10^5 NSCs-CD followed by systemic administration of 5-FC inhibited the tumor growth as assessed by MRI 14 days after treatment start. Furthermore, the survival was significantly prolonged when compared to the animals of the control groups (no NSC implantation, implantation of NSC-mock plus 5-FC treatment or implantation of NSC-CD but no 5-FC). Histological analysis demonstrated the intratumoral distribution of NSCs although the cells were initially implanted distant from the main tumor mass. However, we were not able to detect any NSCs based on X-gal immunohistochemistry in the brains of nude mice analyzed later than 22 days after NSC injection. These results indicate that NSCs represent a potent new delivery system for the local intracerebral treatment of gliomas. However, NSCs may not survive within the tumor environment for a prolonged time, and therefore larger numbers of NSCs or multiple injections should be considered. Future studies need to address the interaction of transplanted NSCs with the tumor environment.

391. THE EFFICACY OF ALGINATE ENCAPSULATED CHO-K1 SINGLE CHAIN-TRAIL PRODUCER CELLS IN THE TREATMENT OF BRAIN CANCER

J.M.A. Kuijlen,[1] B.J. de Haan,[2] J.J.A. Mooij,[1] W. Helfrich,[2] and P. de Vos[1]; [1]Neurosurgery, Groningen; [2]Pathology and Laboratory Medicine, Groningen University Hospital, Groningen; The Netherlands

Patients with astrocytic tumors in the central nervous system (CNS) have low survival rates despite surgery and radiotherapy. Innovative therapies and strategies must be developed to prolong survival of these patients. The alginate microencapsulation method, used to continuously release a certain cytotoxic drug in the vicinity of the tumor, is such a novel delivery strategy. Targeted TRAIL (TNF-related apoptosis-inducing ligand) a death-inducing protein with targeted specificity for a tumor-associated cell receptor seems promising as an antineoplastic drug. TRAIL used in this study was recombinantly coupled to a single chain variable fragment (scFv425) with specificity for the EGF receptor. The biological functionality of the apoptosis inducing scFv425:sTRAIL protein, which was released by the microencapsulation method, was studied in vitro. Analysis of the intracerebral biocompatibility of alginate capsules was performed by implantation of empty alginate capsules in the brain of mice. Chinese hamster ovary cells (CHO-K1) were recombinantly engineered to produce the single chain anti-EGFR-TRAIL (scFv425:sTRAIL). The CHO-K1 producer cells were encapsulated in an alginate capsule with a semipermeable membrane through which the scFv425:sTRAIL could be released. Empty alginate capsules were implanted in the left cerebral hemisphere of C57BL6 mice. In vitro studies show maintained biological functionality of the released scFv425:sTRAIL. There was no tissue response detectable after intracerebral implantation of the alginate capsules in C57BL6 mice brains. Biological functionality of the produced scFv425:sTRAIL is maintained and intracerebral biocompatibility of the capsules is warranted. Alginate encapsulation of CHO-K1–scFv425:sTRAIL producer cells and subsequently their intracerebral implantation is technically feasible. This study justifies further in vivo experiments.

392. MTOR AS A THERAPEUTIC TARGET FOR RADIATION SENSITIZATION OF GLIOMA VASCULATURE

B. Lu; Vanderbilt University, Nashville, Tennessee, USA

It is known that radiation activates the PI3K/Akt pathway and that inhibition of PI3K or Akt sensitizes tumor vasculature to radiotherapy. mTOR is a downstream target of Akt, and we hypothesized that irradiation activates mTOR signaling in both glioma and endothelial cells. By inhibiting this activation, we hypothesized that we could increase radiosensitization of these cell lines. Two compounds which selectively inhibit mTOR, rapamycin and RAD001 (everolimus), were used in this study. Both compounds caused a significant increase in sensitization of vascular endothelial cells with only minor effects on glioma cell radiosensitivity as determined by clonogenic assay. Therefore, we specifically investigated the anti-angiogenic effects of mTOR inhibitors. Increased phospho-mTOR protein was detected in irradiated HUVEC cells with no detectable increase in total mTOR protein. Phospho-S6, a biomarker for mTOR signaling, was also found to be induced following irradiation, and this effect was inhibited by PI3K or mTOR inhibitors. Significant increase in cleaved caspase 3 was detected when Rad001 was combined with radiation. Endothelial tube formation was significantly diminished following treatment with rapamycin and 3 Gy. Power-weighted Doppler of glioma xenografts in mice showed a significant reduction in vasculature and blood flow compared with mice treated with 3 Gy or RAD001 alone. We conclude that irradiation activates mTOR signaling in vascular endothelium and that rapamycin and RAD001 increased apoptosis of endothelial cells in response to radiation. To the authors' best knowledge, this is the first study that demonstrates that mTOR inhibitors may be a way to target the vasculature by radiosensitizing the vascular endothelium resulting in better tumor control, as seen in experiments demonstrating increased tumor growth delay in mice treated with rapamycin

with radiation compared with mice treat with either alone. We conclude that mTOR inhibitors have increased efficacy as antiangiogenics when combined with radiation.

393. HISTONE DEACETYLASE INHIBITOR VALPROIC ACID EXTENDS SURVIVAL OF SCID MICE BEARING ORTHOTOPICALLY HETEROTRANSPLANTED HUMAN MEDULLOBLASTOMA CELLS THROUGH INDUCTION OF APOPTOSIS

Q. Shu,[1] J. Su,[1] B. Antalffy,[2] S. Blaney,[1] C. Lau,[1] and *X. Li*[1]; [1]Pediatrics, Texas Children's Cancer Center; [2]Pathology, Baylor College of Medicine, Houston, Texas, USA

Histone deacetylase (HDAC) inhibitors represent a novel family of anticancer agents, several of which are being tested in clinical trials. Valproic acid (VPA), a widely utilized anticonvulsant with a well-established toxicity profile, has recently been shown to inhibit HDAC. Our previous studies demonstrated that VPA is capable of inducing apoptosis, cellular differentiation, and senescence in vivo in medulloblastoma (MB) cell lines. Additionally, VPA treatment also resulted in potent cell cycle arrest, proliferation inhibition, and tumorigenicity suppression. These anti-tumor activities were mediated through induction of histone (H3 and H4) acetylation and regulation of multiple gene expression. To evaluate VPA's anti-MB effects in a preclinical model that closely recapitulates the biology of human MBs, we injected a total of 10^5 cells from four MB cell lines (DAOY, D283, MHH-MED-1, and MEB-MED-8A) into the right cerebellum of Rag-2 SCID mice. Two weeks later, after formation of a tumor mass, treatment with VPA through subcutaneously implanted osmotic pump (Aztec, model 2001) was initiated. We were able to maintain serum VPA concentrations around 70 Ïg/ml for 7 days, after which a new osmotic pump was implanted to complete treatment for 2 weeks. Our results demonstrated that VPA treatment prolonged the survival of tumor–bearing animals for all four cell lines. Accumulation of histone acetylation, increased apoptosis, and induced cellular differentiation were observed in VPA-treated tumors. Since the heterotransplanted MB cells proliferated in an anatomical site and a microenviroment relevant to human MBs, our results offered compelling preclinical evidence for testing VPA as an alternative or adjuvant therapy for children with MBs. A clinical trial of VPA for children with recurrent tumors has been initiated through the Children's Oncology Group.

394. NON-PATHOGENIC POLIOVIRUS RECOMBINANTS FOR THE TREATMENT OF MALIGNANT GLIOMA

M. Gromeier,[1] E.Y. Dobrikova,[1] M.K. Merrill,[1] S.A. Moore,[1] C. Kaiser,[1] R. Grisham,[1] and L. Selznick[2]; [1]Department of Molecular Genetics & Microbiology and [2]Division of Neurosurgery, Duke University Medical Center, Duke University Brain Tumor Center, Durham, North Carolina, USA

We have developed a novel treatment modality for malignant glioma based on genetically modified polioviruses. Susceptibility to poliovirus depends on the availability of its cellular receptor, the immunoglobulin superfamily molecule CD155. Selective expression of CD155 in spinal cord motor neurons is believed to uniquely render this compartment susceptible to poliovirus infection and ensuing destruction, producing the histopathological hallmarks of paralytic poliomyelitis. We have demonstrated that the *CD155* gene is upregulated ectopically in CNS malignancy, providing a target for therapeutic intervention with poliovirus. However, the inherent neuropathogenic potential of poliovirus would prohibit any therapeutic application. We resolved this obstacle by genetic manipulation of a key viral regulatory element involved in translation control. Poliovirus (+) strand RNA, unlike cellular mRNA, is not equipped with a 5' terminal cap structure and hence uses an alternative mechanism for translation initiation. A complex RNA structure within the 5' untranslated region of the viral genome, the internal ribosomal entry site (IRES), mediates ribosomal entry in a 5' end, cap-independent manner. We exchanged the IRES of poliovirus with its counterpart from human rhinovirus type 2. Unexpectedly, the resulting chimeric virus had completely lost the capacity to propagate in normal neuronal cells. In a comprehensive study conducted at the FDA, injection of the recombinant virus into the spinal cords of *Cynomolgus* macaques failed to induce poliomyelitis. However, the chimera retained wild-type growth potential and cell-killing ability in malignant glioma cells. We have recently deciphered the molecular mechanisms that repress rhinovirus IRES function in neuronal cells and favor viral propagation in malignant glioma. Our observations demonstrate widespread perturbations of the translation initiation apparatus in high-grade gliomas that favor alternative mechanisms of protein synthesis at the IRES and promote virus growth and cytotoxicity. A prototype oncolytic poliovirus/rhinovirus recombinant has been produced for further safety evaluations at the FDA and is scheduled to enter clinical investigation against glioblastoma multiforme in the near future.

395. THE SAFETY OF ADENOVIRAL VECTORS CONTAINING THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE

G. Langford,[1] S. Yla-Herttuala,[2] and A. Boyd[1]; [1]Research and Development, Ark Therapeutics, London, UK; [2]Gene Therapy Unit, Kuopio University Hospital, Kuopio, Finland

Several clinical studies have demonstrated the safety and efficacy of using adenoviral vectors containing the herpes simplex virus thymidine kinase gene (Adv.HSV-tk) for the treatment of high-grade gliomas. To further evaluate the safety of thymidine kinase in an adenoviral vector, a study was performed to assess the immunological and toxicological responses to intravenous or intracerebral administration of Adv.HSV-tk (Cerepro, Ark Therapeutics Ltd). Ad.HSV-tk was administered to rats by intracerebral or intravenous injection at doses of up to 1.2×1011 vps. The animals were sacrificed at days 3, 30, 60, 70, or 90 and the following assessments were performed: macroscopic and microscopic analysis of all major organs, assessment of clinical chemistry and hematological parameters, immunological analysis of inflammatory reactions to Ad.HSV-tk, and PCR analysis of the biodistribution of the viral vector. There was no effect on clinical observations, body weight, food consumption, seminology, or clinical parameters in either group. Minor changes in mean albumin/globulin levels were observed in the intravenous groups compared to controls. At day 3, mean spleen weight was slightly increased, and myeloid hyperplasia was observed in some animals receiving the highest dose of virus. At day 30 and 70 there was complete reversal of the splenic and bone marrow findings. Microscopic changes were seen at the site of injection of the control and treated animals in the intracerebral groups. At day 3 inflammatory cells were seen at the meninges and at remote sites in a few animals. The extent of the infiltration was reduced at day 30, but changes were observed in the neutrophil of some animals. PCR analysis showed that the amount of vector decreased with time and was proportionally related to the dose level (from day 3 to day 90). Vector was only detected at low levels in the urine at day 3. Intravenous or intracerebral administration of Ad.HSV-tk to rats is well tolerated. Any microscopic observations were transient in the intravenous group. The viral vector was cleared over time. Overall the results of this toxicological study support the continued clinical use of Ad.HSV-tk.

396. INHIBITING PERIPHERAL BENZODIAZEPINE RECEPTOR ENHANCES CHEMOTHERAPY-INDUCED CYTOTOXICITY IN GLIOMAS

Y. Zhang,[1] S. Wu-Pong,[1] W. Broaddus,[2] and H. Fillmore[2]; [1]Pharmaceutics and [2]Neurosurgery, Virginia Commonwealth University, Richmond, Virginia, USA

Therapeutic efficacy of adjuvant chemotherapy for the treatment of glioblastomas remains poor in extending patient survival, in part due to cellular resistance to apoptotic death. Peripheral benzodiazepine receptor (PBR) is a putative member of the mitochondrial permeability transition pore which plays a critical role in controlling cellular apoptosis. PBR expression is dramatically upregulated in brain tumors. We hypothesize that overexpression of PBR may play a role in apoptotic resistance. Our aims were to investigate the therapeutic effectiveness of inhibiting PBR expression using antisense (AS) oligonucleotides (ODNs) in combination with chemotherapy to enhance glioma cell death as well as to elucidate the functional role of PBR in apoptosis in non-glioma cells. Two in vivo systems were established, both of which involve altering PBR expression levels and comparing apoptotic susceptibility between control and mis-expressed cells. In the glioma cell model, five 18-mer phosphorothioate-modified AS ODNs were designed. The most potent ODNs were selected based on their ability to inhibit PBR protein expression in T98G human glioma cells using Western blot analysis. Combination treatment of AS ODNs and camptothecin was assessed for changes in apoptosis (caspase-dependent PARP cleavage, TUNEL), and viability (trypan blue exclusion assay). In the lymphoma cell model, an expression vector construct containing PBR cDNA was transfected into Jurkat cells, a PBR-null cell line. Presence of PBR mRNA and protein expression in stable transfectants was confirmed by using RT-PCR and Western blot analysis, respectively. Extent of apoptosis (Annexin V staining, TUNEL) was compared between PBR over-expressing clones and vector control clones. T98G cells did not undergo any significant extent of apoptosis with camptothecin treatment alone. Of the five AS ODNs, AS5 at 100 nM and 200 nM showed most potent inhibition ($34 \pm 5\%$ and $45 \pm 8\%$, $P < 0.05$) of PBR protein expression. Combination treatment of AS

ODNs and camptothecin did not sensitize T98G cells to undergo apoptotic death. However, enhanced cytotoxicity, in an AS ODN dose-dependent manner, was observed. In Jurkat cells overexpressing PBR, apoptotic response following camptothecin treatment was attenuated about 30% in comparison to vector control cells. PBR protein expression was successfully inhibited by AS ODNs. PBR downregulation via AS ODNs enhanced camptothecin-induced cytotoxicity, possibly through apoptosis-independent pathways in glioma cells. PBR overexpression in nonglioma cells partially conferred resistance to apoptotic death, suggesting that targeting PBR presents an attractive therapeutic strategy to potentiate chemotherapy-induced cytotoxicity in both gliomas and other types of cancer.

397. ALKYLGLYCEROL-MEDIATED INCREASE IN DRUG DELIVERY TO THE NORMAL BRAIN AND TO BRAIN TUMORS IN RATS: REGULATION OF DRUG TRANSFER AND COMPARISON WITH HYPERTONIC MANNITOL AND BRADYKININ

B. Erdlenbruch,[1] P. Hülper,[1] W. Kugler,[1] H. Eibl,[2] and M. Lakomek[1]; [1]Universitätskinderklinik, Pädiatrie I, Göttingen; [2]Biophysikalische Chemie, Max-Planck-Institut, Göttingen; Germany

Response of malignant brain tumors to chemotherapy is often poor or transient due to poor penetration of most of anticancer agents across the blood-brain barrier (BBB). The intracarotid administration of short-chain alkylglycerols has been reported to be an effective and low toxic strategy to increase the transfer of various cytotoxic drugs to the brain. We have investigated the delivery of methotrexate (MTX) to the brain of normal and of glioma-bearing rats (C6 and RG2) after i.a. injection of alkylglycerols. Results were compared with those obtained after BBB-opening using hypertonic mannitol or bradykinin. In tumor-free rats, alkylglycerols induced a concentration-dependent increase in the delivery of MTX to the brain. Using 1-O-pentylglycerol (120 and 150 mM) and 2-O-hexyldiglycerol (75 and 100 mM), MTX-concentrations in the ipsilateral hemisphere were increased 4- to 20-fold and 2- to 4-fold, as compared to controls. Osmotic BBB disruption with 1.4 M mannitol resulted in a very strong accumulation of MTX in the ipsilateral normal brain (114-fold), whereas intracarotid infusion of bradykinin (10 μg/kg/min) showed no effects in normal animals. In glioma-bearing animals, 1.4 M mannitol increased MTX concentrations 8.3-fold in the tumor and 15-fold in surrounding brain. In contrast to this, a tumor to brain selectivity of the permeabilizing effect was observed using alkylglycerols and bradykinin; e.g., MTX accumulation in C6 tumors amounted to 10.2 in tumor tissue and to 7.4 in surrounding brain after 2-O-hexyldiglycerol (100 mM). Bradykinin was less effective, resulting in 2.4-fold higher MTX concentrations in tumor tissue as compared to controls. Variations in the concentration and chemical structure of the alkylglycerols as well as in the time schedule of drug administration were identified to be additional instruments to modify the amount of drug delivered to the brain. From these data we conclude that i.a. alkylglycerols are a promising new tool to circumvent the BBB in brain tumor chemotherapy. This research was supported by Deutsche Krebshilfe (10-1554-Er 2 and 10-1995-Er 3).

398. HYPOXIA-DRIVEN CD/5-FC TREATMENT SUCCESSFULLY INITIATES BYSTANDER AND RADIOSENSITIZATION EFFECTS IN A HYPOXIC GLIOBLASTOMA CELL LINE

L. Hu, J. Chen, D. Wang, J. Wang, K. Lamborn, and D. Deen; Department of Neurosurgery, University of California, San Francisco, San Francisco, California, USA

Current therapy for glioblastoma relies on a multimodal approach. However, efficacy of treatment is limited by therapeutic ratio: Doses sufficient to kill tumor cells often prove toxic to normal cells as well. Therefore, it is especially important to develop therapies that are specifically cytotoxic to cancer cells, either directly or through bystander or sensitizing effects. Hypoxic cells would be ideal targets for such an approach since they are specific to diseased tissue and often comprise the most treatment-resistant subpopulation of a tumor. Cytosine deaminase (CD) has been widely studied as a form of suicide gene therapy, acting by deaminating the nontoxic prodrug 5-fluorocytosine (5-FC) to form the highly cytotoxic 5-fluorouracil (5-FU). Previous studies have found that this strategy of treatment can induce a bystander effect and radiosensitization in cancer cells. However, none of these studies were conducted under hypoxic conditions, which are prevalent in solid tumors and are typically resistant to therapeutic efforts. Therefore, in this study, experiments were set to answer these critical questions for our gene therapy strategy. We used a previously made gene construct consisting of the SV40 minimal promoter under the control of 9 copies of hypoxia responsive elements (HRE). Under hypoxia, hypoxia inducible factor-1 (HIF-1) becomes activated and binds to HRE sequences, facilitating transcription of the yeast CD gene downstream. We performed colony-forming efficiency assays to assess survival of clonogenic cells, and found that both a large bystander effect and radiosensitization occurred under hypoxic conditions. This study was supported by CA-85356 and NS-42927.

399. A NOVEL ULTRASONIC DEVICE FOR DISTRIBUTION OF THERAPEUTIC PARTICLES: AN MRI-BASED FEASIBILITY STUDY IN THE RAT BRAIN

Y. Mardor,[1] A. Kanner,[2] H. Ezer,[2] D. Daniels,[1] A. Ocherashvilli,[1] Y. Wollman,[2] D. Massad,[3] M. Goldman,[3] and Z. Ram[2]; [1]Sheba Medical Center-Tel Hashomer, The Advanced Technology Center, Ramat-Gan; [2]Neurosurgery Department, Tel-Aviv Medical Center, Tel-Aviv; [3]Sonenco Ltd, Hedera; Israel

Ultrasonic Dispersion Device (UDD) is a novel tool for interstitial delivery of various therapeutic molecules and particles of various sizes. It is composed of an ultrasound source that transmits ultrasound energy to a probe with a drug-containing tip that is located within the tissue. Activation of the ultrasound (sonication) activates the spring-shaped tip and by controlled vibration drives the particles into the tissue. The purpose of the current study was to optimize UDD design and sonication parameters to achieve a significant homogenous distribution of particles in the normal rat brain. MRI was used to assess the particle distribution in the brain immediately post-treatment and to follow up the particle concentration over time as well as detect cytotoxic effects. The UDD was inserted stereotactically into the rat brain under general anesthesia, with the active region located in the center of the striatum. The probe was loaded with iron oxide (IO) nanoparticles prior to and during treatment. Various UDD design parameters, such as active region size and location along the probe, transmitter and cover materials, and probe diameter and particle loading, as well as various ultrasonic transmission parameters, such as pulsation sequence and bandwidth, were tested. Treatment duration was 1 to 5 min. Each set of parameters was tested in 3 rats up to a total of 36 rats. Rats were scanned by gradient-echo and T1/T2-weighted MRI immediately post-treatment to assess the IO distribution in the brain. Rats with homogenous IO distribution were scanned periodically by MRI for up to 6 weeks to test the IO washout timescale and possible cytotoxic effects. IO distribution in the rat brain was obtained in 22 out of the 36 rats treated so far. The maximal diameter of distribution, as calculated from the gradient-echo MR images, was 8 mm, and the maximal cross section of distribution was 0.42 cm^2. Three rats, in which a homogenous significant IO distribution was observed, were followed by MRI. In the first follow-up scan, 4 days post-treatment, a decrease (estimated 20%–30%) in IO concentration was detected. The remaining IO distribution was stable throughout the 6-week follow-up. No cytotoxic effects were detected on the MR images. The preliminary data demonstrates the unique advantages of the UDD as a minimal invasive tool to achieve homogenous distributions of highly concentrated large particles in brain tissue. Such distribution was achieved in extremely short treatment durations. The long periods in which the large particles remain in the tissue with no apparent toxic effects may enable distribution of large, slow-release therapeutic agents coating the IO particles for effective treatment of CNS pathologies.

400. UPTAKE AND DRUG DELIVERY USING BIODEGRADABLE NANOPARTICLES IN MONOLAYERS AND ORGANOTYPIC BRAIN SLICES

W. Meng,[1] T.L. Parker,[2] P. Kallinteri,[1] G.A. Hutcheon,[4] S. Higgins,[4] D.A. Walker,[3] D.A. Walker,[3] and M.C. Garnett[1]; [1]School of Pharmacy and [2]School of Biomedical Science, University of Nottingham, Nottingham; [3]Queen's Medical Centre, Nottingham; [4]School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool; UK

The efficacy of brain tumor chemotherapy is limited by access of drug to the brain. A therapeutic advantage may be achieved with biodegradable nanoparticle (NP) drug delivery systems targeted to tumors. We aimed to characterize the uptake and fate of NPs and the entrapped drug by a medulloblastoma cell line (Daoy) and organotypic rat brain slice cultures. For these in vivo studies we have used NPs prepared from a novel polymer, which were stabilized by Tween-80, and contained the fluorescent dye rhodamine B isothiocyanate (RBITC) to represent the drug. NPs were incubated with Daoy cells grown in monolayer culture for different incubation times or amounts of NPs. Intracellular fluorescent dye was visualized by fluorescent microcopy and quantified by FACS. Cerebellar and cerebral cortex rat brain slices were prepared from 2 day neonatal rats. After 15 days of culture, RBITC-loaded NPs were added to the surface of slices and incubated for 24 h. Quadruple fluorescent labeling was used to co-label nuclei (DAPI), to co-localize the NPs (RBITC) in the lysosomes (Lyso–tracker), and to label macrophages (OX-42 monoclonal antibody) observed in the

confocal microscope. NPs were taken up by Daoy cells into lysosomes. The uptake of NPs by Daoy cells was concentration and time dependent. Intracellular fluorescence intensity increased sharply within 2 h, and two plateaus were established, one from 2 to 6 h and the other after 10 h of incubation time. In organotypic slices, NP uptake was cell type dependent, and few NPs were taken up by macrophages. Only a few NPs could be seen in the extracellular space. Studying drug release from NPs, a reduction of fluorescence was observed using FACS. A time-series experiment using fluorescence microscopy showed about 50% of the dye had diffused out of cells after 4h. These experiments aid our understanding of drug delivery by nanoparticles. They show that few NPs were taken up by macrophages due to Tween-80 stabilization and that NPs were taken up into the lysosomes, rapidly degraded intracellularly, and showed release of both drug and NPs into the culture medium in a few hours.

401. DEVELOPMENT OF TRAIL THERAPEUTIC STRATEGIES FOR MALIGNANT GLIOMAS

J.H. Song,[1] D. Song,[1] C. Lin,[2] S. Phuphanich,[1] D. Brat,[1] J. Olson,[1] E. Van Meir,[1] and C. Hao[1]; [1]Pathology & Laboratory Medicine, Neurosurgery, Hematology & Oncology, Emory University, Atlanta, Georgia, USA; [2]Medical Research, China Medical University Hospital, Taichung, Taiwan

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) induces apoptosis in malignant glioma cells and thus offers a new potential therapeutic agent for malignant gliomas. Unfortunately, many human malignant glioma cells are resistant to TRAIL, posing a major obstacle in TRAIL therapy. In this study, we investigated inhibitory mechanisms for development of combination treatments that can overcome the resistance. First, we examined a large panel of 26 human malignant glioma cell lines for their sensitivity to TRAIL and grouped them into two phenotypes, TRAIL sensitive and resistant. The resistant cell lines were analyzed for the sensitivity to the combination treatment with TRAIL and chemotherapy drug cisplatin and etoposide and divided into two subtypes, chemotherapy-sensitized resistant and complete resistant. TRAIL induced apoptosis through binding of cell surface death receptor DR4/DR5, leading to the assembly of death-inducing signaling complex (DISC) in the sensitive cells. In the DISC, apoptosis-initiating caspase-8 was cleaved and thus initiated caspase cascade leading to programmed cell death (apoptosis). In contrast, cellular Fas-associate death domain-like, IL-1b-converting enzyme-inhibitory protein (c-FLIP) and phosphoprotein enriched in diabetes (PED) were recruited to TRAIL-induced DISC and inhibited caspase-8 cleavage in the chemotherapy-sensitized resistant cells. Targeting c-FLIP and PED with small interfering RNA (siRNA) and the chemotherapy drugs sensitized the cells to TRAIL-induced apoptosis. The complete resistant cell lines were analyzed by the combined comparative genomic hybridization (CGH), G-banding/spectral karyotyping (SKY), and fluorescence in situ hybridization (FISH) analyses with chromosomal region specific probes used to identify aberration of chromosomal regions that harbor key TRAIL signaling gene *DR4/DR5, caspase-8, capsase-3, caspase-7, caspase-9, Bid, Bax, Bak, Bcl-2*, and *Smac*. Loss or structural aberration of chromosome regions harboring DR4/DR5, caspase-8, Bid and Smac was simultaneously detected in the complete resistant but not sensitive cell lines. In conclusion, this study provides new TRAIL combination therapeutic strategies that target the DISC resistance. In addition, this study identifies genetic markers that could be used to predict the responsiveness of malignant glioma to the TRAIL-based therapies.

402. THE INHIBITION OF THROMBOXANE SYNTHASE ACTIVITY: A NOVEL TARGET FOR THE TREATMENT OF MALIGNANT GLIOMAS

N.O. Schmidt,[1] M. Ziu,[2] T. Cargioli,[2] A. Giese,[3] M. Westphal,[1] P. Black,[2] and R. Carroll[2]; [1]Neurosurgery, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [2]Neurosurgical Oncology Laboratory, Neurosurgery, Brigham & Women's Hospital, Boston, Massachusetts, USA; [3]Department of Neurosurgery, University Schleswig-Holstein, Luebeck, Germany

Thromboxane synthase (TXSA), an enzyme of the arachidonic acid metabolism, is upregulated in human glial tumors and involved in the regulation of glioma cell invasion, which is one of the major limiting factors of current therapeutic strategies in this disease. Recent in vitro studies indicate that the inhibition of thromboxane synthase activity at non-cytotoxic concentrations blocks the invasive phenotype of glioma cells. This in turn increases a pro-apoptotic disposition and therefore the susceptibility to standard apoptosis-inducing chemotherapeutic compounds like BCNU, camptothecin, and etoposide. Here, we evaluated the therapeutic in vivo effects of furegrelate, a clinically tested TXSA inhibitor, in orthotopic U87 human glioblastoma xenografts by using local intracerebral microinfusions at 0.5 and 2 mg/kg/d. Local delivery of furegrelate by osmotic mini-pumps at 2 mg/kg/d for 21 days significantly inhibited the growth of well-established orthotopic gliomas in a nude mice model (76.12%, $P = 0.005$). Furegrelate and BCNU displayed strong synergistic effects on the in vitro induction of U87 glioma cell apoptosis as measured by an ELISA for DNA fragmentation. Therefore, we assessed the effects of furegrelate alone and in combination with BCNU on the survival of human glioma bearing nude mice. While local delivery of furegrelate alone at 2 mg/kg/d for 28 days prolonged the survival only marginally, the combination with systemically administered BCNU (15 mg/kg/d) enhanced the survival more than the single compounds alone. Our results indicate that targeting of the increased TXSA activity in human gliomas inhibits tumor growth in vivo by inducing pro-apoptotic, anti-proliferative, and anti-angiogenic effects. Local treatment with a TXSA inhibitor has the potential to enhance conventional chemotherapeutic schemes for malignant gliomas. Future studies need to evaluate modern modulators of TXSA activity and address their effects in relationship to other metabolites of the arachidonic acid pathway.

403. INHIBITION OF 90-KD HEAT SHOCK PROTEIN POTENTIATES THE CYTOTOXICITY OF DNA-ALKYLATING AGENTS IN HUMAN GLIOMA CELLS

S. Ohba, Y. Hirose, and T. Kawase; Neurosurgery, Keio University, Tokyo, Japan

Since gliomas are not curable surgically, it is necessary to develop an effective regime of adjuvant therapies, such as radio- or chemotherapy. Several ways to potentiate the cytotoxicity of antitumor agents have been reported. These include cell-cycle checkpoint abrogation, depression of anti-apoptotic protein expression, and depletion of DNA repair enzymes. Thus, many pathways can be targeted in an effort to sensitize tumor cells to chemotherapeutic agents. Previous studies revealed that a molecular chaperone 90-kD heat shock protein (Hsp90) is expressed at higher levels in human neoplastic tissues, including gliomas, than in normal tissues. Hsp90 participates in the stability and functions of its client proteins, which are involved in cell-cycle regulation (ex. Wee1, Plk1), cell survival (ex. Akt, survivin), and oncogenesis (ex. raf-1, src), and it is involved in a cytoprotective mechanism against cellular stresses, such as DNA damage. We hypothesized that Hsp90 inhibitors might act as antitumor agents against gliomas and potentiate the cytotoxicity of DNA-damaging agents. In the present study, we found that at a low concentration (3 nM) the Hsp90 inhibitor geldanamycin (GA), an ansamycin derivative, reduced the clonogenicity of U87MG human glioma cells in a p53-independent manner, and that GA potentiated the cytotoxicity of DNA-alkylating agents temozolomide and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on human glioma cells at a lower concentration (<1 nM). This GA-induced potentiation of DNA-alkylating agent-induced cytotoxicity was not a consequence of G_2 checkpoint abrogation or degradation of the anti-apoptosis proteins Akt or survivin, and exogeneous Akt overactivation did not overcome GA-induced sensitization of U87MG cells to DNA-alkylating agents. Experiments using another Hsp90 inhibitor, radicicol, a macrocyclic antibiotic, showed the results similar to those mentioned above. Although the mechanism of the GA-induced enhancement of the cytotoxicity of DNA-alkylating agents is still unclear, since two different types of Hsp90 inhibitors showed potentiation of the cytotoxicity of DNA-alkylating agents, and since this effect of Hsp90 inhibitors was clearly recognized with very low concentration of compounds that can be toxic to normal cells at much higher concentration, we conclude that Hsp90-targeted therapy may provide an effective strategy for the chemosensitization of human gliomas.

404. ADULT HUMAN MESENCHYMAL STEM CELLS: VEGF-DRIVEN INTERACTION WITH GLIOMA CELLS IN VITRO

C. Schichor,[1] N. Etminan,[1] S. Miebach,[1] S. Grau,[1] T. Birnbaum,[2] C. Padovan,[2] J. Tonn,[1] and R. Goldbrunner[1]; [1]Neurosurgery; [2]Neurology, Clinic Univers. LMU Munich Grosshadern, Munich, Germany

Much effort has been put into establishing human multipotent cells as carriers for malignant glioma therapy. The aim of our study were (1) to characterize factors that influence active movement of stem cells in the environment of a tumor infiltrated brain and (2) to test human adult mesenchymal stem cells (MSCs), which are easily available through bone marrow biopsy, for their migratory and invasive behavior and their interaction with human gliomas. Human MSC were isolated from bone marrow biopsies carried out for hematological indications. Migration of human adult MSC- and rodent embryonal NSC-spheroids (cell line C17.2) was studied on different matrices: laminin, tenascin, and plastic. Tumor-conditioned medium as well as VEGF were added in order to evaluate the role of glioma derived factors. To assess invasion, confrontational co-cultures of glioma (U373 GFP, C6 GFP, C6 VEGF sense, C6 VEGF antisense transfected) and stem cell spheroids (human MSC and rodent NSC, respectively) were investigated. Invasion

was visualized by light and confocal microscopy. Migration of both rodent embryonal NSC and human adult MSC was fastest on laminin, when compared to tenascin and plastic. VEGF as well as tumor-conditioned medium significantly increased NSC and MSC migration. Human MSCs showed an extensive invasion into glioma spheroids, even more than embryonal rodent NSCs. Invasion of NSC into VEGF sense C6 spheroids was much more rapid than invasion into VEGF antisense spheroids. Both NSC and MSC show intensive migratory behavior in the presence of glioma cells and glioma-conditioned medium. Obviously, VEGF is a crucial factor in enhancing stem cell motility.

405. IN VITRO SAFETY AND EFFICACY OF A NOVEL CB2-SELECTIVE CANNABINOID CHEMOTHERAPEUTIC AGENT, KM-233, FOR THE TREATMENT OF HIGH-GRADE GLIOMA

C. Duntsch,[1] M. Divi,[1] T. Jones,[1] G. Wood,[1] B. Moore,[1] and *A. Sills*[1]; [1]Neurosurgery and [2]Pharmaceutical Sciences, University of Tennessee, Memphis, Memphis, Tennessee, USA

Currently accepted therapeutic adjuvants to surgery for the treatment of high-grade gliomas such as radiotherapy and chemotherapy provide only a minor improvement in the disease course and life expectancy. Recently, it has been demonstrated that ligands of the CB1/CB2 receptors have varying degrees of cytoxicity against a variety of cancer cell lines. We have completed in vitro studies designed to test the efficacy and safety of a novel chemotherapeutic agent, KM-233, for the treatment of high-grade glioma. KM-233 is a classical cannabinoid with good blood-brain barrier penetration that possesses a 2.3- and 27-fold higher affinity for the CB-1 and CB-2 receptors, respectively, relative to THC. In vitro tissue culture cytotoxicity assays were used to measure the anti-tumor effects of KM-233 against human U87 glioma cells. KM-233 was found to have significant cytotoxic effects against U87 human glioma cells and reproducibly demonstrated a fifty-percent inhibitory concentration (IC50) of 1.42 mM. Similar assays were used to compare the cytotoxic efficacy of KM-233 to Δ^8-tetrahydrocannabinol and BCNU. In these studies, KM-233 was as efficacious in its cytotoxicity as Δ^8-tetrahydrocannabinol, and far superior to the commonly used anti-glioma chemotherapeutic agent BCNU. Kinetic studies of the onset of activity of KM-233 demonstrated that cytotoxic effects of KM-233 against human glioma cells in vitro occur as early as two hours after administration. Furthermore, we found that the dosing of KM-233 can be cycled on a daily basis without compromising its cytotoxic efficacy, thereby limiting potential toxicity from excessive exposure in vivo. To test the safety and toxicity of KM-233 against healthy adult brain tissue, an organotypic brain slice coculture model of cortex, striatum, and substantia nigra was used for dose-escalation studies. We found that, while there is some minimal toxicity associated with continuous administration of KM-233 in an organotypic brain slice culture model, cycling of KM-233 at doses that are exquisitely cytotoxic to glioma cells were well tolerated by healthy cultured brain tissue. These studies provide in vitro evidence that KM-233 shows promising efficacy against cultured glioma cell lines, shows minimal toxicity to healthy cultured brain tissue, and should be considered for preclinical development in animal models of glioma.

406. CONVECTION-ENHANCED DELIVERY OF LIPOSOMAL DOXORUBICIN ERADICATES U251MG INTRACRANIAL BRAIN TUMORS IN RATS

R. Saito,[1] M. Krauze,[2] C. Noble,[3] T. Kumabe,[1] T. Tominaga,[1] J. Park,[3] M. Berger,[2] and K. Bankiewicz[2]; [1]Neurosurgery, Tohoku University, Sendai, Japan; [2]Neurological Surgery and [3]Hematology-Oncology, University of California San Francisco, San Francisco, California, USA

Convection-enhanced delivery (CED) of liposomes into brain and brain tumor xenograft models resulted in robust tissue distribution and could be detected by MRI (Saito, Cancer Res., 2004). Image-guided CED of therapeutic liposomes for treatment of brain tumors is a lofty goal. The efficacy of well-characterized clinically available liposomal doxorubicin liposomal drug delivered by CED was evaluated in U251MG human glioblastoma intracranial xenografts. CED of liposomal doxorubicin at a dose safe to the normal brain (0.2 mg/ml doxorubicin) was significantly more effective than systemic administration at the maximum tolerable dose. When CED of liposomal doxorubicin was compared with free drug, liposomal drug demonstrated a higher therapeutic index, resulting from improvements in both efficacy and safety. When used at a nontoxic dose, liposomal doxorubicin demonstrated improved survival over free doxorubicin at the same dose. In addition, at a tenfold higher dose (2 mg/ml doxorubicin), liposomal doxorubicin was less toxic than free doxorubicin. A study of the tissue distribution following CED revealed liposomal doxorubicin distributed over larger regions in the brain parenchyma and resulted in longer tissue retention of the drug at the site of initial distribution. Free drug, when infused by CED, did not distribute as well as liposomal doxorubicin and induced early onset tissue damage, which led to increased tissue toxicity. CED of liposomal doxorubicin shows promise for treating brain tumors. The combination with imaging may provide an effective strategy for brain tumor therapy.

407. SYNERGISTIC INTERACTION BETWEEN 17-AAG AND PHOSPHATIDYLINOSITOL 3-KINASE INHIBITION IN HUMAN MALIGNANT GLIOMA CELLS

D. Premkumar, B. Arnold, A. Engram, and *I. Pollack*; Neurosurgery, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA

The PI3K/Akt pathway is often constitutively activated in malignant glioma cells, in many cases as a result of mutation of PTEN, an endogenous inhibitor of Akt, which renders tumor cells resistant to cytotoxic insults, including those related to anticancer drugs. Pharmacological inhibition of this pathway may potentially restore or augment the effectiveness of conventional chemotherapy or other signaling-targeted agents. Because the heat shock protein (HSP) is involved in the conformational maturation of a number of signaling proteins critical to the proliferation of malignant glioma cells, we hypothesized that the combination of the PI3K inhibitor LY294002 and the HSP90 inhibitor 17-AAG would promote glioma cytotoxicity by decreasing both the activation status and levels of Akt, as well as downregulating the levels of other relevant signaling effectors. We therefore examined the effects of the LY294002 and 17-AAG, alone and in combination, on signal transduction and apoptosis in a series of malignant glioma cell lines. Simultaneous exposure to these inhibitors significantly induced cell death and irreversibly inhibited proliferative activity and colony-forming ability of the glioma cell lines. Quantitative analysis revealed that enhancement by LY294002 of 17-AAG-induced cytotoxicity was synergistic, leading to a pronounced increase in active caspase-3 and PARP cleavage. No significant growth inhibition or caspase activation was seen in control cells. The enhanced cytotoxicity of this combination was associated with diminished Akt activation and a significant downregulation of epidermal growth factor receptor (EGFR), Raf-1, and mitogen-activated protein kinase (MAPK). Combination of 17-AAG and LY294002 did not modify phospho JNK/SPK, or p38 MAPK. Cells exposed to 17-AAG and LY294002 displayed a significant reduction in cell cycle regulatory proteins, such as pRb, CDK4, CDK6, and cyclin D1. Taken together, these findings suggest that the PI3K/Akt pathway plays a critical role in regulating the apoptotic response to 17-AAG and that targeting this pathway could provide a potent strategy to treat patients with malignant gliomas.

408. DOWNMODULATION OF E1A PROTEIN EXPRESSION AS A NOVEL STRATEGY TO DESIGN CANCER SELECTIVE ADENOVIRUSES

H. Jiang,[1] D. Medrano,[1] C. Gomez-Manzano,[1] M. LeMoine,[1] R. Alemany,[2] M. Olson,[1] M. Alonso,[1] C. Conrad,[1] W. Yung,[1] and J. Fueyo[1]; [1]Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA; [2]Institut Catala d'Oncologia, Barcelona, Spain

Oncolytic adenoviruses are being tested as potential therapies for human malignant tumors, including gliomas. Here we report for the first time that the deletion of a 48-60 aa region in the CR1 domain of E1A resulted in low levels of E1A protein, conditioning the replication of the mutant adenoviruses specifically to cancer cells. In this study, we compared the oncolytic potencies of three mutant adenoviruses encompassing deletions within the CR1 (Delta-39) or CR2 (Delta-24) regions or in both regions (Delta-24/39) of E1A protein. Analyses of cell viability showed a comparable cytopathic effect of Delta-24 and Delta-39, and viral replication studies revealed similar replication capability for both adenoviral constructs in glioma cells, although Delta-24/39 displayed more attenuated potency. Importantly, the activity of Delta-39 was significantly attenuated compared to Delta-24 in proliferating normal human astrocytes. Direct analyses of the activation of E2F-1 promoter demonstrated the inability of Delta-39 to induce S-phase-related transcriptional activity in normal cells. Interestingly, immunoblotting analysis showed that E1A protein levels in cells infected with Delta-39 were remarkably downmodulated. Further, protein stability studies through inhibiting protein synthesis with anisomycin revealed enhanced degradation of CR1-mutant E1A proteins, and block of the proteasome activity with lactacystin resulted in the striking rescue of the E1A levels. Collectively, our data showed that, compared to Delta-24, the deletion in Delta-39 resulted in suboptimal expression level of E1A protein, leading to significant attenuation of the virus in normal astrocytes but still maintaining efficient replication in glioma cells. We conclude that the level of E1A protein is a critical determinant of the selectivity of oncolytic adenoviruses, and we propose a completely novel strategy for the design and construction of conditionally replicative adenovirus.

409. ENHANCED CELLULAR RETENTION OF AN INTERNALIZING ANTI-EGFRVIII MONOCLONAL ANTIBODY RADIOIODINATED USING LYS5-[*I]IODOBENZOYL GLY1-MALEIMIDO GEEEK ([*I]IB-MAL-D-GEEEK), A PROSTHETIC GROUP CONTAINING NEGATIVELY CHARGED D-GLUTAMATES

G. Vaidyanathan, K.L. Alston, P.C. Welsh, and M.R. Zalutsky; Radiology, Duke University Medical Center, Durham, North Carolina, USA

Monoclonal antibodies (mAbs) reactive to EGFRvIII, a mutant form of the epidermal growth factor receptor expressed by gliomas but not by normal tissues, are rapidly internalized and degraded after binding to EGFRvIII-expressing cells. If mAbs are radioiodinated directly on the tyrosine residues, the radiolabeled catabolite iodo-tyrosine is rapidly washed out of the tumor. Therefore, if anti-EGFRvIII mAbs are to be useful in radioimmunotherapy, radiohalogenation methods with which enhanced tumor retention of radioactivity can be achieved are necessary. Earlier, we evaluated a radioiodinating agent [*I]D-KRYRR containing a tyrosine for labeling, 3 arginines for positive charge, and a lysine for mAb coupling via a maleimido bifunctional agent. In this study, we evaluated a novel agent with 3 negatively charged D-glutamates for lysosomal trapping, an iodobenzoyl moiety for minimizing dehalogenation, and a maleimido group for conjugation to mAb. Maleimidoglycine was prepared as a precursor for Gly^1-maleimido GEEEK preparation by solid-phase peptide synthesis. The lysine side chain of Gly^1-maleimido GEEEK was derivatized with 3-iodobenzoyl and 3-(tri-n-butylstannyl)benzoyl moieties by treatment with the respective *N*-succinimidyl benzoyl esters to obtain the iodo standard and tin precursor, respectively. Radioiodination of the tin derivative yielded [^{125}I]IB-Mal-D-GEEEK in 90.3 ± 3.9% radiochemical yields. This radioiodinated agent was conjugated to iminothiolane-treated L8A4, an anti-EGFRvIII mAb in 54.3 ± 17.7% conjugation yields. The protein-associated radioactivity (methanol precipitation) of the labeled mAb was 94.3 ± 5.8%, and the immunoreactive fraction was 82.3 ± 2.2% (Lindmo method). In vitro assays with the U87*f*EGFR glioma cell line indicated that internalized radioactivity for [^{125}I]IB-Mal-D-GEEEK-L8A4 conjugate increased from 14% at 1 h to 45% at 24 h. On the other hand, from a paired-label study, only 7% of the radioactivity from the directly radioiodinated L8A4 was internalized at 1 h, and the value steadily decreased, reaching 3% at 24 h. In comparison, internalized radioactivity for L8A4 radioiodinated with [*I]D-KRYRR in the same cell line decreased from about 40% at 2 h to about 20% at 24 h. These results suggest that [^{125}I]IB-Mal-D-GEEEK is a promising reagent for the radioiodination of internalizing mAbs. We are currently evaluating L8A4 radioiodinated using this new method in EGFRvIII-expressing tumor xenograft models.

410. *S*-FARNESYLTHIOSALICYLIC ACID TARGETS RAS SIGNALING IN A MOUSE MODEL OF GLIOMA

Y. Lyustikman,[1] Y. Kloog,[3] and E. Holland[1,2]; [1]Cancer Biology and Genetics, [2]Surgery (Neurosurgery), Memorial Sloan-Kettering Cancer Center, New York, New York, USA; [3]Neurochemistry, Tel Aviv University, Tel Aviv, Israel

The Ras signal transduction cascade is commonly activated in high-grade gliomas. Evidence suggests that farnesylation is critical for recruitment of Ras to the membrane and its subsequent activation. However, treatment with several farnesyltransferase inhibitors (FTIs) has failed to achieve therapeutic benefit. This failure may result from resistance by evolving alternative prenylation mechanisms. By contrast, *S*-farnesylthiosalicylic acid (FTS) is a competitive inhibitor of Ras that likely acts by displacing Ras from the plasma membrane and that does not require inhibition of farnesylation for its effect. Therefore, it may be more effective than FTI therapy. We explored the mechanism of action of FTS in vitro and the therapeutic efficacy of FTS on a mouse model of glioma in vivo. We transformed neural progenitor cells with activated forms of Kras, Akt, Kras + Akt, or PDGF-B by somatic gene transfer. Transformed progenitors were exposed to increasing doses of FTS and analyzed for changes in morphology, induction of apoptotic cell death by flow cytometry, and signaling effects by Western blotting. Synergistic effects with other drugs were addressed by combined treatments with a MEK inhibitor, an mTOR inhibitor, or an Akt inhibitor. Subcellular localization of Kras was determined by immunofluorescence microscopy. Finally, we assessed the potential therapeutic effects of FTS in vivo in a Kras-driven glioma model. FTS induced rapid onset of apoptosis in a dose-dependent and cell line–dependent manner. Specifically, at low concentrations of the drug, only the Ras transformed cells underwent apoptosis, whereas at high doses FTS exhibited a generalized toxicity toward all cell lines. This suggests specificity for FTS activity against Ras at low concentrations. Addition of constitutively active Akt protected against FTS-induced apoptosis at lower doses, and combined treatment with an Akt inhibitor restored sensitivity. The rescue provided by Akt was mTOR independent because addition of an mTOR inhibitor had no effect. Experiments are underway to examine the effects of FTS in vivo. FTS induces apoptosis in cells that are reliant on Ras signaling to maintain their transformed characteristics in a dose-dependent manner. Activation of Akt provides a rescuing effect that is abolished by treatment with an Akt inhibitor. These results suggest that FTS may potentially be a novel therapeutic agent for treatment of tumors with increased Ras signaling. As activation of Akt is also common in high-grade gliomas, combined therapy with FTS and an Akt inhibitor may be clinically useful and more effective than with either drug alone.

411. SYNERGISTIC AUGMENTATION OF VINCRISTINE-INDUCED CYTOTOXICITY BY PHOSPHATIDYLINOSITOL 3-KINASE INHIBITOR IN HUMAN MALIGNANT GLIOMA CELLS: EVIDENCE FOR THE INVOLVEMENT OF P38 AND ERK SIGNALING PATHWAY

I. Pollack, D. Premkumar, J. Mathas, and B. Arnold; Neurosurgery, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA

Microtubule-interfering agents (MIAs), such as vincristine, are widely used for the treatment of cancer, and are included in many treatment regimens for childhood brain tumors. The anticancer properties of MIAs have been attributed in part to interference with microtubule assembly, impairment of mitosis, and cytoskeletal changes, with additional effects on mitogen-activated protein kinase signaling and caspase activation. Because malignant gliomas commonly have dysregulation of PI3K/Akt signaling, which can promote cell survival and potentially limit the activity of such agents, we questioned whether PI3K inhibition with LY294002 could potentiate the efficacy of vincristine in a panel of glioma cell lines versus normal astrocytes. We therefore examined the effects of the LY294002 and vincristine, alone and in combination, on cell survival, signal transduction, and apoptosis in a series of malignant glioma cell lines versus normal astrocytes. Simultaneous exposure to these inhibitors significantly induced cell death and inhibited proliferation and clonogenicity of the glioma cell lines. Quantitative analysis revealed that enhancement by LY294002 of vincristine-induced cytotoxicity was synergistic, leading to pronounced caspase activation at concentrations that had no significant effects on control cells. The enhanced cytotoxicity of this combination was associated with significant activation of p38 MAPK signaling and induction of G2/M blockade on cell cycle analysis. Pretreatment with either SB203580 or z-VAD.fmk, selective inhibitors of p38 MAPK and caspase signaling, respectively, abrogated the apoptotic response to the combination of LY294002 and vincristine. Taken together, these findings demonstrate that PI3K/Akt inhibition can potentiate the effects of vincristine and that the combination of molecularly targeted therapies and conventional agents could provide a potent strategy to treat patients with malignant gliomas.

412. COMBINATION OF AN ANGIOGENESIS INHIBITOR WITH RADIOTHERAPY FAILED TO SHOW SYNERGISM IN AN ORTHOTOPIC MURINE GBM MODEL

J. Verhoeff,[1] L. Stalpers,[2] J. Haveman,[2] D. Troost,[3] M. Ramkema,[3] D. Bosch,[1] and W. van Furth[1]; [1]Neurosurgery, [2]Radiation Therapy, and [3]Neuropathology, Academic Medical Centre, Amsterdam, The Netherlands

Clinical effects of irradiation in GBM patients are likely the result of a direct anti-neoplastic effect, as well as radiation-induced damage to the tumor vasculature. We hypothesized that additional damage to the tumor vessels, by combining radiotherapy with an angiogenesis-inhibitor, will enhance radiotherapy effectiveness. An orthotopic murine GBM (U251-NG2) model was used, which we modified to allow focal brain irradiation. The antibody against murine VEGF-R2 (DC101) was given in a dose of 40 mg/kg every 3 days, starting on the same day as the irradiation (2 mCi iodine seeds). Treatment started 7 days after stereotactic injection of the cells in the right frontal lobe. Both treatment modalities were controlled with sham treatment, resulting in 4 groups. Mice were sacrificed when losing >20% weight, showing neurological signs, or after 13 weeks after cell inoculation. Tumor take in 96 athymic nude mice was 90%. Control mice, getting only sham treatments, had a mean survival of 5.9 weeks. Focal brain irradiation improved mouse survival statistical significantly, with a median survival of 9.9 weeks. DC101 did not affect the median survival (5.1 weeks), although histological analysis did show a changed vascular pattern in these tumors. By combining DC101 with focal brain irradiation the favorable effects of irradiation on survival were lost, with a median survival of 5.9 weeks. In our orthotopic murine GBM model we could not find a cooperative effect of DC101 with radiotherapy. In fact, a contrary effect was shown, with a survival similar to control groups. Histology showed altered tumor vasculature in the mice that received DC101, but no explanation for the worsened outcome of the combination therapy. We suggest that receptor

blockage may have resulted in increased local VEGF concentrations and physiological effects through the VEGF-R1. New experiments will be conducted to explore the result of combining radiation therapy with a therapy that directly binds to the VEGF molecule.

413. EFFICACY AND SAFETY OF A REPLICATION-RESTRICTED VSV FOR THE TREATMENT OF GLIOBLASTOMA USING ORGANOTYPIC BRAIN SLICE AND RODENT MODELS OF INTRACRANIAL GLIOMA

A. Sills,[1] C. Duntsch,[1] H. Jayakar,[4] Q. Zhou,[1] Z. Xiang,[1] L. Pfeffer,[2] J. Robertson,[1] and M. Whitt[3]; [1]Neurosurgery, [2]Pathology and Laboratory Medicine, and [3]Molecular Sciences, University of Tennessee at Memphis, Memphis, Tennessee; [4]GTX, Inc., Memphis, Tennessee; USA

Vesicular stomatitis virus (VSV) is an enveloped negative strand RNA virus being evaluated for use in treatment a variety of tumors. A sensitive organotypic brain tissue slice-glioma coculture system was used to evaluate use of recombinant, wild-type VSV (wt-VSV) in the treatment of glioma. Previous work has shown that even when replication of wt-VSV was blocked by pretreatment of the slice culture with interferon-b, the integrity of neuronal tissues were significantly damaged following exposure to wt-VSV. This neurotoxicity would pose a major limitation for clinical use of wt-VSV. In this report, we describe the use of a recombinant, replication-restricted second-generation VSV, GTx-v401, as a potential candidate for glioma therapy. In contrast to the results observed with wt-VSV, we found that GTx-v401 exhibited minimal cytotoxicity in the organotypic slice culture while displaying high levels of oncolytic activity toward glioma cells growing within the slice culture. We also observed no virus-induced loss of neuronal integrity as measured by MAP-2 staining and no change in the electrophysiological properties of the slice culture. We further developed these studies with in vivo experiments designed to compare and contrast the safety and efficacy of replication competent recombinant wt-VSV and replication restricted GTx-v401 in reducing tumor volume in a rat model of glioma. In these studies, we found that the use of wt-VSV to treat intracranial glioma resulted in an overwhelming encephalopathy and caused unacceptable morbidity and mortality. In sharp contrast to these results, administration of GS-VSV directly to the intracranial tumor bed was well tolerated and effective at reducing the tumor load. In the highest plaque-forming unit dose of GS-VSV tested (10^9 pfu, 5 logs higher than the lowest dose of wt-VSV tested), there was little morbidity and no mortality associated with administration to the tumor site. A 71% reduction of tumor surface area was noted in the GS-VSV treated group versus controls. We also found no significant differences between treated and control animals with respect to weight loss, neurological changes, or neurohistopathological changes at all doses tested. GS-VSV appears to be safe and effective when used in vivo to treat intracranial glioma and warrants further development as an adjuvant therapy.

414. COMINATION OF IMATINIB MESYLATE (STI-571, GLEEVEC) AND TEMOZOLOMIDE (TEMODAR) DISPLAYS INCREASED ACTIVITY AGAINST GLIOMA XENOGRAFTS

J. Rich,[1] S. Keir,[2] S. Sathornsumetee,[2] D. Reardon,[2] D. Bigner,[3] and H. Friedman[2]; Departments of [1]Medicine, [2]Surgery, and [3]Pathology, Duke University Medical Center, Durham, North Carolina, USA

Temozolomide is an orally available methylator now widely used in glioma therapy. Although temozolomide has activity against malignant gliomas, development of resistance is common. We have sought to increase the activity of temozolomide in these cancers. Imatinib mesylate is a novel low-molecular-weight ATP-mimetic inhibitor of several tyrosine kinases, including platelet-derived growth factor receptors (PDGFRs). Although imatinib mesylate has been relatively inactive in monotherapy trials against gliomas, imatinib mesylate may offer a benefit in increasing the efficacy of temozolomide through increased tumor accumulation by decreasing interstitial fluid pressure and blocking retrograde blood-brain barrier transporters as well as disrupting tumor survival mechanisms. The combination of imatinib mesylate (200 mg/kg × 5 days starting two days before temozolomide dosing) and temozolomide (single dose at 0.1 LD_{10}) was well tolerated by athymic mice without significant weight loss. Athymic nude mice bearing established subcutaneous human glioma xenografts displayed additive benefit in time to reach 5× original tumor volume (24.8 days temozolomide alone, 2.6 imatinib alone, 26.3 with the combination). The impact of the combination was significantly greater on the survival of mice bearing intracranial xenografts (control 22 days, imatinib mesylate 25 ($P > 0.1$), temozolomide 49 ($P < 0.001$), combination 60 ($P < 0.001$ vs. control, $P < 0.018$ vs. temozolomide alone). Additionally, only mice treated with the combination became long-term survivors (3/10). Current studies are confirming PDGFR inhibition and exploring mechanism of the additional benefit. These results suggest that gliomas may exhibit greater sensitivity to temozolomide when combined with imatinib mesylate. The combination of imatinib mesylate and temozolomide offers combinatorial benefit and is now in development as a clinical trial. This work was supported by the Pediatric Brain Tumor Foundation of the United States, Accelerate Brain Cancer Cure, Southeastern Brain Tumor Foundation, and NIH grant NS047409 (J.N.R.). J.N.R. is a Damon Runyon-Lilly Clinical Investigator and a Sidney Kimmel Cancer Foundation Scholar.

415. IMATINIB MESYLATE (STI571) INHIBITION OF MALIGNANT GLIOMA CELLS IS INCREASED BY MODULATION OF THE MAPK PATHWAY

H. Ren,[1] C. Walker,[3] L. Pazmany,[4] S. Pelech,[5] and *N. Rainov*[1,6]; [1]Department of Neurological Sciences, University of Liverpool, Liverpool, UK; Department of Immunology, Harbin Medical University, Harbin, PR, China; [3]JK Douglas Laboratories, Clatterbridge Cancer Research Trust, Wirral, UK; [4]Academic Rheumatology Unit, University of Liverpool, Liverpool, UK; [5]Kinexus Bioinformatics Corporation, Vancouver, Canada; [6]Neurosurgery, The Walton Centre for Neurology and Neurosurgery NHS Trust, Liverpool, UK

This study investigated mechanisms of action of imatinib mesylate (STI571, Gleevec), a novel receptor tyrosine kinase signal transduction inhibitor, in human malignant glioma cells. Real-time PCR (RT-PCR) was carried out to quantitate the expression of PDGFa and PDGFβ receptors (PDGFRa and PDGFRβ) and the stem cell factor receptor c-Kit, putative targets of the drug, during imatinib treatment. Multi-immunoblot (Kinetworks) differential protein kinase and phosphorylation-specific profiling was performed on imatinib-treated and control glioma cells. The malignant human glioma cell lines U87MG and LNZ308 expressed PDGFa and PDGFβ receptors, but not c-Kit. T98G human malignant glioma cells expressed neither PDGF receptors nor c-Kit. RG human malignant glioma cells expressed both PDGF receptors and c-Kit. Treatment with imatinib caused dose-dependent downregulation of PDGFR, in all expressing cell lines, while PDGFRa expression was less affected. Expression of c-Kit in RG cells was also downregulated by imatinib treatment in a dose-dependent manner. Phosphorylation-specific multi-immunoblot (Kinetworks KPSS) in U87MG glioma cells treated with imatinib showed significant functional activation (up to 325% of control) of MAP kinases ERK1 and ERK2, compared with untreated control cells. Phosphorylation activity of other kinases in the MAPK signaling pathway, such as MEK1 and MEK2, and of kinases outside this pathway, was downregulated by imatinib. Quantitative RT-PCR in U87MG cells showed upregulation of the ERK phosphatase MKP-1 and downregulation of the ERK phosphatase MKP-3 after treatment with imatinib. Finally, simultaneous specific inhibition of ERK kinases during imatinib treatment using the MAPK inhibitor PD098059 significantly increased the toxicity of imatinib in U87MG glioma cells. In conclusion, our data show that inhibitory effects of imatinib on malignant glioma cells are mediated at least in part by the MAPK signaling pathway. Pharmacological inhibition of components of the MAPK signaling pathway, as suggested by data from a phosphorylation-specific assay, can result in increased toxicity of imatinib and in improved killing of glioma cells.

416. NOVEL POLYMERIC MICELLE DRUG CARRIER SYSTEMS FOR BRAIN TUMOR THERAPY

Y. Ino,[1] Y. Guan,[1] N. Nishiyama,[2] H. Cabral,[3] K. Kataoka,[3] and T. Todo[1]; [1]Department of Neurosurgery, Hospital; [2]Center for Disease Biology and Investigative Medicine, Graduate School of Medicine, and [3]Department of Material Science and Engineering, Graduate School of Engineering, The University of Tokyo, Tokyo, Japan

The use of polymeric micelles is one of the promising modalities of macromolecular carrier systems. The particle size of polymeric micelles (~50 nm) is smaller than that of other macromolecular carriers, and they are therefore not easily trapped by the reticular systems during circulation. The long circulation time will allow them to accumulate effectively in the solid tumor through the enhanced permeability and retention (EPR) effect. Doxorubicin or cisplatin carried by polymeric micelles has shown higher antitumor activities than the drugs alone. Diaminocyclohexane platinum (Dach-platin) is a second-generation platinum based anticancer drug which is highly hydrophobic and is toxic when administered systemically. We have developed a new polymeric micelle incorporating Dach-platin (Dach-Pt/m) via the polymer-metal complex formation between Dach-platin and poly(ethylene glycol)-poly(aspartic acid) block copolymers (PEG-P[Asp]). The Dach-Pt/m was designed so that it would accumulate in the tumor and be released only after reaching the tumor. The efficacy of Dach-Pt/m was tested in Neuro2a (murine neuroblastoma) subcutaneous and intracerebral tumor models. Oxaliplatin, a less toxic derivative of Dach-platin, was also used as a control. All animal experiments have been approved by the review committee of the Tokyo University. The maximum tolerated

dose (MTD) of Dach-Pt/m in A/J mice was 1.75mg/kg and similar to that of oxaliplatin when administered from the tail vein three times every other day. The toxicity was presumably due to a spontaneous decay of the micelle causing accumulation of Dach-Pt in the liver followed by liver dysfunction. Nonetheless, Dach-Pt/m was more effective in inhibiting the Neuro2a subcutaneous tumor growth than oxaliplatin at the MTD level. Dach-Pt/m was also effective in the Neuro2a intracerebral tumor model and prolonged the survival of tumor-bearing mice compared with oxaliplatin. These results suggest that the polymeric micelle macromolecular carrier system may be useful for the treatment of brain tumors. Optimization of dosing schedules may further enhance the efficacy of Dach-Pt/m.

417. SPECIFIC TRANSLOCATIONS OF CHROMOSOMES 11 AND 22 IN RECURRENT MALIGNANT GLIOMAS

A.C. Scheck,[1] L. Panyon,[1] N.C. Hank,[1] and G. Martinez[2]; [1]Neuro-Oncology Research, Barrow Neurological Institute; [2]Biological Sciences, Paradise Valley Community College, Arizona State University West, Phoenix, USA

Malignant gliomas are typically treated with surgery, radiation, and chemotherapy. Despite this, these tumors recur and are resistant to additional treatment. We have previously demonstrated that cells selected for resistance to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in vitro or in vivo (recurrent tumor from patients treated with BCNU) are near-diploid with over-representation of chromosome 7 and regions of chromosome 22. To identify the over-represented regions of chromosome 22, we analyzed cells from several primary/recurrent tumor pairs prior to, and following selection for resistance to 10 μg/ml of BCNU. Fluorescent in situ hybridization (FISH) using bacterial artificial chromosome (BAC) probes allowed us to map specific chromosomal aberrations in these cells. FISH analyses allowed us to map the over-represented region to 22q12.3-13.31. In addition, we have identified 3 specific translocations in cells from recurrent tumor involving chromosomes 22 and 11. We mapped the chromosome 11 breakpoints to within 1.5 Mbp and the chromosome 22 breakpoints to less than 82 kbp. The first translocation occurs between the telomeric side of 22q12.3 and centromeric edge of 11q23.1. The second translocation involves sequences on the 22q12.1/22q12.2 and 11q23.1/11q23.2 borders. The third translocation occurs between the telomeric sides of 22q11.1 and 11q23.3. Additional work has shown that these translocations are frequently reciprocal and found in addition to normal copies of the chromosomes. We have also found them in paraffin-embedded tissue from recurrent tumor but not in tissue from the same patient's primary tumor. Further, in vitro selection for cells resistant to BCNU also selects for cells with these translocations; however, in vitro treatment of cells from primary tumor cannot induce these translocations. Preliminary work suggests that radiation treatment may be a causative event in the formation of these translocations. This work suggests that one or more of these translocations provides the cell with a selective advantage that contributes to therapy resistance or to the growth of therapy resistant cells.

418. ENCOURAGING RESULTS FOR A NOVEL CHEMOTHERAPEUTIC REGIMEN IN NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

E. Arenson, J. Bank, M. Pierick, C. Greenwald, T. Fullagar, and J. McVicker; Colorado Neurological Institute and Swedish Medical Center, Englewood, Colorado, USA

Substantial benefit from chemotherapy of cancer requires effective combinations; nevertheless, monotherapy with temozolomide (TMZ) has emerged as the "standard" treatment of glioblastoma multiforme (GBM). We report results of treatment of GBM with the novel combination of BCNU, irinotecan (CPT11) and TMZ (BITE). Four patients were excluded from this treatment because of expected survival <3 months, absence of caregiver, or 24-h care requirement. Thirty-seven patients with newly diagnosed GBM were treated between August 1999 and October 2002. Mean age was 53.4 years. Thirteen patients had bilateral and/or multifocal disease. Nine had gross total resection, 27 subtotal resection, and 1 biopsy, as determined by early post-operative contrast MRI. Treatment consisted of three courses of CPT11 (400 mg/m^2 × 1) and TMZ (200 mg/m^2 × 5) given every 21 days during standard radiotherapy (RT) (phase I) to which BCNU (40 mg/m^2 × 3) was added after RT for up to 6 monthly courses as tolerated (phase 2). Three patients did not complete phase 1 because of patient choice, neurological decline, or death from intratumoral hemorrhage unrelated to treatment. Two additional patients did not proceed to phase 2 because of poor performance status. Thirty-two patients received 115 courses of BITE (mean 3.4 courses) and had episodes of grade 3/4 toxicities as follows: GI 12%, neutropenia 42%, and thrombocytopenia 11%. There were 2 deaths during phase 2 unrelated to disease progression: one disseminated CMV and one bacterial pneumonia. One patient survived an atypical mycobacterial pneumonitis, and one survived an episode of BCNU pneumonitis. With minimum follow-up of 30 months, mean survival is 19 months. Overall survival is 59% 1 year, 30% 2 years, and 19% 3 year3. Relapse-free survival is 46% 1 year, 22% 2 years, and 11% 3 years. Six patients are alive (16%), five without evidence of disease and one with stable disease from 37 to 48 months post-diagnosis. For patients with initial gross total resection (24%), relapse-free survival was 89% at 1 year, 89% 2 years, and 56% 3 years. We conclude that (1) BITE is toxic, but effective, (2) polychemotherapy should not be abandoned in GBM, (3) BITE deserves further study with efforts to reduce toxicity, and (4) patients with gross total resection may survive long-term with aggressive post-surgical treatment.

419. SALVAGE CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE FOR RECURRENT TEMOZOLOMIDE-REFRACTORY ANAPLASTIC ASTROCYTOMA

M. Chamberlain, D. Tsao-Wei, and S. Groshen; Preventive Medicine, University of Southern California, Los Angeles, California, USA

We conducted a prospective phase 2 study of cyclophosphamide (CYC) in adult patients with recurrent temozolomide-refractory anaplastic astrocytoma (AA) with a primary objective of evaluating 6-month progression-free survival (PFS). Forty patients (28 men; 12 women) ages 26 to 57 years (median 43), with recurrent AA were treated. All patients had previously been treated with surgery and involved-field radiotherapy. Additionally, all patients were treated adjuvantly with temozolomide (TMZ) chemotherapy. All patients were treated at first recurrence with CYC administered intravenously on 2 consecutive days (750 mg/m^2/day) every 4 weeks (operationally defined as a single cycle). Neurological and neuroradiographic evaluation were performed every 8 weeks. All patients could be evaluated. A total of 215 cycles of CYC (median 2 cycles; range 2–12) was administered. CYC-related toxicity included alopecia (all patients, 100%), anemia (5, 12.5%), thrombocytopenia (6, 15%), and neutropenia (8, 20%). Four (10%) patients required transfusion. Nine patients (22.5%; 95% CI, 11%–39%) demonstrated a neuroradiographic partial response, 16 patients (40.0%; 95% CI, 25%–57%) demonstrated stable disease and 15 patients (37.5%; 95% CI, 23%–54%) had progressive disease following two cycles of CYC. Time to tumor progression ranged from 2 to 19 months (median, 4 months; 95% CI, 2–6 months). Survival ranged from 2 to 26 months (median, 8 months; 95% CI, 6–10) months). Six-month and 12-month PFS was 30% and 8%, respectively. CYC demonstrated modest efficacy with acceptable toxicity in this cohort of adult patients with recurrent anaplastic astrocytoma, all of whom had failed prior TMZ chemotherapy.

420. HIGH-DOSE BCNU WITH AUTOLOGOUS BLOOD STEM CELL RESCUE: DEFINITIVE RESULTS OF A PHASE 3 MULTICENTRIC STUDY IN SUPRATENTORIAL COMPLETELY RESECTED GLIOBLASTOMA PATIENTS TREATED WITH POST-OPERATIVE RADIOTHERAPY

M. Ben Hassel,[1] C. Linassier,[2] D. Figarella-Branger,[3] J.O. Bay,[4] H. Bourgeois,[5] T. Lesimple,[1] J.P. Cottier,[6] L. Taillandier,[8] R. Delepine,[2] and D. Frappaz[7]; [1]Centre Eugène Marquis, Rennes; [2]CHRU Bretonneau, Tours; [3]CHU La Timone, Marseille; [4]Centre Jean Perrin, Clermont-Ferrand; [5]CHU La Mileterie, Poitiers; [6]Centre Alexis Vautrin, Nancy; [7]Groupe Hospitalier Pitié-Salpêtrière, Paris; [8]Centre Léon Bérard, Lyon; Neuro-Oncology Group of the National French Federation of Compréhensive Cancer Centers, FNCLCC ; France

A previously published phase 2 study with high-dose BCNU and autologous bone marrow graft added to post-operative radiotherapy suggested a favourable trend in extensively resected adult glioblastoma patients. In the present study patients were randomized after extensive surgery between two groups: arm A, BCNU at 800 mg/m^2 followed by stem cell rescue on day 3 and radiotherapy 4 weeks later (60 Gy and classical fractionation), and arm B, radiotherapy (60 Gy, classical fractionation) followed by 80 mg/m^2 BCNU at 6-week intervals during one year. Criteria of inclusion were as follows: age 16–65, supratentorial tumor site, glioblastoma histology, gross total tumor resection, OMS performance status (PS) = 0-2, adequate liver, kidney, and lung functions, no pregnancy, no other malignant tumor, and written consent. Sixty-nine patients only (median age = 50.8 years) instead of the 140 expected were included in the study between September 1997 and November 2001: 36 in arm A and 33 in arm B. Both post-operative imaging and histology were subjected to review. Follow-up lasted up to a data analysis made in December 2004 (3 years after the last inclusion). Toxicity was recorded according to WHO classification. The study was closed because of a prohibitive toxicity death rate above 10%: 1 case of septic shock and 3 cases of severe lung fibrosis were recorded in arm A. One reversible grade 4 liver toxicity was recorded in arm A. Definitive results will be presented in terms of early and late toxicity, time to progression,

and survival. We conclude that favorable prognosis factors like age, extensive resection, and PS, have a stronger influence than a high-dose BCNU chemotherapy regimen.

421. CARBON ION RADIOTHERAPY FOR MALIGNANT GLIOMAS

J. Mizoe,[1] H. Tsujii,[1] A. Hasegawa,[1] R. Takagi,[1] T. Yanagi,[1] and K. Takakura[2]; [1]National Institute of Radiological Sciences, Research Center for Charged Particle Therapy, Chiba; [2]Neurosurgery, Tokyo Women's Medical University, Tokyo; Japan

We conducted a study to investigate the effects of combined X-ray, ACNU, and carbon ion radiotherapy for malignant gliomas. Between October 1994 and February 2002, 48 patients with histologically confirmed malignant gliomas (16 anaplastic astrocytoma and 32 glioblastoma multiforme) were enrolled into the phase 1/2 clinical study of combined X-ray, ACNU, and carbon ion radiotherapy. Their age range was from 18 to 78, and the mean age was 53 years. By gender, they comprised 29 males and 19 females. The loci included 22 frontal lobe, 10 temporal lobe, and so on. Twenty-seven patients underwent partial resection, 8 subtotal resection, and 8 macroscopic total resections prior to radiotherapy. Treatment involved the application of 50 Gy/25 fractions/5 weeks of X-ray, followed by carbon ion radiotherapy at 8 fractions/2 weeks. ACNU of 100 mg/m^2 were administrated concurrently in the first and fourth or fifth weeks of X-ray therapy. Carbon ion dose was escalated from 16.8 to 24.8 GyE on 10% incremental steps after confirmation of the safety of each dose given previously. There were 9 cases with grade 2 acute reaction in the skin but there was no grade 2 or higher reaction in the brain. The late reactions included 2 cases of grade 2 brain morbidity (RTOG/EORTC) and 3 cases of grade 2 brain reaction (LENT/SOMA, MRI) out of 48 cases. There were no grade 3 or higher-grade reactions until the date of analysis. Median survival time (MST) of AA was 35 months and that of GBM 17 months. In the AA patients, MST was 20 months for the low-dose group (16.8 ~ 20.0 GyE, 6 patients) and 40 months for the high-dose group (22.4 ~ 24.8 GyE, 10 patients) ($P = 0.0382$). MST of GBM patients was 7 months for the low-dose group (16.8 GyE, 7 patients), 19 months for the middle dose group (18.4 ~ 22.4 GyE, 23 patients), and 24 months for the high-dose group (24.8 GyE, 5 patients) ($P = 0.031$). In recursive partitioning analysis (RPA), two years overall survival rate of class I (5 patients) was 80%, class II (5 pts) 60%, class III (12 pts) 58%, class IV (14 pts) 21%, class V (6 pts) 50%, and class VI(6 pts) 33%. Results of combined therapy of X-ray, ACNU, and carbon ion radiotherapy showed the potential efficacy of carbon ion radiotherapy for malignant gliomas in terms of the improved survival rate in those patients who received higher carbon doses. Based on these results, a new protocol for malignant gliomas using carbon ion radiotherapy alone was initiated.

422. EFFICACY OF RADIATION THERAPY ON SEIZURES IN LOW-GRADE ASTROCYTOMAS

R. Soffietti, M. Borgognone, A. Ducati, P. Gaviani, E. Laguzzi, M. Nobile, U. Ricardi, and R. Rudà; Neuroscience, University of Torino, Torino, Italy

The role of radiation therapy in low-grade astrocytomas is still controversial, and seizure control represents the most relevant clinical problem. There are some reports in the literature suggesting that radiation therapy might be effective in reducing seizure frequency in patients with inoperable low-grade gliomas. The objective of this study was to analyze, in a retrospective series of patients with supratentorial histologically verified grade II astrocytomas and persisting seizures despite conventional AEDs, the response of epilepsy to conventional radiotherapy. The study population (1985–2001) included 25 patients, ages 20 to 54 years (median 35), 16 men and 9 women. Previous surgery consisted of a biopsy in 9 patients, a partial/subtotal resection in 13 patients, and a total resection in 3 patients. The seizure frequency varied from >1 per day to >1 per month, and the type was as follows: partial simple in 9, partial complex in 9, and generalized in 7. Twenty-two tumors were non-enhancing on CT or MRI, whereas 3 only were enhancing. Patients received external radiotherapy either adjuvantly or at tumor progression in conventional fractionation, with total doses ranging from 40 to 60 Gy (median 54). Overall, 19 patients (76%) had a significant reduction (>50% decrease) in seizure frequency, with 2 patients seizure free, whereas 5 patients (20%) had no significant change, and 1 (4%) had an increase in seizures. Basing on Macdonald's criteria of response, we observed 6 PR (24%), 17 SD (68%), and 2 PD (8%). When correlating the clinical with the radiological response, among patients who had a significant reduction of seizure frequency (19), 4 (21%) had a PR, 13 (68%), an SD, and 2 (11%) a PD, whereas among patients who had no significant change of seizure frequency (5), 2 (40%) had a PR, and 3 (60%) a SD. In conclusion, this study confirms in a larger series of patients that conventional radiotherapy is able to significantly reduce the seizure frequency in a high proportion of patients with grade II astrocytomas. Moreover, a correlation does not seem to exist between the seizure reduction and tumor response on CT or MRI.

423. OBSERVATIONS ON THE RELATIONSHIP BETWEEN RADIOTHERAPY AND SURVIVAL IN PATIENTS WITH LOW-GRADE GLIOMAS FROM THE SURVEILLANCE, EPIDEMIOLOGY, AND END RESULTS (SEER) PROGRAM DATA

B. Lally,[1] J. Baehring,[2] D. Zelterman,[3] and *J. Knisely*[1]; [1]Therapeutic Radiology; [2]Neurosurgery & Neurology; [3]School of Public Health, Yale University School of Medicine, New Haven, Connecticut, USA

Despite trials providing class I data, radiotherapy's use in the management of low-grade gliomas (LGG) remains controversial. The utilization of radiotherapy and its relationship to survival was examined in the Surveillance, Epidemiology, and End Results (SEER) program data. SEER is a national cancer surveillance program that covers ~26% of the U.S. population. SEER database patients with supratentorial, pathologically confirmed LGG diagnosed between 1983 and 2001 who were 21 to 60 years of age were evaluated. The relationship between early postoperative radiotherapy (yes or no) and various other prognostic variables was evaluated. These included age (≤40 vs. >40), extent of surgery (biopsy vs. subtotal vs. total resection vs. surgery, NOS), and histology (oligodendroglioma, oligoastrocytoma, and astrocytoma—which included fibrillary, protoplasmic astrocytomas and astrocytoma, NOS). A total of 4210 patients were in the final pool for analysis. SAS version 8.1 (SAS Institute, Cary, N.C.) was used for all analyses. The ~^{2} test was used to test for interaction between radiotherapy and other variables. Kaplan-Meier methods were used to estimate crude survival from time of diagnosis until death from any cause. Multivariable Cox proportional hazards models were used to calculate adjusted hazard ratios (HR) to assess the importance of radiotherapy as an independent predictor of survival. The HR for age >40 was 2.124; radiotherapy was more frequently used in patients >40 ($P < 0.0001$). The HR for oligodendroglioma was 0.658 ($P < 0.0001$), and the HR for astrocytoma was 1.613 relative to oligoastrocytoma; radiotherapy was more frequently used for astrocytomas ($P < 0.0001$). Survivals were increased for complete and subtotal resections compared to biopsy and surgery, NOS. Radiotherapy's use was associated with an HR of 1.509; it was more commonly used with less complete surgical resection. Median survivals for astrocytoma, oligoastrocytoma, and oligodendroglioma histologies with or without the use of postoperative radiotherapy were (in years) 3.2 versus 7.5; 6.4 versus not yet reached; and 10.0 versus 14.6, respectively. It is difficult to discern any benefit from early radiotherapy as a result of this retrospective analysis.

424. ENHANCEMENT OF RADIOSENSITIVITY OF HUMAN MEDULLOBLASTOMA CELLS BY HISTONE DEACETYLASE INHIBITOR VALPROIC ACID IS ASSOCIATED WITH THE ACTIVATION OF P21 AND DOWN-REGULATION OF C-MYC AND SURVIVIN GENES

X. Li,[1] Q. Shu,[1] A. Munshi,[2] Y. Xia,[3] E. Chang,[1,2] S. Woo,[2] and C. Lau[1]; [1]Pediatrics and Texas Children's Cancer Center, Baylor College of Medicine, Houston, Texas, USA; [2]Experimental Radiation Oncology and Division of Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA; [3]Cancer Center, Radiation Oncology, Sun Yat-sen University, Guangzhou, China

Histone deacetylase (HDAC) inhibitors are emerging therapeutic agents capable of disrupting critical cellular processes in cancer cells. One particular novel HDAC inhibitor that has great potential of being quickly translated into clinical trials is valproic acid (VPA), which is an established anti-convulsant drug with well-known safety profiles. VPA can also pass through the blood-brain barrier, making it a more attractive agent for treatment of malignant brain tumors such as medulloblastoma (MB). Our previous study has demonstrated that VPA at clinically achievable doses of 0.6 and 1 mM, suppressed cell proliferation, arrested cells in G_0/G_1 phases, induced apoptosis, reduced tumorigenicity in SCID mice, and is also active in MB subcutaneous xenograft models. To investigate the ability of VPA in modulating cellular responses to ionizing radiation in MB cells, four MB cell lines (D283-MED, DAOY, MHH-MED-1, and MEB-MED-8A) were pretreated with VPA (0.1, 0.2, 0.6, and 1 mM) for 7 days, followed by irradiation at doses of 2, 4, and 6 Gy. Our results showed that VPA (0.2, 0.6, and 1 mM) not only suppressed the colony-forming efficiency by itself, but also enhanced the radiosensitivity in all four MB cell lines ($P < 0.01$). Complete suppression of colony formation was achieved by 4 Gy irradiation in D283-MED and MEB-MED-8A cells pretreated with 1 mM VPA, an effect that was not seen with 6 Gy irradiation alone. The mechanisms underlying VPA-induced radiosensitization were further investigated by

examining the expressions of genes involved in cell cycle regulation and apoptosis. Upon treatment with VPA, the expression of p21 was increased, while that of c-Myc and the anti-apoptosis gene survivin were significantly reduced. Taken together, these results suggest that activation of cell cycle inhibitor and inhibition of DNA repair and anti-apoptosis genes are the mechanisms underlying radiation-enhancing effects of VPA and support the use of VPA as a radiosensitizer in order to reduce the radiation doses and thereby the toxicities on developing brains.

425. LOCAL RADIOTHERAPY AFTER RESECTION OF SINGLE BRAIN METASTASIS

S. Kinhult,[1] A. Gustavsson,[1] and E. Kjellén[1]; [1]Department of Oncology, Lund University Hospital, Lund, Sweden

Patients with a single, resectable brain metastasis can have a relatively good prognosis, with median survival of approximately 1 year. Radiotherapy to the brain can have serious side effects with high risk of leukencephalopathy and dementia after 0.5 to 1 year. So far, the value of postoperative whole-brain radiotherapy has not been established. Therefore, to minimize these late side effects of radiotherapy, patients operated for single brain metastasis were given postoperative local radiotherapy instead of whole-brain radiotherapy. From 2000, patients who were referred for postoperative radiotherapy after resection of single brain metastasis were given a planned dose of 25 Gy in 5 fractions to the metastasis site with a margin of 2 cm. Twenty-four patients were treated according to the protocol, 11 men and 13 women. The primary tumors were 8 lung cancer, 4 breast cancer, 4 unknown primary, 2 renal cancer, 2 malignant melanoma, and 4 gastrointestinal tumors. Median age at treatment was 58.5 (range, 48–79) years. Median time from primary diagnosis to brain metastasis was 22.2 (0–160) months. Median time from diagnosis of brain metastasis to surgery was 32 (6–83) days and from surgery to radiotherapy 36.5 (20–80) days. The planned dose was delivered to 22 patients, one patient had 20 Gy in 4 fractions, and one had 20 Gy in 5 fractions. The radiotherapy was given with mask fixation and in 7 patients after 3D dose planning. Intracranial recurrence was seen in 14 patients; only 3 of these patients had recurrence only outside the irradiated volume. Six patients did not have any intracranial recurrence, and 3 were lost to follow-up, but 1 of these 3 was alive after 3 years. Median time from radiotherapy to intracranial progression was 6.7 (1.5–25.3) months. Three patients had a second operation for the same or a second brain metastasis. Eight patients were given cranial radiotherapy a second time, 30 Gy in 10 to 15 fractions to the whole brain. Median survival from surgery was 10.8 (1.6–47.4) months, and from start of radiotherapy 9.8 (0.2–45.9) months. Seven patients were alive at time of analysis. Five of them had no intracranial recurrence, with a median survival from surgery of 11.0 (1.6–47.4) months. A majority of the patients with a resected brain metastasis recur within the irradiated volume after postoperative radiotherapy. This does not support giving whole-brain radiotherapy postoperatively after resection of single brain metastasis. Reducing the irradiated volume diminishes the risk of late side effects, especially cognitive disturbances.

426. STEREOTACTIC RADIOSURGERY FOR ATYPICAL/ANAPLASTIC MENINGIOMAS

J.A. Takahashi, H. Kano, N. Katsuki, M. Shirahata, M. Ohno, N. Hashimoto, N. Araki, and M. Hiraoka; Neurosurgery, Kyoto University Hospital, Kyoto, Japan

Atypical and anaplastic meningiomas frequently recur in the relatively short-term after surgery, even if they are radically resected. We have followed such postoperative cases by short-interval repeated MRI and have performed stereotactic radiosurgery (SRS) toward progressive tumors as salvage therapy. We report these results of SRS in high-grade meningioma in comparison with low-grade meningiomas. We reviewed 15 meningioma patients with 28 lesions treated by SRS at Kyoto University Hospital between 1997 and 2001, all of whom underwent initial surgery and received SRS for tumor progression as postoperative salvage therapy. They included 5 low-grade meningiomas, 9 atypical ones, and one anaplastic type. The mean tumor volume was 6.35 cm^3 (0.51–18 cm^3), and the mean marginal dose was 18.1 Gy (12–20 Gy). After a mean follow-up period of 30.9 months (24–72 months), 8 cases had tumor progression within the SRS field, and 6 had out of the SRS field. Cases with tumor progression had atypical/anaplastic meningiomas except one case with low-grade meningioma in the skull base. In atypical/anaplastic meningiomas, the mean time to progression after SRS was 12.3 months, and the mean marginal dose was 17.3 Gy. Five of 9 lesions, which were treated by less than 20 Gy SRS, had local recurrence within the SRS field. The marginal dose less than 20 Gy was a statistically significant predictor for a short-term progression in high-grade meningiomas ($P < 0.0029$). The two-year progression-free survival ratio in cases <20 Gy and >20 Gy was 40% and 57%, respectively. Extra-field tumor progression was due to CSF dissemination, meningiomatosis, and diffuse dural invasion. While tumor progression of low-grade meningiomas was controlled by relatively low-dose SRS (15–18 Gy), cases of high-grade meningiomas were recommended to receive SRS with marginal dose of more than 20 Gy.

427. BIODISTRIBUTION AND INTERNAL DOSIMETRY OF THE 188-RE LABELED HUMANIZED MONOCLONAL ANTIBODY H-R3 ADMINISTERED LOCO-REGIONALLY TO PATIENTS WITH HIGH-GRADE MALIGNANT GLIOMAS: PRELIMINARY RESULTS

L.A. Torres Aroche,[1] M. Coca,[1] A. Casaco,[2] G. Lopez,[3] I. Garcia,[3] A. Perera,[1] J. Batista,[1] A. Hernandez,[1] and Y. Peña[1]; [1]Nuclear Medicine, Center for Clinical Research, [2]Clinical Research, Center of Molecular Immunology, and [3]Neurosurgery, International Center of Neurological Restoration, Havana, Cuba

Favorable clinical response after direct infusion of 90-Y or 131-I labeled anti-tenascin Mabs into the postoperative tumoral bed of patients with malignant gliomas has been found. A clinical trial was performed to evaluate the biodistribution, internal radiation dosimetry, toxicity, the maximal tolerated dose (MTD), and any clinical effect after the loco-regional radioimmunotherapy (RIT), using the humanized MAb h-R3, labeled with188-Re. A phase 1 dose escalation trial was performed by administrating into the post-operative cavity through an indwelling catheter a single dose of the h-R3 MAb directed against epidermal growth factor receptors (EGFR). The study was reviewed and approved by the ethics committees of all the involved institutions. Five patients have been included; they had partial tumor resections and overexpressed the EGFR. Patients were treated with 3 mg of MAb labeled with 10 or 15 mCi of 188-Re after signing the written informed consent. SPECT and planar images as well as multiple blood and urine samples were collected up to 24 h after injection. Biodistribution was computed from scintigraphic images, and the absorbed doses were estimated using the MIRD methodology at organ and voxel level. Data processing and statistical analyses were performed using the SPSS and Microcal Origin v6.0 software packages. The effective half-life of the 188-Re H-R3 in the tumoral bed ranged from 7.3 to 14.4 h (mean, 8.4 ± 2.8 h). The liver, kidneys, and urinary bladder showed the highest uptake of the compound leaving the tumoral bed. The mean absorbed dose in the tumor ranged from 13.9 Gy to 68.4 Gy, and the maximum doses ranged from 26.9 Gy to 136.2 Gy. The maximum absorbed dose for liver, kidneys, and urinary bladder was less than 2 Gy in all patients. Transitory acute side effects following treatment were headache, seizures, and worsening of pre-existing neurological symptoms. Two patients developed stable disease during 3 months, and 2 GBM patients are practically asymptomatic and in complete remission after one year of treatment. The other GBM patient cannot yet be evaluated after one month of treatment. The preliminary results of this study strongly suggest that loco-regional radioimmunotherapy of high-grade glioma using the h-R3 MAb labeled with 188-Re may be safe and constitute a promising therapeutic approach for these patients.

428. BRAINSTEM GLIOMAS IN ADULT: PROGNOSTIC FACTORS AND RESULTS IN A SERIES OF WESTERN ITALIAN COOPERATIVE GROUP

L. Fariselli, A. Salmaggi, I. Milanesi, E. Lamperti, A. Boiardi, C. Maccagnano, E. Laguzzi, R. Ruda, and R. Soffietti; Radiotherapy, Istituto Nazionale Neurologico "C. Besta," Milan, Italy

The charts of 35 patients with brainstem gliomas were reviewed to define prognostic factors and to evaluate the effect of combined radiochemotherapy treatment. Mean age at onset was 39 years (16–55). The main presenting symptoms were diplopia (40%), paresis (30%), and ataxia (10%). At MRI, 18 patients (51%) had diffuse infiltrative tumor, in 15 patients (43%) diffuse or local contrast-enhancement, and in 2 patients (6%) focal cystic-necrotic areas were described. MR-spectroscopy was positive in all analyzed patients, and quantitative PET was performed in 9 patients. Twenty-four patients had surgical intervention; conformal radiotherapy was performed in all patients (total dose range, 48–54 Gy, 1.8 Gy/fraction) associated to chemotherapy (temodal 250 mg/day weekly, max 5 cycles) in 20 patients. Overall median survival was 40 months. On multivariate analysis, the duration of symptoms before diagnosis, the absence of contrast-enhancement, and histological diagnosis were confirmed to have prognostic significance on survival. Neurological conditions and/or radiological response were improved in 50% of the cases, while they were stable in 35% after treatment. Adult brain stem gliomas resemble supratentorial gliomas: Radiotherapy associated to chemotherapy seems to have potential chances of temporary improvement of neurological functions.

429. RADIOTHERAPY FOR PRIMARY BRAIN TUMORS: CLINICAL POTENTIAL OF NOVEL TREATMENT TECHNIQUES

C. Niël, H. Struikmans, and J. van Santvoort; Radiation Oncology, Medisch Centrum Haaglanden, The Hague, The Netherlands

Radiation dose to the normal brain restricts the total radiation treatment dose given for a primary brain tumor. In the last decade radiation techniques have evolved to an approach which reduces the normal tissue irradiation by optimizing the shape and beam direction of a given radiation beam. These techniques are known as 3-dimensional conformal radiotherapy (3dCRT) and are the current standard in radiation treatment for primary brain tumors. The latest technical development in radiotherapy is, apart from optimal shaping of the beam contour, the computerized modulation of the "dose content" of a radiation beam, so-called intensity modulated radiation therapy, or IMRT. The aim of these techniques is to increase the effect of sparing normal tissue that has been achieved by 3dCRT. We evaluated the possible clinical advantage of IMRT by comparing the actually given 3dCRT plan with an IMRT plan for 8 patients with low-grade glioma, mean tumor volume 174 cm^3 (72–329). We compared the volumes of normal brain tissue irradiated to a total dose of respectively 20%, 40%, and 60% of the prescribed treatment dose. A substantial reduction of brain tissue irradiated to these dose levels (for 3dCRT 69 [42–87], 40 [18–54], and 21 [4–30] cm^3, respectively) was achieved by IMRT (42 [27–55], 15 [8–24], and 5 [2–10] cm^3), even for larger tumor diameters. The impact of IMRT on the dose distribution for the primary tumor was small (mean volume receiving at least 95% of the therapeutic dose: 93% [90–98] vs. 100% for 3dCRT) and, in light of the therapeutic gain by reducing the radiation dose to the normal tissue, not of any clinical relevance. Achievable radiation doses to the tumor, given by IMRT without an increase of risk in neurotoxicity are calculated from our data and the known data from the literature. The clinical use of IMRT in the treatment for primary brain tumors may lead to a substantial dose escalation without increasing the potential radiation toxicity to the normal brain. Phase 2 studies, carefully undertaken, should be performed to investigate the therapeutic potential of IMRT in the treatment for primary brain tumors.

430. THE POSITIVE EFFECT OF RADIOTHERAPY DOSE ESCALATION AND CHEMOTHERAPY ON THE SURVIVAL OF GLIOBLASTOMA PATIENTS

L. Mangel,[1] L. Sipos,[2] J. Julow,[3] J. Havel,[3] T. Major,[1] G. Németh,[1] and J. Fodor[1]; [1]Department of Radiotherapy, National Institute of Oncology, Budapest; [2]National Institute of Neurosurgery, Budapest; [3]Department of Neurosurgery, St. John's Hospital, Budapest; Hungary

The aim of our retrospective study was to investigate novel therapeutic approaches on the survival of glioblastoma patients. We analyzed the data of 102 glioblastoma patients who were treated with definitive radiotherapy (RT) between 1997 and 2003; the short palliative RT courses were excluded. We examined the effect of age (70 vs. KPS $\leqslant$ 70), type of surgery (radical vs. partial), T-stage (T1 vs. T2–4), adjuvant chemotherapy (CHT) with Temodal and/or BCNU (yes vs. no), and RT dose escalation (biological equivalent dose >60 Gy vs. $\leqslant$60 Gy) on survival. The RT dose escalation consisted of hypofractionated regimen of 60/2.5 Gy, or interstitial AL boost, or conventional RT of 66 Gy total dose. The treatment selection was based upon patient and tumor characteristics, individually. Uni- and multivariate analyses were performed. Good performance status and T1 stage had positive effect on survival. The median survival times (MST) were 14 versus 9 months ($P = 0.0004$) and 14 versus 10 months ($P = 0.0024$), respectively. The type of surgery and the age showed a non-significant tendency, the MSTs were 13 versus 10 months ($P = 0.0724$) and 12 versus 10 months ($P = 0.0724$), respectively. The addition of CHT and RT dose escalation significantly prolonged the survival, the MSTs were 14.5 versus 8 months ($p<0.0001$) and 13 versus 8.5 months ($P < 0.0001$). Seven patients were alive 2 years after initial diagnosis, 6 of them had been treated with CHT, and all of the 2-year survivors received higher RT doses. Multivariate analysis revealed that good KPS ($P = 0.0280$; RR, 0.59; CI, 0.36–0.94), low T-stage ($P = 0.0383$; RR, 0.63; CI, 0.4–0.98), RT dose escalation ($P = 0.0009$; RR, 0.46; CI, 0.29–0.73), and the addition of CHT ($P = 0.0017$; RR, 0.48; CI, 0.3–0.76) had independent positive effect on survival. This study indicates that in addition to CHT the RT dose escalation may have therapeutic benefit. However, further investigation is required to define the optimal forms of RT dose escalation in glioblastoma patients with different prognostic factors.

431. MAINTENANCE OF NEUROPSYCHOLOGICAL FUNCTION IN CHILDREN AND YOUNG ADULTS WITH BENIGN AND LOW-GRADE BRAIN TUMORS TREATED PROSPECTIVELY WITH HIGH-PRECISION FOCAL CONFORMAL RADIOTHERAPY

R. Jalali,[1] S. Goswami,[2] N. More,[1] R. Sarin,[1] M. Siddha,[3] R. Kamble,[1] and S. Parab[3]; [1]Radiation Oncology; [2]Clinical Psychology; [3]Brain Tumor Foundation of India, Mumbai, India

The aim of our study was to present prospective neuropsychological data at baseline and follow-up in children and young adults with benign and low-grade gliomas treated with focal stereotactic conformal radiotherapy (SCRT). Twenty-six patients (age 4–25 years) with residual/progressive benign and low-grade tumors considered suitable for SCRT underwent a detailed neuropsychological and cognitive testing at baseline before starting RT and subsequently at 6 months and 24 months (and every year thereafter) after SCRT. Intelligence quotient (IQ) was measured by an age-adjusted and validated WISC giving verbal quotient (VQ), performance quotient (PQ), and global quotient (GQ). For patients more than 17 years, memory quotient (MQ) was measured by Wechsler memory scale (WMS). VITHOBA battery was employed for blind children to assess PQ. Anxiety was measured by C1 and C2 scales and Hamilton anxiety rating scale (HARS; for adults), and depression was measured by Hamilton depression rating scale (HDRS; for adults). Cognition was measured by LOTCA battery (max value, 119) and quality of life by Health Utility Index (HUI; normal score: very good, 7; poor, 31). Mean baseline global-IQ (normal 90–109) of patients before starting RT was 80 (range, 33–129), which improved to 87 and 92 respectively at 6 and 24 months following SCRT. The corresponding mean values for VQ and PQ at similar timescale were 82, 87, and 89 and 82, 93, and 106 respectively. Memory remained maintained at 6 months and 2 years after SCRT, with mean values of 97 and 103 (range, 100–106) compared to mean baseline value of 83 before RT. In 3 blind children, PQ recorded by Vithoba battery revealed a reduction of mean value 94 at 6 months and 77 at 2 years after RT compared to mean pre-SCRT value of 97. LOTCA battery used for patients aged >6 revealed respective average baseline, 6-month, and 2 year follow-up values of 93, 102, and 98. Anxiety assessments with C1, C2 (no anxiety <35) & HARS (normal <17) showed mean baseline values of 39, 36, and 23 reducing to 29, 17, and 17 at 6-month and 23, 24, and 14 at 2-year follow-up. Mean depression values using HDRS (normal <17) was 23 (range, 3–41) before and 14 after 2 years following SCRT. For QOL in children, mean pre-SCRT HUI values of 9.5 improved to 8.8 at 2 years following SCRT. Barthel's ADL were also maintained at follow-up. Preliminary analysis of this relatively small cohort of young patients treated with high-precision SCRT reveals maintained neuropsychological profile assessed prospectively up to 2 years following treatment. However, it clearly needs mature data in larger number of patients at a longer follow-up to derive firm conclusions.

432. THE INCIDENCE OF CEREBROVASCULAR ACCIDENTS (CVA) AND SECONDARY BRAIN TUMORS IN PATIENTS WITH PITUITARY ADENOMA

P. Brown,[1] C. Brown,[1] R. Kline,[1] B. Pollock,[2] S. Stafford,[1] M. Link,[2] P. Schomberg,[1] and R. Foote[1]; [1]Radiation Oncology and [2]Neurosurgery, Mayo Clinic Rochester, Rochester, Minnesota, USA

The aim of this study was to assess the risk of CVA and secondary brain tumors in patients with pituitary adenoma. A cohort of 144 patients (median age 36.4 years; range, 12.3–86.8) from Olmsted County, Minnesota, diagnosed with pituitary adenoma between 1935 and 2000 was studied. Only patients from Olmsted County were included because of the unique nature of medical care in Olmsted County allowing the ascertainment of virtually all cases of pituitary adenoma for this community's residents during these years. Additionally, the patients with pituitary adenoma represent a cohort from a much larger, well-studied population (i.e., the entire population of Olmsted County), providing opportunities for future comparisons. Seventy-seven patients (53.5%) underwent surgery for their pituitary tumor, with 4 of these patients undergoing 2 operations. Twenty-eight (19.4%) underwent radiation therapy (median dose 45 Gy; range, 17.6–60 Gy) with twenty of these patients (13.9%) undergoing both surgery and radiation therapy (RT). Five patients received repeat RT (median 11.3 Gy; range, 8.4–50 Gy). Fifty-nine patients (41%) were simply observed after their diagnosis of a pituitary tumor. Thirty -five (24%) patients have died with a median follow-up of 10.4 years (range, 0.1–53.1). The RT group had significantly ($P < 0.01$) longer follow-up than the other patients, with a median follow-up of 18.6 years versus 8.7 years, respectively. There were no significant differences in CVA rates between the 3 groups, observation, surgery, and radiotherapy (10-year CVA rate 13.9%, 6.7%, 11.7%, respectively). The combination of surgery and RT did not lead to an increased risk of CVA when compared to surgery or RT alone. Analyzing only the RT patients revealed a significantly increased risk of CVA at 20 years for patients after repeat RT (60%) compared to a single course of RT (22%, $P = 0.05$) but no difference with fraction sizes $\geqslant$2 Gy ($P = 0.24$). Only

one secondary tumor was diagnosed, a meningioma 18 years after surgery only for a prolactinoma. CVA is a significant risk for patients with pituitary tumors. Except for repeat RT, treatment does not seem to impact the risk. Second malignancies in this population even with long-term follow-up are a rare event. Future analyses will compare the rate of CVA in pituitary patients to the general community.

433. SAFETY OF HIGH-PRECISION FOCAL CONFORMAL RADIOTHERAPY EMPLOYING CONSERVATIVE MARGINS IN CHILDHOOD BENIGN AND LOW-GRADE BRAIN TUMORS

R. Jalali, B. Budrukkar, S. Sarin, and D. Sharma; Radiation Oncology, Tata Memorial Hospital, Mumbai, India

The purpose of our study was to report safety of employing conservative margins in treatment planning and delivery of high-precision conformal radiotherapy for childhood brain tumors. Between December 1999 to December 2003, 31 children (22 boys and 9 girls, median age 12 years) with incompletely excised or recurrent benign and low-grade brain tumors (14 craniopharyngiomas, 10 chiasmal/hypothalamic gliomas, 5 low-grade gliomas [LGG], and 2 others) were treated with three-dimensional conformal radiotherapy (CRT) (12 patients) and stereotactic conformal radiotherapy (SCRT) (19 patients). Gross tumor volume (GTV) included neuro-imaging based visible tumor and/or resected tumor bed. Clinical target volume (CTV) consisted of GTV + 5 mm margin, and planning target volume (PTV) consisted of additional 5 mm margin for CRT and 2 mm for SCRT. Treatment was delivered with 3 to 9 conformal fixed fields to a median dose of 54 Gy/30 fractions. The actuarial 2-, 3-, and 4-year disease-free and overall survival was 96%, 100%, and 100%, respectively (median follow-up, 30 months; range, 12–58 months). Radiological follow-up available in 30 patients revealed complete response in 1, partial regression in 12, stable disease in 17, and progression in 1 patients (within the CTV). One patient with craniopharyngioma on a routine imaging revealed a mild asymptomatic cyst enlargement, which resolved with conservative management. A patient with chiasmatic glioma developed cystic degeneration and hydrocephalus 9 months after SCRT requiring cyst drainage and placement of a VP shunt. Pre-irradiation evaluation showed hormonal dysfunction in at least one endocrine axis in 16 patients. On follow-up, 2 out of the remaining 15 patients also had hormonal impairment. Serial visual assessments revealed impaired vision (acuity/fields) in 26 patients before starting RT, which showed improvement in 16, stable in 14, and mild deterioration in 1 patient. Focal conformal radiotherapy techniques delivering irradiation to a computer-generated target volume employing 7- to 10-mm 3D margins beyond the visible tumor and/or resected tumor bed appear to be safe in children with incompletely resected or recurrent benign/low-grade brain tumors. Because of the ability of these techniques to achieve sharp dose differential between the target volume and adjacent normal brain, long-term prospective studies are required to test their potential objectively in minimizing treatment related late morbidity and sustaining local control.

434. SPINAL RE-IRRADIATION AFTER SHORT-COURSE RT WITH 1 × 8 GY OR 5 × 4 GY FOR METASTATIC SPINAL CORD COMPRESSION

D. Rades,[1] L. Stalpers,[2] T. Veninga,[3] and P. Hoskin[4]; [1]Radiation Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]Radiation Oncology, Academic Medical Center Amsterdam, Amsterdam, The Netherlands; [3]Radiation Oncology, Dr. B. Verbeten Institute Tilburg, Tilburg, The Netherlands; [4]Radiation Oncology, Mount Vernon Cancer Centre, Northwood, UK

This study investigated the feasibility and the effectiveness of re-irradiation (re-RT) for in-field recurrence of metastatic spinal cord compression (MSCC) in long-term survivors (follow-up of at least 12 months), initially treated with 1 × 8 Gy or 5 × 4 Gy. Of a total series of 540 MSCC patients, who were irradiated with 1 × 8 Gy (n = 261) or 5 × 4 Gy (n = 279) between 1/1995 and 8/2003, 56 patients (1 × 8 Gy, n = 28, and 5 × 4 Gy, n = 28) could be identified who had a follow-up ⩾12 months and were treated with re-RT for in-field recurrence of MSCC. Median follow-up in these 56 patients was 17 (12–58). Median time to recurrence was 6 (3–40) months. Re-RT was performed with 1 × 8 Gy (after 1 × 8 Gy or 5 × 4 Gy, n = 32), 5 × 3 Gy (after 5 × 4 Gy, n = 13), or 5 × 4 Gy (after 1 × 8 Gy, n = 11). The cumulative (primary RT plus re-RT) biologically effective dose (BED) was 80 to 100 Gy2. Median follow-up after re-RT was 10 (4–23) months. Motor function was evaluated up to 6 months after re-RT with a 5-point-scale (grade 0, normal strength; grade 1, ambulatory without aid; grade 2, ambulatory with aid; grade 3, not ambulatory; grade 4, paraplegia). Twenty-four patients (43%) showed improvement of motor function, 29 (52%) no change, and 3 (5%) deterioration. Five of 6 previously non-ambulatory patients regained the ability to walk. No second in-field recurrence in the same spinal region was observed after re-RT. Outcome was not significantly influenced by the radiation schedule. Radiation-induced myelopathy was not observed. Spinal re-irradiation with 1 × 8 Gy, 5 × 3 Gy, or 5 × 4 Gy for in-field recurrences of MSCC appears safe and effective. Myelopathy seems unlikely, if the cumulative BED is ⩽ 100 Gy2.

435. RADIOTHERAPY FOR MOTOR DEFICITS DUE TO METASTATIC SPINAL CORD COMPRESSION: IS 1 × 8 GY AS EFFECTIVE AS 10 × 3 GY?

D. Rades,[1] and L.J.A. Stalpers[2]; [1]Radiation Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; [2]Radiation Oncology, Academic Medical Center Amsterdam, Amsterdam, The Netherlands

Since life expectancy is markedly reduced in patients with metastatic spinal cord compression (MSCC), a short and effective radiation schedule is desired. This study investigates a reduction of the overall treatment time to only one day by comparing 1 × 8 Gy to the multi-fractionated 10 × 3 Gy for functional outcome. Data of 204 patients, treated for MSCC with either 1 × 8 Gy (n = 96) or 10 × 3 Gy (n = 108), were analyzed retrospectively. Motor function and ambulatory status were evaluated before and up to 24 weeks after RT. A multivariate analysis (nominal regression) was performed, including radiation schedule, performance status, age, irradiated vertebra, and the three relevant prognostic factors (type of primary tumor, pre-RT ambulatory status, and time of developing motor deficits prior to RT). Improvement of motor deficits was selected as basic category and compared with no change. The univariate analysis showed no significant difference between the two radiation schedules, either for improvement of motor function ($P = 0.89$ at 6 weeks after RT, $P = 0.89$ at 12 weeks after RT, and $P = 0.89$ at 24 weeks after RT) or for post-treatment ambulatory rates ($P = 0.75$ at 6 weeks after RT, $P = 0.52$ at 12 weeks after RT, and $P = 0.67$ at 24 weeks after RT). The multivariate analysis demonstrated a significant effect on functional outcome for the ECOG performance status ($P = 0.01$), for the pre-RT ambulatory status ($P = 0.01$), for the type of primary tumor ($P = 0.01$), and for the time of developing motor deficits prior to RT ($P < 0.001$), but not for the radiation schedule ($P = 0.85$). In the whole series, 23/68 (34%) initially non-ambulatory patients regained the ability to walk after RT, 11/30 patients (37%) after 1 × 8 Gy, and 12/38 patients (32%) after 10 × 3 Gy ($P = 0.82$). Our data suggest the two fractionation schedules to be comparably effective for functional outcome. Thus, 1 × 8 Gy should be considered for patients with a poor survival prognosis.

436. ROLE OF SURGICAL RESECTION IN COMBINATION WITH FRACTIONATED STEREOTACTIC RADIOTHERAPY FOR MANAGEMENT OF VESTIBULAR SCHWANNOMA

Y. Sawamura,[1] H. Shirato,[2] and T. Sakamoto[3]; [1]Neurosurgery, [2]Radiology, and [3]Otology, Hokkaido University Hospital, Sapporo, Japan

The goal of this study was to clarify the role of surgery in combination with fractionated stereotactic radiotherapy (SRT) analyzing outcomes in patients with vestibular schwannoma (VS). A total of 165 vestibular schwannomas were treated with surgery and SRT. Twenty-four large tumors of T4b (Hanover classification) were partially removed, and then residual mass was treated by SRT. T4a and smaller tumors, 141 cases, were treated with SRT alone at a radiation level of 40 to 50 Gy administered in 20 to 25 fractions. At the time of SRT, the median tumor size was 16.2 mm (range, 3–36 mm). The median follow-up period was 42 months. The actuarial 7-year rate of tumor control (no growth >2 mm and no requirement for salvage surgery) was 91.8% (95% CI, 87%–96%). Three patients with progressive tumors underwent salvage tumor resection. All 24 tumors that were partially removed before SRT were well controlled. The actuarial 5-year rate of hearing preservation (Gardner-Robertson Class I–IV) was 71.5%. The observed complications of fractionated SRT included transient facial nerve palsy (3% of patients), trigeminal neuropathy (9% of patients), and balance disturbance (9% of patients). No new permanent facial weakness occurred after fractionated SRT. Fractionated SRT resulted in an excellent tumor control rate and produced a high rate of hearing preservation. The role of surgery is only to reduce mass volume to the amount that can be adequately treated by fractionated SRT. Only the large tumors of Hanover Class T4b are the target of neurosurgical resection.

437. EFFECT OF MICROBEAM RADIATION EXPOSURE TO THE MICROVASCULATURE OF HEALTHY MOUSE BRAIN

C. Rémy,[1] R. Serduc,[1] P. Vérant,[2] J. Vial,[2] R. Farion,[1] E. Brauer,[3] A. Bravin,[3] J. Laissue,[4] C. Segebarth,[1] and B. van der Sanden[1]; [1]INSERM U594 and [2]CNRS UMR 5588, Université Joseph Fourier, Grenoble, France; [3]ESRF, Grenoble, France; [4]University of Bern, Institute of Pathology, Bern, Switzerland

One of the important side effects of brain tumor radiotherapy is the breakdown of the blood-brain barrier (BBB), which may cause cerebral edema and high intracranial pressure. A new technique, microbeam radiation therapy (MRT) using synchrotron radiation X rays, has recently been developed and is based on the idea that radiation damage in normal brain tissue can be decreased by spatial microfractionation of the absorbed dose. The aim of this study was to assess the early effects (2 h–1 month) of MRT on the microvasculature (BBB and blood volume) in the cortex of nude mice using intravital two-photon microscopy. The upper part of the left hemisphere of Swiss nude mice (5 weeks old) was irradiated in an anteroposterior direction by a 3-mm-high, ~3.1-mm-wide array of 16 vertically oriented, quasiparallel microplanar beams (width ~ 25 μm, center-to-center spacing ~ 207 μm; entrance dose, 312 or 1000 Gy). At different time intervals after MRT (2 h, 12 h, 24 h, 48 h, 4 d, 7 d, 12 d, 30 d), 3 mice were anesthetized and placed on the motorized step stage of the two-photon microscope after craniotomy (3 mm in diameter) and intravascular injection of 2 fluorescent probes, FITC-dextran (70 kDa) and sulforhodamine B (577 Da). The vascular volume in the irradiated portion of the brain was estimated from z-scans at the FITC wavelength over a maximum distance of 650 μm starting from the dura. The vascular permeability was detected as extravasations of sulforhodamine B in the irradiated microplanar tissue slices. For all time intervals after MRT and both tested radiation doses, the FITC-dextran remained in the functional vessels, and no significant change in vascular volume was observed. From 12 h until 12 days after MRT with a 1000-Gy radiation entrance dose, diffusion of sulforhodamine B in microbeam stripes was observed. No diffusion was detected one month after MRT. It seems that a BBB breakdown occurs between 12 h and 12 days and is repaired between 12 and 30 days after irradiation. After 312 Gy, no leakage of sulforhodamine B in the microbeam stripes of normal brain tissue was detected at any time after MRT. Up to one month after a dose of 312 Gy applied in the microbeam mode, no radiation damage to the microvasculature was detected in the irradiated microplanar slices of normal brain tissue. This entrance dose would therefore be more appropriate for the treatment of gliomas using crossfired microbeams than a dose of 1000 Gy.

438. SINGLE-FRACTION, IMAGE-GUIDED, INTENSITY-MODULATED RADIATION THERAPY (IG IMRT) FOR OLIGOMETASTATIC LESIONS OF THE SPINAL COLUMN

Y. Yoshiya,[1] M. Lovelock,[2] M.H. Bilsky,[2] M. Hunt,[2] and Z. Fuks[1]; [1]Radiation Oncology; [2]Medical Physics, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Stereotactic radiosurgery (SRS) utilizing high-dose, single-fraction radiation has been shown to be very effective in the management of intracranial metastases. Image-guided techniques were developed to deliver precise, high-dose, intensity-modulated radiotherapy (IMRT) in a single fraction to metastatic lesions of the spinal column. Twenty-one oligometastatic patients with spinal metastases near the spinal canal were immobilized in a noninvasive cradle. IMRT was utilized to provide spinal cord dose-sparing treatment plans (dmax $<$ 1000 cGy) while delivering 1800 to 2400 cGy to the lesion (median, 2100 cGy) in a single fraction. Two-dimensional and/or three-dimensional (cone beam CT) image-guided verification was performed. Each patient was followed every three months with clinical and radiographic (including MRI) assessment. No patient was lost to follow-up. The noninvasive cradle coupled with IG IMRT provided set errors ± 1 mm. The median maximum dose (dmax) to the gross tumor volume (GTV) and spinal cord was 2640 cGy and 1042 cGy, respectively. The median average cord dose was 434 cGy (range, 345–768 cGy). With a median follow-up of 5 months (range, 3–15 months), 95% of patients have demonstrated durable radiographic control and palliation of presenting symptoms. No significant treatment-related toxicity has been encountered, including myelopathy and radiculopathy. High-dose, single-fraction IG IMRT is safe and effective. Treatment precision approaches that expected from fixed-frame SRS for intracranial metastases, with similar rates of local control and minimal toxicity.

439. COMPLICATIONS OF STEREOTACTIC RADIOSURGERY IN PATIENTS WITH BRAIN METASTASES: CONSIDERATIONS FOR DEEP AND FUNCTIONAL BRAIN TUMOR LOCATIONS

D. Suki,[1] F.F.L. Lang,[1] M. Maldaun,[1] E.L. Chang,[2] and R. Sawaya[1]; [1]Neurosurgery and [2]Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, USA

Stereotactic radiosurgery (SRS) has become a common and popular treatment for metastatic brain tumors over the past decade. The appeal of SRS for the treatment of metastatic brain tumors stems from many factors related to the inherent nature of the tumors themselves, as well as factors related to the procedure. Among those factors is the widely held assumption that SRS-associated morbidity is much less common than its neurosurgical and other radiation counterparts, and that the procedure can be performed in any brain location regardless of depth or regional brain function. We performed a review of the published literature on SRS from 1990 to 2004 to assess the adequacy of the evidence on the relationship between brain tumor location and the risk of complications. We also reviewed our radiosurgery experience among patients with metastatic brain tumors at M.D. Anderson, correlating the risk of complications with deep and functional brain tumor locations. The review of published literature failed to reveal adequate representation of various brain tumor locations, including eloquent and/or deep ones, or adequate description and analysis of the role of tumor location as a predictor of SRS-related complications. Most published series either do not mention location or provide the distribution of locations without correlating these with complications. There is no report formally assessing the threshold between maximum tolerated SRS doses and toxicity within specific tumor locations. The review of the M.D. Anderson SRS history showed that the majority of patients with lesions in eloquent areas experienced complications following the procedure. Complications were more frequent in lesions located in eloquent brain areas (58% of 93 lesions) compared to noneloquent areas (31% of 58 lesions) or near eloquent areas (39% of 110 lesions) ($P < 0.05$, log-rank test). Complications were also more frequent in tumors located in the motor/sensory area or brain stem area compared to others (57%, 65%, and 37%, respectively). The same trends were seen when only severe complications (RTOG >3) were included, and when failures, seizures, early deaths, headaches, nausea, and DVT were excluded from the list of complications. Significant multivariate predictors of complications included eloquent tumor location, progressing primary, and a melanoma primary cancer. The finding of a significant independent role of tumor location is especially compelling given the lower dose received at some of these deep-seated locations. We propose a review of the notion of safety of SRS in deep-seated and eloquent brain tumor locations based on the following two premises: (1) the limited availability of published data investigating the issue and (2) the recent data from our Center, which, owing to longer and more complete follow-up, a detailed classification of tumors by tumor functional location and grade, and adequate numbers in various categories, support the hypothesis that the radiosurgical treatment of metastatic brain tumors located in eloquent areas of the brain is associated with an increased risk of severe complications.

440. ANALYSIS OF HISTOPATHOLOGICAL AND RADIOLOGICAL PARAMETERS TO PREDICT RESPONSE TO RADIOTHERAPY IN LOW- AND HIGH-GRADE GLIOMAS

S. Al-Haddad,[1] D. du Plessis,[1] K. Kopitzky,[1] T. Smith,[2] D. Husband,[3] B. Haylock,[3] C. Walker,[3] and P. Warnke[1]; [1]Neurosciences, University of Liverpool, Liverpool; [2]Radiology, Walton Centre of Neurology and Neurosurgery, Liverpool; [3]Neurooncology, Clatterbridge Cancer Research Trust, Wirral; UK

Limited information is available correlating radiographic response seen after radiotherapy and survival outcome among patients with low- and high-grade gliomas. There are no studies comparing radiographic response between low- and high-grade tumors. The most widely used radiological response measurement is based on the changes seen on contrast-enhanced scans. However the majority of low-grade gliomas do not enhance, and there is no established methodology applicable to both low- and high-grade gliomas. The aim of this study was to analyze whether clinical parameters or radiological changes observed after radiotherapy correlate with time to progression (TTP) and overall survival (OS). Sixty adult patients with WHO grade II and III supratentorial gliomas who had undergone stereotactic or burr hole biopsy and were treated by primary radiotherapy between 1994 and 2000 were included in this analysis. Area and volume analysis of both the contrast-enhanced regions and hypodense regions on CT or hyperintense regions on T2 MR images was conducted by using NIH Scion image analyzer to quantify the response in both low- and high-grade tumors. Radiographic response to radiotherapy was correlated with time to progression (TTP) and overall survival (OS). Of the 60 patients evaluated for imaging response, the complete, partial response, stable disease, and progressive disease rates were 5.0%, 26.7%, 43.3%, and 25% respectively. Changes seen in hypodense area on CT or hyperintense regions on T2-

weighted image did not correlate with changes of the contrast-enhanced regions. A significant correlation between radiographic response and survival was observed (Cox regression, $P < 0.05$). Grade II tumors showed greater volume reduction after radiotherapy compared to grade III gliomas (Mann-Whitney U test, $P < 0.05$). Our findings indicated that the presence of contrast enhancement was significantly associated with poorer prognosis. Almost a third of low-grade and two thirds of high-grade gliomas showed evidence of contrast enhancement. However, we found no evidence to support the association between area or volume changes based on contrast-enhanced regions after radiotherapy with time to progression (TTP) or overall survival (OS). Instead, the data gathered suggested that there was a good correlation between volume changes seen on hypodense on CT or hyperintense regions on T2-weighted image (CT/T2) with TTP and OS in both low- and high-grade gliomas. Reduction in the volume of CT/T2 after radiotherapy was significantly associated with survival. The volume reduction was more evident in low-grade than high-grade gliomas. We found that seizures at the time of presentation and radiographic response based on Modified Macdonald's criteria to be independent prognostic factors for time to progression. For overall survival, WHO histological grade and radiographic response based on Modified Macdonald's criteria were found to be independent prognostic factors. We recommend the use of volume based on CT/T2 to standardize response measurement to treatment especially after radiotherapy.

441. PATTERN OF CARE AND SURVIVAL IN A RETROSPECTIVE ANALYSIS OF 1866 PATIENTS (PTS) WITH GLIAL TUMORS TREATED WITH RADIOTHERAPY (RT) IN TWELVE ITALIAN CENTERS FROM 1985 TO 2003

M. Buglione,[1] R. Santoni,[2] L. Pirtoli,[3] V. Tombolini,[4] R. Bagnoli,[5] M.F. Osti,[6] L. Lupattelli,[7] A. Somensari,[8] U. Ricardi,[9] M. Krengli,[10] P. Giacobazzi,[11] and G.P. Biti[12]; [1]Radiation Oncology, University of Brescia, Brescia; [2]Radiation Oncology, University Tor Vergata of Rome, Rome; [3]Radiation Oncology, University of Siena, Siena; [4]Radiation Oncology, University of L'Aquila, L'Aquila; [5]Radiation Oncology of Arezzo, Arezzo; [6]Radiation Oncology, University La Sapienza of Rome, Rome; [7]Radiation Oncology, University of Perugia, Perugia; [8]Radiation Oncology of Cremona, Cremona; [9]Radiation Oncology, University of Torino, Torino; [10]Radiation Oncology, University of Piemonte Orientale, Novara; [11]Radiation Oncology of Modena, Modena; [12]Radiation Oncology, University of Firenze, Firenze; Italy

The end points of our study were to analyze patterns of clinical presentation, care, and outcome in a multi-institutional series of radiotherapy-treated malignant glioma pts and to evaluate actuarial overall survival (OS) in the different clinical and therapeutic subsets. Histology was reclassified by using the WHO system; performance status was defined according to the Karnofsky index and Order scale. Type of surgery, RT volumes, RT techniques and doses, supportive care, and chemotherapy were analyzed also according to the accrual period (1985–1990, 1991–1996, and 1997–2003). The OS was calculated only for the pts with G3–4 astrocytoma (1466 pts), using the Kaplan-Meier method. Differences in actuarial overall survival (OS) were analyzed with the log-rank test and the Cox regression test. Statistically significant differences ($0.000 < P < 0.02$) in clinical, diagnostic, and therapeutic features according to the accrual period are evident. In the last period, many more pts were treated who were aged >60 years (27%, 42.3%, and 50.7%, respectively, in the 3 groups), with worse Order score (78%, 66%, and 89%, respectively), with lesions 3 to 5 cm large (35%, 45%, and 50%, respectively) with G4 disease (60%, 73%, and 73%, respectively). As for the diagnostic workup, the number of pts submitted to MRI or CT and MRI significantly increase in the more recent periods, both in the presurgical and in the postsurgical setting ($P = 0.000$). In the last period, more pts were submitted to radical surgery ($P = 0.037$) and to conformal radiotherapy ($P = 0.000$), mainly on more limited volumes ($P = 0.000$). The majority of the pts were treated with RT doses >60 Gy (53.3%). Median OS of the entire series was 9 months. The univariate analysis showed a better survival for young pts ($P = 0.0000$), in those with better Order score ($P = 0.0000$), with G3 histology ($P = 0.0000$), and small disease ($P = 0.0027$). Among treatment variables, radical surgery ($P = 0.0001$), high RT dose ($P = 0.0000$), limited treatment volumes ($P = 0.0000$), and the use of chemotherapy ($P = 0.0000$) were related with a better survival. The multivariate analysis confirmed the importance of histology, tumor size, age, neurological performance status, radical surgery, dose of RT, and volumes of treatment. In Italy, patterns of practice for malignant gliomas changed significantly during the last two decades. Staging procedures were increasingly accurate, surgery more aggressive, and RT techniques more sophisticated. The relevance for OS of age, NPS and WHO histology, radical surgery, high-dose radiotherapy on limited volumes is confirmed.

442. DETECTION AND DIFFERENTIATION OF LACTATE AND LIPIDS BY SINGLE VOXEL PROTON MR SPECTROSCOPY

Y. Kajiwara, F. Yamasaki, T. Saito, K. Sugiyama, K. Arita, and K. Kurisu; Department of Neurosurgery, Hiroshima University, School of Medicine, Hiroshima, Japan

In single voxel proton MR spectroscopy ([1]HMRS), lactate and lipids signals partially overlap, and it is sometimes difficult to differentiate lactate and lipids signals in clinical settings. Our aim in this study is to identify lactate and lipid by varying the TE, and we hypothesize that accurate detection of lactate and lipid has a powerful value in diagnosis of brain tumors. Between August 1999 and February 2004, [1]HMRS was performed, and meaningful spectra following in our protocol were obtained on 163 brain tumor patients (101 men and 62 women; mean, 44.3 years old). [1]HMRS is done with TE of 144 and then following TE of 30 and/or TE of 288 if necessary. Of the 163 patients, the level of lactate was "negative" in 31 patients, "positive" in 107 patients, and "strongly positive" in 25 patients. The level of lipids was negative in 68 patients, positive in 45 patients, and strongly positive in 50 patients. In glioneuronal tumors, lactate was detected in 54.5% of WHO grade 2 (positive 54.5%, strongly positive 0%), 93.1% of grade 3 (positive 86.2%, strongly positive 6.9%), and 100% of grade 4 (positive 66.7%, strongly positive 33.3%). Lipids were detected in 3.0% of WHO grade 2 (positive 3.0%, strongly positive 0%), 58.6% of grade 3 (positive 31.0%, strongly positive 27.6%), and 100% of grade 4 (positive 52.8%, strongly positive 47.2%). Detection rate of lactate was a significant factor for discriminating between WHO grade 2 and 3 ($P = 0.0239$) and grade 3 and 4 ($P = 0.0347$). The detection rate of lipids was a more significant factor to discriminate between WHO grade 2 and 3 ($P = 0.0073$) and between grade 3 and 4 ($P = 0.0048$) in glioneuronal tumors. With our varying TE method, it is possible to accurately and efficiently detect lactate and lipids in brain tumors. We showed a significant correlation between lactate/lipid expression and WHO grade in glioneuronal tumors.

443. QUANTIFICATION OF EARLY BRAIN TUMOR THERAPEUTIC RESPONSE USING FUNCTIONAL DIFFUSION MAPS

B.D. Ross,[1] B.A. Moffat,[1] T.L. Chenevert,[1] T.S. Lawrence,[2] C.R. Meyer,[1] T.D. Johnson,[3] L. Junk,[4] and A. Rehemtulla[2]; [1]Radiology, [2]Radiation Oncology, [3]Biostatistics, and [4]Neurology, University of Michigan, Ann Arbor, Michigan, USA

Assessment of the efficacy of radiation and chemotherapy for brain cancer patients is traditionally accomplished by measuring changes in tumor size several weeks after therapy has been administered. The ability to use noninvasive imaging during early stages of fractionated therapy to determine if a particular treatment will be effective would provide an opportunity to optimize individual patient management and avoid unnecessary systemic toxicity and expense. We investigated whether changes in the Brownian motion of water within tumor tissue as quantified by diffusion magnetic resonance imaging (MRI) could be used as an early, surrogate marker for early prediction of treatment response in brain cancer patients. Twenty brain tumor patients were examined by standard and diffusion MRI before initiation of treatment. Additional images were acquired at 3 weeks post-initiation of chemo- and/or radio-therapy. Images were co-registered to pretreatment scans, and changes in tumor water diffusion values were calculated and displayed as a functional diffusion map (fDM) for correlation with clinical response. Of the 20 patients imaged during the course of therapy, 6 were classified as having a partial response (PR), 6 as stable disease (SD), and 8 as progressive disease (PD). FDMs were found to predict patient response at 3 weeks from the start of treatment revealing that early changes in tumor diffusion values could be used as a prognostic indicator of subsequent volumetric tumor response. The use of fDM provides an early surrogate marker for predicting treatment response in brain tumor patients.

445. VALIDATION OF MOTOR PATHWAY TRACTOGRAPHY BY COMPARISON WITH HEMIPARESIS IN PATIENTS WITH INTRACRANIAL TUMORS

T.J.D. Byrnes, T.R. Barrick, B.A. Bell, and C.A. Clark; Clinical Neurosciences, St. Georges Hospital Medical School, London, UK

Radiation necrosis is a dose-related consequence of radiotherapy and necrosis within eloquent brain, including the motor pathways, causes permanent neurological deficits. Conventional magnetic resonance imaging gives limited information regarding the position of the white mater tracts, especially when displaced by tumor. Diffusion tensor imaging (DTI) tractography can demonstrate white matter tracts in vivo, and it has been shown that incorporation of tractography into treatment planning can

reduce radiation dosage to white matter tracts, which may reduce the risks of symptomatic radiation necrosis. Previously tractography required a large amount of user interaction, prior knowledge, and time, placing it beyond the scope of routine clinical practice. A new algorithm dramatically reduced the time required to perform white matter tractography and allows motor pathway segmentation with virtually no prior knowledge or interaction, using a single standardized seed point. White matter tractography has been validated by comparison with anatomical brain images, but there is a lack of in vivo functional validation. We present the results from 25 patients with intracranial tumors, 10 with a hemiparesis, comparing motor pathway segmentation with motor function. Between March and October 2004, 25 patients, 18 male and 7 female, with an average age of 48 (25–73) years, underwent DTI prior to treatment for their supratentorial tumors. Tractography was initiated from every voxel, and the geometry of the individual fibers from each voxel was computed. A single voxel was chosen bilaterally within the anterior medulla. The geometry of the fiber computed for that voxel was compared with every other fiber in the entire image and grouped together if they were statistically similar, to segment the motor pathways. There were 6 glioblastomas, 5 metastatic tumors, 6 grade 2 astrocytomas, 2 meningiomas, and 6 other tumor types. Ten patients had a clinically demonstrable hemiparesis. All of those patients with a hemiparesis were shown to have a clearly distorted and/or disrupted motor pathway. There were 3 patients who appeared to have normal motor function but had a reduction in the size of the motor pathways on tractography. This represents 100% sensitivity of motor tract disruption for hemiparesis with a specificity of 80%. This study has demonstrated a semiautomatic segmentation of the descending motor pathways with minimal user interaction or prior anatomical knowledge, bringing the technique closer to being practical within a clinical setting. We have shown that motor pathway disruption is highly sensitive to hemiparesis, validating motor pathway segmentation by tractography and clearly demonstrating its potential for radiotherapy treatment planning.

446. IS THERE AN ASSOCIATION BETWEEN LOSS OF HETEROZYGOSITY (LOH) ON CHROMOSOMES 1p/19q AND IMAGING FEATURES OF LOW-GRADE OLIGODENDROGLIOMAS (LGO)?

S. Lieberman,[1] I. Lavon,[2] J. Gomori,[1] and *T. Siegal*[2]; [1]Neuroradiology Unit, Department of Radiology; [2]Leselie and Michael Gaffin Center for Neuro-Oncology, Hadassah Hebrew University Hospital, Jerusalem, Israel

LGO are diagnosed by their histologic features. Genetic analysis has improved the distinction between subgroups of this tumor, and the combined allelic loss on chromosomes 1p and 19q was found to be predictive of favorable outcome. It is not known whether imaging features may correlate with the biologic behavior of the tumor as predicted by current chromosomal analysis. The objective of our study was to test the hypothesis that genetic profiles might be associated with typical imaging characteristics identified in LGO. We retrospectively reviewed the MRI studies of 31 patients with histologically verified oligodendroglioma (WHO II) whose median age was 39 years (range 21–61). MRI images were assessed for the following: rCBV grade, the presence of small cysts, the presence and relative extent of non-cystic regions of FLAIR hypointensity which are hyperintense on T_2, for location and for ^{1}H-MRS findings. A standard integral algorithm calculated the peak areas of NAA, Cho, Cr, Lac, and myoinositol, and each peak area was normalized for the Cr and the unsuppressed water peak. LOH on chromosomes 1p and 19q was evaluated in paired tumor-blood DNA samples, using PCR-based microsatellite analysis. LOH on chromosomes 1p/19q was found in 21 pts (68%) (group 1), and no deletions were observed in 10 pts (32%) (group 2). Data on rCBV was available for 29 pts. High rCBV were detected in 10/20 pts (50%) in group 1 and in 6/9 pts (67%) in group 2 (P = NS). Small cystic lesions were more common in group 1 (67%) than in group 2 (40%), but it did not reached statistical significance. Hypointense FLAIR, hyperintense T2 foci engaged more than 50% of the tumor cut surface in 19% of group 1 patients and in 50% of group 2 (P = NS). Frontal lobe location was less frequent in group 1 (48%) as compared with group 2 (7/10 pts [70%]), but it was not statistically significant. NAA, Cho, Cr, Lac and myoinositol ratios did not differ significantly between the two groups. MRI characteristics cannot differentiate reliably between subgroups of LGO when stratified according to their chromosomal profile that frequently predicts chemosensitivity. Yet, high rCBV was observed in 55% of pts with LGO, regardless of their 1p/19q status, and was not associated with an unfavorable disease course. There is some tendency for LGO with no loss on 1p/19q to have higher rCBV, less cystic lesions, and more extensive manifestation of hypointense FLAIR. Still, this requires a larger study to be verified.

447. PERFUSION-WEIGHTED IMAGING IN THE ASSESSMENT OF BRAIN GLIOMAS

C. Chaskis, A. Michotte, T. Stadnik, K. Van Rompaey, and J. D'Haens; Neurosurgery, Academisch Ziekenhuis, Vrije Universiteit Brussel, Brussels, Belgium

Because brain gliomas are histologically heterogeneous neoplasms, biopsy targeting must be based on adequate identification of malignant areas on imaging. Considering the reported differences in relative cerebral blood volume (rCBV) among tumor grades, we evaluated whether preoperative perfusion-weighted imaging (PWI) could improve the detection of areas bearing a higher yield for malignancy. We studied a series of 55 consecutive patients with newly diagnosed brain glioma. All patients underwent preoperative MR imaging and PWI. Surgery consisted of stereotactic biopsy in 29 cases and open craniotomy in 24 cases. Patients were followed up to 5 years after the diagnosis to evaluate their final survival. In our experience, PWI added to standard MR imaging improved the choice of targets for biopsy and the diagnostic accuracy, as confirmed by the clinical follow-up. Despite a less precise demarcation of the tumor borders, it allowed a better localization of its most malignant parts. Among the PWI parameters, we found that only maximal rCBV ratios were clinically useful. PWI was a valuable implement in the imaging assessment of brain gliomas, discriminating high- from low-grade tumors. PWI correlated significantly with the tumor grade and the final outcome ($P < 0.01$).

448. CORRELATION OF HYPOXIC CELL FRACTION WITH GLUCOSE METABOLIC RATE AND MARKERS OF ANGIOGENESIS AND PROLIFERATION IN GLIOMAS

L. Cher,[1] C. Murone,[4] S. Ramdave,[2] A. Hannah,[2] G. O'Keefe,[2] K. Pathmaraj,[2] H. Tochon-Danguy,[2] J. Sachinidis,[2] J. Chan,[2] S. Berlangieri,[2] G. Fabinyi,[3] and A. Scott[4]; Departments of [1]Oncology, [2]Nuclear Medicine and Centre for PET, and [3]Neurosurgery, Austin Health, Parkville, Victoria; [4]Ludwig Institute for Cancer Research, Tumor Targeting Program, Heidelberg, Victoria; Australia

In vitro studies suggest ^{18}F-fluorodeoxyglucose (FDG) uptake in tumor cells may reflect both aerobic and anaerobic glycolysis. ^{18}F-Fluoromisonidazole (FMISO) has been shown to selectively identify hypoxic but viable tissue, which may contribute to chemoradiotherapy resistance in gliomas. The aim of this study was to map and correlate tumor hypoxia with glucose metabolic rate, angiogenesis, and proliferation in patients with low- and high-grade gliomas. Seventeen patients with newly diagnosed primary brain tumors were studied prospectively with ^{18}F-FMISO and ^{18}F-FDG positron emission tomography (PET), and MP-RAGE MRI, prior to surgery. All FMISO and FDG PET images were co-registered with the MRI for stereotactic comparison of relative uptake. Biopsy samples at surgery were histologically examined for expression of GLUT3, Ki67, HIF1a, VEGF, VEGFR1, and microvessel density (MVD). In the 17 patients studied there were 7 grade IV, 3 grade III, 4 grade II gliomas, and 2 metastatic adenocarcinoma. ^{18}F-FMISO uptake was found to correlate with tumor grade and was often heterogeneous in the tumor. In high-grade gliomas ^{18}F-FMISO uptake overlapped regions of maximal ^{18}F-FDG uptake. Low-grade gliomas uniformly did not take up ^{18}F-FMISO and were negative on ^{18}F-FDG PET. GLUT3 expression correlated with ^{18}F-FDG uptake. Both ^{18}F-FMISO and ^{18}F-FDG uptake was associated with a significant increase in Ki67 and VEGFR1 markers in tumor tissue (^{18}F-FMISO, $P < 0.0001$ and < 0.0014; and ^{18}F-FDG, $P < 0.00001$ and < 0.0012, respectively). There was a trend for increased HIF1a expression with high ^{18}F-FMISO uptake. Expression of HIF1a was associated with increased Ki67, VEGF, and VEGFR1 and reduced MVD ($P < 0.05$). ^{18}F-FMISO uptake correlates with glioma tumor grade and ^{18}F-FDG uptake. Cellular maWrkers of hypoxia, proliferation, and angiogenesis are strongly associated with hypoxic cell fraction and glucose metabolic rate in gliomas. Further studies are planned to define the biology of hypoxia in high-grade gliomas and the predictive ability of ^{18}F-FMISO scans in glioma patients during therapy.

449. DIFFUSION-WEIGHTED MRI IN THE EARLY DIAGNOSIS OF MALIGNANT GLIOMA

J. Baehring,[1] W. Bi,[2] and J. Piepmeier[2]; [1]Neurology and Neurosurgery and [2]Neurosurgery, Yale University School of Medicine, New Haven, Connecticut, USA

The diagnosis of glioblastoma multiforme is based upon imaging findings and histopathological features that are usually readily identified in incisional or stereotacic biopsy specimens. A small subset of patients comes to medical attention before the masses show rim enhancement and central necrosis. As in those cases the tumors are typically located in eloquent areas of the brain, tissue diagnosis is obtained through stereotactic biopsy and not uncommonly is unsuccessful or does not allow for accurate grading. We

conducted this study in order to identify imaging characteristics of early stages of malignant gliomas. This is a retrospective analysis of patients with newly diagnosed malignant glioma seen on the Neuro-Oncology Service at the Yale Brain Tumor Center between 2002 and 2004. Patients with typical radiographic presentation (large rim-enhancing masses on T1-weighted MR images after administration of gadolinium) were excluded. Medical records and magnetic resonance imaging studies of patients were reviewed. Eight patients meeting the above inclusion criteria were identified. Diffusion-weighted imaging (DWI) showed areas of increased signal intensity in all cases. Low signal on apparent diffusion coefficient maps (ADC) indicative of restriction of proton diffusion in corresponding areas was present in six patients, whereas in the other two, ADC maps were not obtained. In five patients, patchy or small nodular enhancing lesions without central necrosis were identified. Biopsy was performed in six patients at the time of the above-described imaging findings. Diagnosis of a malignant glioma could only be established prior to further tumor growth in two cases. Glioblastoma can be a challenging diagnosis in a small subset of patients. Those may particularly benefit from early diagnosis and initiation of treatment, as tumors are frequently located in a functionally important area in which even minimal progression can give rise to substantial disability. Information obtained through DWI and ADC maps should be incorporated in the clinical decision-making process. For patients with masses displaying restricted proton diffusion indicating high cellularity, even in the absence of contrast enhancement, a biopsy should not be delayed.

450. PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA – VOLUMETRIC ANALYSIS OF RESPONSE TO CHEMOTHERAPY: IS THERE PREDICTIVE VALUE TO A "RESPONSE NOMOGRAM"?

M.M. Mrugala,[1] S. Schreiber,[1] C.B. Knobbe,[1] A. Muzikansky,[3] G.J. Harris,[2] J. Rabinov,[2] and F.H. Hochberg[1]; [1]Stephen E. and Catherine Pappas Center for Neuro-Oncology, [2]Radiology, and [3]Biostatistics Center, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Primary central nervous system lymphoma (PCNSL) is a non-Hodgkin's B-cell lymphoma. Over 52% of patients achieve a complete response (CR) following receipt of parenteral high-dose methotrexate (HD-MTX). We provide HD-MTX (8 g/m^2) every 14 days until a complete response (CR) is achieved or disease progression is observed. Of importance to us is the separation of early responder patients from those who will not achieve CR with this therapy. We have been unable to use as predictors of CR the following variables: age, performance status at diagnosis, or MTX pharmacokinetics. The development of a "response nomogram" would provide early identification of patients likely to achieve a CR and to separate these individuals from those with either progressive disease (PD) or partial response (PR) for whom phase 1/2 trials would be most appropriate. We performed a retrospective analysis of 20 patients whose PCNSL completely responded to MTX in the absence of XRT or other chemotherapeutic agents. Ten male and 10 female immunocompetent patients with median age at diagnosis of 64.5 years were included. All patients received MTX therapy, in the setting of diminishing steroid dosing, for measurable disease (MRI-T1 gadolinium-enhancing masses). Contrast-enhanced MRI scans were performed prior to treatment and thereafter every 2 cycles. Tumor volumes (T1 gadolinium-enhanced images and FLAIR images) were calculated by using Vitrea2 software and a semi-automated edge detection system. The median baseline, pretreatment, T1 post-gadolinium-enhancing tumor volume was 7.28 cm^3, and median baseline FLAIR volume was 64.49 cm^3. At CR the enhancing tumor volume was 0 and the median FLAIR volume was 29.52 cm^3. Twenty percent of patients achieved CR after 4 cycles, a further 15% after 3 cycles, and an additional 15% after 5 cycles. Ten percent achieved CR following 6, 7, or 8 cycles. The remaining 5% of completely responsive patients required 9 to 12 cycles to achieve remission. The median time to achieve 50%, 75%, and full reduction of the enhancing tumor volume from the start of therapy was 1 cycle (14.5 days), 2 cycles (28 days), and 5.5 cycles (76 days), respectively. We present (A) the relationship of baseline tumor volume to therapeutic response, (B) the relationship between response criteria based on T1-gadolinium and FLAIR volumes, and (C) correlates of MRI volumetric response including initial tumor volume, number of lesions, and patient age. We conclude that construction of a nomogram, based on volumetric analysis, can permit (A) rapid identification of non-responsive patients who will be eligible for early-phase drug trials and (B) identification of populations of MTX-responsive and non-responsive patients as the basis for pharmacogenomic and chromosomal studies of drug response correlates.

451. SYSTEMATIC REVIEW OF THE DIAGNOSTIC ACCURACY OF THALLIUM-201 SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY IN THE DETECTION OF RECURRENT GLIOMA

M. Vos,[1] B. Tony,[2] O. Hoekstra,[2] T. Postma,[1] J. Heimans,[1] and L. Hooft[3]; [1]Neurology, [2]Nuclear Medicine, and [3]Clinical Epidemiology and Biostatistics, VU Medical Centre, Amsterdam, The Netherlands

The aim of this study was to determine the diagnostic accuracy of thallium-201 single-photon emission computed tomography (201Tl SPECT) in the detection of tumor recurrence in patients with previous radiotherapy for supratentorial glioma. The databases of PubMed and Embase were searched for relevant studies. Two reviewers independently selected and extracted data on study characteristics, quality, and accuracy of studies. Studies were included if they comprised at least six eligible patients, who underwent 201Tl SPECT (index test) and in whom (histo)pathological confirmation (reference test) of the suspected brain lesion was obtained. Because of the methodological and statistical heterogeneity of the included studies, a quantitative meta-analysis was not performed. Instead, for every individual study, the sensitivity, specificity, and diagnostic odds ratio (DOR) of 201Tl SPECT was calculated. Eight studies met the inclusion criteria. Only one study was considered of high methodological quality. Methodological limitations referred most notably to blinding and patient selection. The DOR was greater than 1 in all included studies, with a broad range (2.1–350.9) and relatively wide 95% confidence intervals. The sensitivity of 201Tl SPECT ranged from 0.43 to 1.00, and the specificity from 0.25 to 1.00. We conclude that 201Tl SPECT is a valuable method in the detection of tumor recurrence in patients treated with radiotherapy for supratentorial glioma. However, the evidence is not very robust because of the low quality and high heterogeneity of the studies included. Future studies are warranted to further explore the diagnostic potential of 201Tl SPECT, and to determine optimum thresholds for the detection of glioma recurrence.

452. TAURINE DETECTED BY 1H MAGNETIC RESONANCE SPECTROSCOPY IS INDICATIVE OF SUPRATENTORIAL PRIMITIVE NEUROECTODERMAL TUMORS (PNETS)

H.G.J. Krouwer,[1,2] R.W. Prost,[3] W.M. Mueller,[2] G.P. Sinson,[2] C.J. Schultz,[4] and K.-C. Ho[5]; Departments of [1]Neurology, [2]Neurosurgery, [3]Radiology (Neuroradiology), [4]Radiation Oncology, and [5]Pathology (Neuropathology), Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Proton (1H) magnetic resonance spectroscopy (MRS) is a noninvasive imaging technique to characterize intracranial lesions. With this modality, medulloblastomas have recently been found to demonstrate elevated taurine levels. Taurine is a non-proteinogenic amino acid present in high concentrations in fetal neural tissues and decreasing thereafter. We extend this observation to include other primitive neuroectodermal tumors (PNETs) with 2 cases of disseminated pineoblastomas. Spectra were acquired on a 0.5 T clinical system with a quadrature transmit/receive head coil using an elliptical excitation chemical shift imaging (CSI) sequence with TE = 46 ms and TR = 1000 ms. Taurine resonance was detected at 3.36 ppm. This resonance becomes more detectable at 0.5T because of the collapse of its complicated resonance in a manner similar to what has been shown for glutamate (Prost et al., Magn. Reson. Med. 37, 615, 1997). Two male patients, 32 and 47 years of age, presented with pineal region masses, both with extensive leptomeningeal dissemination (4th ventricle and anterior cervical spinal cord at C3-C4; auditory internal canals bilaterally, cerebellar folia, along cervical and thoracic spinal cord and conus medullaris, as well as all nerve roots) as assessed by conventional imaging studies. In both, beta-HCG levels in CSF were slightly elevated, and AFP levels in CSF were normal. MRS studies demonstrated, in addition to the resonances assigned to myo-inositol, choline, creatine, *N*-acetylaspartate, glutamine/glutamate, and lipid/lactate, a resonance at 3.36 ppm, compatible with taurine. Histopathological specimens obtained from a drop metastasis at T10-T11, and from the 4th ventricular mass, respectively, showed pineoblastoma. These findings support the hypothesis that detection of taurine by 1H-MRS in an intracranial mass, supra- or infratentorial, may indicate a PNET histology. Whether taurine as detected with the 0.5 T system is indeed specific for PNETs requires further study.

453. COMPARISON OF GENOTYPE, HISTOPATHOLOGICAL GROWTH PATTERNS, AND THE MR IMAGING CHARACTERISTICS OF OLIGODENDROGLIAL NEOPLASMS

M. Jenkinson,[1] D. du Plessis,[1] E. Smith,[2] K. Joyce,[3] P. Warnke,[1] and C. Walker[3]; [1]Neuroscience, University of Liverpool, Liverpool; [2]Neuroradiology, The Walton Centre for Neurology and Neurosurgery, Liverpool; [3]Molecular Biology, Clatterbridge Cancer Research Trust, Bebington; UK

Oligodendroglial neoplasms with loss of chromosomes 1p and 19q are recognized to be more indolent with prolonged survival and increased responsiveness to therapy compared with their morphologically equivalent counterparts with intact 1p/19q. However, the biological basis of these clinical differences is not known. Recent research suggests that genotype influences the magnetic resonance (MR) imaging characteristics of oligodendroglial neoplasms, supporting the hypothesis that these tumors may differ in their growth characteristics. The aim of this study was to examine the histopathological growth pattern and MR imaging characteristics in oligodendroglial neoplasms classified by genotype. Tumor imaging features were assessed on T1 (±gadolinium) and T2-weighted axial images: (1) sharp versus indistinct border, (2) smooth versus irregular contour, (3) homogenous versus heterogenous signal intensity, and (4) contrast enhancement, present versus absent. The histopathology of each case was assessed for calcification and the growth pattern (solid, mixed, infiltrative). In the 45 cases diagnosed with CT-guided serial stereotactic biopsy, the position of each sample was recorded in relation to the biopsy tract, enabling characterization of transitions in cellularity within the tumor margin. Allelic imbalance in chromosomes 1p36 and 19q13 was determined. Eighteen oligodendrogliomas (14 with 1p/19q loss) and 35 oligoastrocytomas (12 with 1p/19q loss) were investigated. Intact 1p/19q alleles correlated with a sharp/smooth border in the whole group and grade II tumors (Fisher exact: $P = 0.037$ and $P = 0.007$, respectively). Of the 7 cases with a sharp/smooth border, 6 had intact 1p/19q. WHO grade III tumors were more likely to have a solid growth pattern and WHO grade II tumors a mixed or infiltrative growth pattern (χ^2: $P = 0.001$). Tumor genotype did not correlate with growth pattern or transition in cellularity, but correlated with calcification (Fisher exact: $P = 0.031$). Solid and mixed tumor growth patterns were associated with contrast enhancement, and infiltrative growth with no contrast enhancement (χ^2: $P = 0.008$). All tumors with a homogenous signal on T2 ($n = 4$) had a mixed growth pattern (χ^2: $P = 0.041$). A sharp T1, but not T2 tumor border was associated with sudden transition in cellularity (Fisher exact: $P = 0.048$). In the absence of genetic testing, the observation that oligodendroglial tumors with a sharp/smooth MRI border are more likely to have intact 1p and 19q may be diagnostically useful. Growth pattern, although related to contrast enhancement, was not associated with MR tumor border appearance or genotype. These data suggest that growth characteristics are unlikely to contribute to the differences in clinical behavior of oligodendroglial neoplasms with or without loss of 1p and 19q.

454. IN VIVO MAGNETIC RESONANCE IMAGING OF β-GALACTOSIDASE IN A RAT C6 GLIOMA MODEL

S. Assadian,[1,2] B.J. Bedell,[2,3] S. Mzengeza,[2,4,5] A.C. Evans,[2,4,5] and R.F. Del Maestro[1,4,5]; [1]Brain Tumor Research Center, [2]McConnell Brain Imaging Center, and Departments of [3]Pathology, [4]Neurology, and [5]Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Canada

With recent developments in tumor-targeted gene therapy, it has become increasingly important to monitor the successful delivery of the gene to the tumor. Current methods for determining reporter gene expression suffer from serious limitations, including the invasive acquisition of tissue and the inability to perform longitudinal studies on individual subjects. The potential to assess reporter gene expression using high-resolution, non-invasive, in vivo imaging techniques would abrogate these limitations. As such, we have developed a novel MRI probe which allows for in vivo measurement of the expression of β-galactosidase (β-gal), which is commonly used as a reporter of gene expression. Upon activation by tissue-localized β-gal, the probe increases the local blood-tumor permeability. Quantitative measurement of the change in blood-tumor permeability, via the determination of extravasation of Gd-DTPA (Magnevist) into the tumor, provides an estimate of relative β-gal expression. In this study, we have evaluated the ability of this probe to measure β-gal expression in a rat intracerebral C6 glioma model in which tumor cells have been transfected to secrete β-gal into the extracellular space. C6 glioma cells were transfected in vitro with the *lacZ* gene following an Igk secretion tag. Spheroids of the transfected cell line were implanted into cerebral hemispheres of male Sprague-Dawley rats. Animals implanted with non-transfected spheroids served as the control group. Two weeks post-implantation, the animals were imaged using a Siemens 1.5 T whole-body MRI scanner. T1-weighted coronal images were acquired following intravenous administration of probe and Gd-DTPA. Following completion of imaging, the animals were sacrificed and brains snap-frozen in isopentane. X-gal staining was performed on cryostat sections to confirm the expression of β-gal. MR data was processed using software developed in-house to generate quantitative maps of β-gal expression. Quantitative MR image maps successfully demonstrated the β-gal expression within the transfected tumors, but not the control tumors. The secretion of β-gal by the transfected tumor was confirmed by X-gal staining of the corresponding cryosections. We have successfully imaged the expression of β-galactosidase in vivo in a rat C6 glioma model using MRI. Our probes will provide a powerful means for assessing the efficacy of in vivo gene transfer and can also be manipulated to image the expression of other proteins involved in targeted therapies of tumors.

455. CT-PERFUSION IN PRIMARY AND SECONDARY BRAIN TUMORS: PRELIMINARY EXPERIENCE

A. Vidiri, A. Mirri, A. Fabi, S. Telera, A. Pace, M. Carosi, F. Portieri, *C. Carapella*, and M. Crecco; Regina Elena National Cancer Institute, Rome, Italy

CT-perfusion has been used to evaluate microvascular characteristics of brain tumors and monitoring treatment effects. In the present study, CT-perfusion has been performed with Multisections-CT (Volume Zoom Siemens Medical Solutions); the images are post-processed in a dedicated workstation, which yields the maximum intensity projection (MIP) images and the maps of cerebral blood volume (CBV), cerebral blood flow (CBF), and perfusion permeability (PTC). This technique has been presently used in 18 patients affected by brain metastases and 4 by malignant gliomas, mainly with the goal of differentiating between radiation necrosis and tumor recurrence; the results have been related with FDG PET and SPECT scans and subsequent surgical specimens. In some cases the tumors have been studied before and after radiotherapy for defining tumor vascularization, and correlating it with treatment-induced variations in tumor volume. Preliminary data show good correlation between CT-perfusion and PET-SPECT results, mainly in detecting the presence of radiation necrosis in metastatic lesions submitted to stereotactic radiotherapy; in 3 patients affected by metastatic lesions (two from breast cancer and one from melanoma) the CT-perfusion showed the presence of the tumor in agreement with surgical reports; in one case, CT-perfusion and SPECT allowed early documentation of relapse in a patient affected by GBM who had had previous surgery and chemoradiation therapy. MR-perfusion is the gold standard in the evaluation of brain vascularization; however, CT-perfusion represents an easy technique with short acquisition and post-processing time. This technique is useful for patients for whom MR is not suitable.

456. THE ROLE OF 111 INDIUM-OCTREOTIDE BRAIN SCINTIGRAPHY IN THE DIAGNOSIS OF MENINGIOMAS FROM OTHER CRANIAL BASED DURAL LESIONS

N. Nathoo,[1] K. Ugokwe,[1] A. Chang,[2] L. Li,[3] J. Ross,[2] J. Suh,[4] M. Vogelbaum,[1] and G. Barnett; Departments of [1]Neurosurgery, [2]Neuroradiology, [3]Biostatistics and Epidemiology, and [4]Radiation Oncology, The Taussig Cancer Center and Brain Tumor Institute, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

Meningiomas are common extra-axial brain tumors with somatostatin receptors that bind octreotide. We report the use of 111 indium-octreotide brain scintigraphy (ObS) in the non-invasive differentiation of meningiomas from other cranial dural-based pathology. A retrospective analysis of our experience with ObS in the non-invasive differentiation of meningiomas was performed. A neuroradiologist, blinded to the clinical data, used a standardized grading scheme to define the uptake of octreotide and the pattern of enhancement performed at 6 and 24 h postadministration. Correlation of ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), MRI scans, and octreotide uptake was performed. The cohort consisted of 49 patients (34 female; 15 males) with a mean age 62.4 years and a median follow-up of 24 months. Management consisted of biopsy ($n = 3$), resection ($n = 10$), observation ($n = 15$), and radiation therapy (radiosurgery $n = 21$; external beam $n = 3$). ObS was correlated with FDG-PET brain studies ($n = 37$), histology ($n = 13$), and angiography ($n = 1$). The sensitivity, specificity, accuracy, and positive predictor value for ObS were 100%, 60%, 85%, and 80%, respectively. The test successfully differentiated meningiomas from a dural-based venous anomaly except for 2 false patients (metastasis; chronic inflammation). The addition of PET did not improve the specificity of ObS to detect meningiomas. Correlation of ObS vs. MRI ($P < 0.001$), MRI vs. PET ($P = 0.0218$), and octreotide vs. PET ($P = 0.0071$) revealed a significant relationship. Octreotide scintigraphy together with FDG-PET scanning increases the diagnostic specificity of conventional neuroimaging when differentiating meningioma from other dural-based pathology.

457. SENESCENCE, AUTOPHAGY, MORPHOLOGICAL PLASTICITY, AND SURVIVAL OF GLIOMA CELLS EXPOSED TO IONIZING RADIATION

W. Dos Santos, H. Fillmore, and W.C. Broaddus; Neurosurgery, Virginia Commonwealth University, Richmond, Virginia, USA

Resistance to ionizing radiation (IR) and apoptosis is one of the key factors contributing to the poor prognosis of patients with glioblastoma. However, the mechanism underlying this resistance remains poorly understood. Two approaches were used in this study to better identify relevant events involved in radioresistance of glioma cells. First, glioma cells with different p53 status (U87 wt, T98 mut, LN-Z308–null) were distributed into 100-mm plates, in triplicate. Cells were treated with single doses of IR (6 Gy), administered five consecutive times every six days. For each time, samples were separated for protein extraction and further maintenance in culture prior to analysis. A second approach aimed to select highly resistant cells. Thus, cells were treated with high-dose radiation (60 Gy), administered twice within a period of 6 days. Cells were analyzed for morphological changes by optical and electronic microscopy; proliferation rate and viability were assessed by MTS assay and trypan blue; IR resistance was monitored by clonogenic assay; changes in cell cycle were determined by flow cytometry, and alterations in protein profile followed by Western blot and immunocytochemistry. Antibodies to both p53 and p53-induced proteins, as well as to differentiation markers and radiation resistant-related proteins, were used. U87 glioma cells (wt p53) were more radioresistant than T98G (mut p53). Exposure to consecutive doses of 6 Gy IR decreased proliferation rate in a dose-dependent manner. However, with continuous passage, irradiated cells were able to resume their normal rate of proliferation comparable to cells that did not receive IR. Resistant U87 cells exposed to high-dose IR exhibited a striking change in morphology characterized by increase in size (10–15-fold larger than untreated control), increase in cytoplasm vacuolization that preceded the formation of neurite-like projections and aneuploidy. These cells entered into a senescence state and survived for at least one year with reduced proliferation. At the molecular level, resistant U87 cell population showed an increased expression of p53 and $p21^{cip/waf}$ protein, decreased expression of the undifferentiated marker nestin, reduced levels of Rb protein but increased levels of survivin. These cells also showed aberrant expression of the neuronal marker beta III tubulin in addition to the astrocytic marker GFAP. In contrast, T98 cells showed no alteration in the levels of these proteins but did not survive as long as U87. In conclusion, our results suggest that radiation resistance may be in part associated with the plasticity of some glioma cells to undergo senescence and terminal differentiation followed autophagy. Furthermore, the p53 and Rb pathway with participation of DNA repair and anti-apoptotic proteins such as survivin are important players in radioresistance.

458. BIOLOGICAL EFFECTS OF PROTON BEAM IRRADIATION ON GLIOMA CELL LINES IN VITRO

A. Gee,[1] A. Kacperek,[2] D. Spiller,[4] E. Shaw,[1] J. Dunn,[1] P. Warnke,[3] and C. Walker[1]; [1]Clatterbridge Cancer Research Trust, JK Douglas Laboratory, Bebington; [2]Clatterbridge Centre for Oncology, Douglas Cyclotron, Bebington; UK

Gliomas are considered to be one of the most radioresistant and least curable tumors, and although many patients show an initial response to radiotherapy, the majority recur within or adjacent to the target volume. Proton therapy used in the treatment of some gliomas enables higher doses of conformal irradiation to be given, while sparing critical normal tissue, and offers slightly better linear energy transfer and radiobiological effects than photons. However, cellular responses to protons have not yet been extensively investigated. In this study, four glioma cell lines with differing molecular genetic characteristics were used to investigate the in vitro biological effects of proton irradiation. Cells plated in monolayers at 5×10^4 cells per 35-mm dish were positioned in the modulated Bragg peak and received 0, 2, 4, 6, 8, or 10 Gy proton irradiation (60 MeV) at a dose rate of 30 Gy min^{-1}. Post-irradiation, Annexin-V and propidium iodide were added to the media to assess apoptosis, and time-delay confocal microscopy was performed over 65 h with an image recorded every 15 min. Flow cytometric analysis was performed 65 h post-irradiation. Monolayer cultures were maintained post-irradiation to determine whether populations surviving sequential fractions of proton irradiation could be established. T98G with mutant p53 was the most resistant cell line, with little change in proliferation and apoptosis and cells accumulating in G_1 of the cell cycle following 10 Gy irradiation. In contrast, U373, also with mutant p53, showed complete inhibition of proliferation, and cells accumulated in G_2M after 65 h. U-87 MG with wild-type p53 showed a similar cell cycle distribution and rate of apoptosis to the unirradiated control, yet proliferation was inhibited. Hs683 ceased proliferating and demonstrated the greatest level of apoptosis, with a 9-fold increase in Annexin-V staining at 65 h post-irradiation, and surviving cells accumulated in G_1 of the cell cycle. All cell lines produced populations that survived a single fraction of 10 Gy protons. Surviving populations were established following 2×10 Gy for U-87MG and 3×10 Gy for T98G. Expression profiling using oligonucleotide microarrays to investigate differential gene expression in these cell lines in response to irradiation is currently being undertaken. The four cell lines with their different molecular genetic profiles showed very different responses to proton beam irradiation, suggesting that depending on their genetic subtype, gliomas may display differential intrinsic radiosensitivity. Through expression profiling we aim to identify factors that contribute to radiation resistance.

459. GROWTH DELAY OF HUMAN GLIOBLASTOMA MULTIFORME SPHEROIDS AFTER THERMAL NEUTRON IRRADIATION WITH GADOLINIUM (GDNCT) OR BORON (BNCT)

R. Ciftci,[1] J.J. Hofstra,[1] F. Stecher-Rasmussen,[2] K. Franken,[1] R. Kuperus,[2] R. Huiskamp,[2] J. Haveman,[1] and L. Stalpers[1]; [1]Radiotherapy, Academic Medical Center, Amsterdam; [2]Nuclear Research Group (NRG), Petten; The Netherlands

The survival of patients with GBM continues to be dismal despite various modalities of treatment. Neutron capture therapy (NCT) is an experimental treatment modality: Following thermal neutron irradiation of nuclear elements with a high propensity to capture neutrons, such as boron-10 (^{10}B) or gadolinium-157 (^{157}Gd), a high dose of secondary radiation is released according to the following nuclear reactions.

$^{157}Gd + nth$	à $^{158}Gd + g + e^-$	Q = 7.94 MeV
$^{10}B + nth$	à $^{4}He + {}^{7}Li + g$	Q = 2.79 MeV

Certain compounds, such as boron phenylalanine fructose (BPA-F) and Gd-DTPA, can accumulate locally in a brain tumor. Neutron irradiation of a patient receiving boron (BNCT) or gadolinium (GdNCT) may reach a tumoricidal local irradiation. In this study we investigated if the theoretical effectiveness of BNCT and GdNCT works in the in vitro GBM tumorspheroid model. Multicellular spheroids of the human GBM cell line Gli-6 were cultivated up to a diameter of about 300 microns. Clinically achievable concentrations of gadolinium-DTPA (Magnevist; 2.5 mmol) and BPA-F (30 ppm) were used as neutron capture agents. The spheroids were irradiated with thermal neutrons in a dose of 0.3 Gy and 0.6 Gy at the Low Flux Reactor (NRG, Petten) or with cesium-137 X rays (photons) up to 8 Gy (AMC, Amsterdam). The size of the spheroids was measured for some weeks. Irradiation of spheroids with X-ray-photons shows a dose-dependant growth delay; Gd-DTPA or BPA-F did *not* alter the tumor response to X rays. Irradiation of spheroids with thermal neutrons gives a dose-dependant growth delay. The radiobiological effectiveness (RBE) of thermal neutrons is about 5- to 10-fold that of X-ray photons; combination with Gd-DTPA or BPA-F caused a significant prolonged growth delay. The prolongation is somewhat more pronounced in Gd-DTPA treated spheroids, both after 0.3 Gy neutron irradiation, and more pronounced after 0.6 Gy neutron irradiation. Both Gd and B, at a clinically feasible concentration, produce a strong growth delay of GBM spheroids after neutron irradiation. This radiosensitization by Gd and B was, as expected, not observed after spheroids to photon irradiation. The NCT-effect of Gd cannot be explained by the release of secondary Auger electrons (as these do not penetrate more than 1 or 2 cell layers), and should be attributed to the secondary release of longer range photons and nuclear conversion electrons.

460. ABSCOPAL EFFECT ON SUBCUTANEOUSLY IMPLANTED N29 RAT GLIOMA TUMORS, FROM CONTRALATERAL TREATMENTS WITH PULSED ELECTRIC FIELDS, RADIATION THERAPY, AND IMMUNIZATION WITH SYNGENEIC INTERFERON-GAMMA-SECRETING TUMOR CELLS

B.R.R. Persson,[1] C. Bauréus Koch,[1] G. Grafström,[1] C. Ceberg,[1] B. Widegren,[2] and L.G. Salford[3]; Departments of [1]Radiation Physics, [2]Tumor Immunology, and [3]Neurosurgery, Rausing Laboratory, Lund University, Lund, Sweden

Effects of radiation therapy on cancer tumors outside the radiation field are a phenomenon known as the abscopal effect. The aim of the present study is to study the effect of immunization with syngeneic interferon-gamma (IFNg)-secreting cells on abscopal regression of contralateral, subcutaneously implanted rat glioma tumors treated with radiation therapy in combination with pulsed electric field treatment. The study was performed on rats of the Fischer-344 strain with rat glioma N29 tumors implanted subcutaneously on the flank or on both the right treated hind leg and the left untreated hind leg. Pulsed electric field with 16 exponentially decaying pulses with a maximum electric fields strength of 1300 V/cm and $t_{1/e} = 1$ ms were first applied to the tumors. Then within 5 min radiation treatment was given in daily fractions of 5 Gy, to a total absorbed dose of 20 Gy. The animals were arranged into one group of controls and 3 groups of various

treatments: pulsed electric fields only (PEF), radiation therapy (RT) with ^{60}Co-gamma radiation only (5 Gy per session), or combination of PEF and RT. Tumors were treated 4 consecutive days at about 4 weeks after inoculation, when a solid tumor has grown to a diameter of 1 to 1.5 cm. Once weekly for three weeks, the animals were given intraperitoneal injections of irradiated, modified N29 tumor cells, secreting IFN-g. The abscopal effect was evaluated by comparing the growth rate of the untreated contralateral tumors with the treated tumors. Fitting the data obtained from consecutive measurements of tumor volume (*TV*) of each individual tumor to an exponential model $TV = TV_0 \times exp[TGR \times t]$ estimated the tumor growth rate (*TGR %per day*) after the day of treatment (t = 0). In total, 5 experimental series were performed with total number of 141 rats, of which 45 were controls and the number of animals in each treatment group was around 30. The *TGR* of both types of tumors treated with the combination of PEF and RT are significantly decreased compared to the controls. The tumor growth rate for both types of tumors is significantly decreased by combined treatment with PEF and RT compared to RT alone. There was no significant difference in contralateral tumor growth in animals without treatment and those treated with IFN-g-secreting cells only. But a significantly decreased growth rate of the untreated tumor was observed in animals given PEF and RT combined with IFN-g-secreting cells. These results indicate that the most pronounced abscopal effect was achieved using pulsed electric fields and radiation therapy combined with immunization using syngeneic tumor cells.

461. CORRELATIVE MR IMAGING WITH GBM CANCER GENOMICS

M. Kuo,[1] C. Nardini,[2] S. Cha,[3] and M. Diehn[4]; [1]Radiology, University of California San Diego, San Diego, California, USA; [2]Computer Science, University of Bologna, Bologna, Italy; [3]Radiology, University of California San Francisco, San Francisco, California, USA; [4]Medicine, Stanford University, Stanford, USA

Glioblastoma multiforme (GBM) is characterized by genetic instability and an extremely variable appearance on magnetic resonance imaging (MRI). We sought to determine whether characteristic GBM imaging defining features seen on MRI could capture significant alterations in intrinsic gene expression signatures identified by gene expression microarrays. Four imaging parameters were defined a priori to reflect fundamental GBM-defining MR imaging characteristics (degree of contrast enhancement, necrosis, T2 heterogeneity, mass effect). MR images (T1, T2, contrast enhanced) of 20 GBMs in 20 patients were evaluated and scored across these 4 parameters by 2 radiologists in consensus and without knowledge of the matching GBM genomic information. Unsupervised and supervised analyses were performed for correlation of the imaging parameters against the corresponding matched GBM microarray data (each microarray containing 23,000 clones) to identify relationships between imaging traits and tumor gene expression. Four of four imaging traits demonstrated statistically significant correlations with large-scale alterations in gene expression: degree of contrast enhancement ($P < 0.01$), necrosis ($P < 0.01$), T2 heterogeneity ($P < 0.01$), and mass effect ($P < 0.005$). Further, there was significant enrichment of genes identified by 3/4 imaging traits in several fundamental GBM gene expression signatures: Degree of contrast enhancement was enriched for the hypoxia/angiogenesis ($P = 3.9 \times 10^{-43}$), extracellular matrix ($P = 1.6 \times 10^{-48}$), and immune infiltration ($P = 9.1 \times 10^{-56}$) signatures, and necrosis and mass effect were both enriched for the proliferation signature ($P = 1.6 \times 10^{-14}$ and $P = 3.2 \times 10^{-51}$, respectively). Radiogenomic evaluation of GBM on MRI can potentially co-localize several characteristic imaging findings to fundamental GBM gene expression signatures and may provide plausible insights into GBM tumor biology based on imaging alone.

462. IMAGING PRIMARY BRAIN TUMORS WITH [18F]-3-FLUORO-AMINOCYCLOBUTANE CARBOXYLIC ACID [FACBC] PET: A PILOT STUDY

A. Demopoulos, T. Akurst, B. Beattie, J. Pena-Gomez, H. Schoder, N. Pillarsetty, S. Larson, R. Finn, and R. Blasberg; Neurology, Memorial Sloan Kettering Cancer Center, New York, New York, USA

In the management of primary brain tumors, new or expanding lesions observed on MRI may represent recurrent tumor, treatment effect, or necrosis. ^{11}C-methyl-methionine (^{11}C-MET) PET imaging has several advantages over [18F]-fluoro-2-deoxyglucose PET, but is confounded by the presence of radiolabeled metabolites. Logistically, ^{11}C is more challenging because of a short half life (20 min vs. 110 min for ^{18}F). ^{11}C requires on-site production and is less widely available. For these reasons and substantial cost savings, 3-fluoro-aminocyclobutane carboxylic acid (18F-FACBC) PET studies may be superior to both [18F]-fluoro-2-deoxyglucose and ^{11}C-MET PET. We report interim results of a pilot study evaluating dosimetry and imaging characteristics of ^{18}F-FACBC in comparison to ^{11}C-MET PET. Patients with previously treated primary brain tumors and a suspicion for recurrent or progressive disease were included. All patients had ^{18F}-FACBC, ^{11}C-MET PET, and recent MRI with gadolinium. Because of the shorter half-life, ^{11}C-MET PET imaging was performed first, followed by ^{18}F-FACBC imaging 2 h later. ^{18}F-FACBC brain and body dosimetry information were collected at t = 0 (brain only), 1, 2, and 3 h. Blood and urine sampling was performed for radioactivity concentration and metabolite analysis via HPLC. MRI coregistration was performed in all patients. Descriptive SUVmax statistics were recorded and compared with tissue pathology and clinical outcome, where available. In this small series, we found good concordance between ^{11}C-MET and ^{18}F-FACBC PET imaging. Isotope localized to tumor as visualized on MRI. Uptake of ^{18}F-FACBC was delayed in non-contrast enhancing regions. No metabolites were detected in blood or urine. In conclusion, ^{18}F-FACBC PET imaging is a promising and cost-effective imaging modality for primary brain tumors. Larger studies will further delineate its role in the management of these difficult neoplasms.

463. IMAGING CORRELATES OF MOLECULAR SIGNATURES IN OLIGOASTROCYTOMAS

M. Eoli, C. Maccagnano, L. Bissola, A. Silvani, B. Pollo, T. De Simone, D. Bianchessi, A. Boiardi, G. Finocchiaro, and M. Bruzzone; Clinical Neuro-Oncology, Neurological Institute C. Besta, Milan, Italy

In order to improve therapeutic management of oligoastrocytomas (OA) we investigated the relationship between magnetic resonance imaging (MRI) features on the pre-operatory scan, the histopathological diagnosis, and the genetic signatures of these tumors. Sixty-two gliomas were classified by histological analysis as grade II (n = 31) or grade III OA (anaplastic OA, AnOA; n = 31). Loss of heterozygosity (LOH) with different microsatellite markers was studied on chromosomes 1p, 10q, 17p, and 19q. On each MRI examination the following parameters were analyzed: location of the tumor, sharpness of tumor border on T1-weighted images, homogeneity of the tumor signal on T1 and T2 weighted images, mass effect and presence of contrast enhancement. In a series of 62 cases we observed that 1p and/or 19q LOH correlates with homogeneous T1 and T2 appearance (25/38, $P = 0.008$) and with decreased frequency of temporal location (3/38, $P = 0.002$). Indistinct border, lack of T1 and T2 homogeneity, and presence of enhancement were significantly associated with LOH on 10q ($P < 0.001$). These findings indicate that molecular alterations associated with cancer may confer physical or biochemical characteristics to the tumor that can be imaged. In oligoastrocytomas, LOH on 10q, a marker of astrocytoma progression, correlates well with MRI findings of high-grade gliomas (indistinct border, T1 and T2 lack of homogeneity, and presence of enhancement). Genotypic assessment, in addition to MRI and histological evaluation, may improve the management of patients with oligoastrocytomas.

464. IMAGING TUMOR PROLIFERATION: VALIDATION OF 3'-DEOXY-3'-[F-18]FLUOROTHYMIDINE (FLT) POSITRON EMISSION TOMOGRAPHY IN CEREBRAL GLIOMAS

S. Price,[1] D. Wang,[2] T. Fryer,[2] A. Dean,[5] P. Hutchinson,[1] N. Burnet,[3] J. Pickard,[1] and J. Gillard[4]; [1]Academic Neurosurgery Unit, [2]Wolfson Brain Imaging Centre, and Departments of [3]Oncology and [4]Radiology, University of Cambridge, Cambridge; [5]Department of Neuropathology, Addenbrooke's Hospital; Cambridge; UK

Uncontrolled cellular proliferation is one of the cardinal features of malignant tumors. 3'-Deoxy-3'-[F-18]fluorothymidine (FLT) is a compound that is taken up into dividing cells and phosphorylated by thymidine kinase 1 (TK1), which leads to intracellular trapping. FLT activity is therefore a measure of cellular TK activity and hence cellular proliferation. Its location within the body can also be imaged by using positron emission tomography (PET). Twelve patients with cerebral gliomas (mean age, 49.6; 4 WHO grade IV, 4 WHO grade III, and 4 WHO grade II tumors) were studied preoperatively; 200 MBq of FLT was given intravenously and a 3D dynamic PET study performed. Venous blood sampling was performed throughout the study. Maps of FLT uptake were generated and coregistered to an MR study used for biopsy planning. All patients underwent an image-guided biopsy, with biopsies taken at intervals along the biopsy tract. Biopsies were paraffin embedded and sections used for regular histopathological analysis and immunohistochemistry using the MIB-1 antibody as a marker for cellular proliferation. The coordinates for all biopsy sites were determined, and uptake values for FLT were determined. There was little uptake of FLT in either the normal brain or the low-grade gliomas, except in one case where the MIB-1 labeling index was 12% without features of anaplastic transformation. In 3 of the WHO grade III tumors the MRI showed little enhancement, but there was focal increase in FLT in these cases. In all grade IV tumors there was marked uptake of FLT. The maximum FLT uptake from all biopsy regions correlated with the maximum MIB-1 labeling index

(Pearson's correlation coefficient r = 0.69; $P = 0.03$). By using a threshold of 1.5×10^3 $ml_{plasma}\,min^{-1}\,ml_{brain}^{-1}$) for the individual biopsy sites, FLT could detect tumor with a sensitivity of 92% and a specificity of 64%; it was marginally more sensitive but less specific than either T_2- or contrast-enhanced T_1-weighted MRI. FLT is a good imaging marker of tumor proliferation. It may be especially useful in monitoring response to therapy and guiding image-guided biopsies in minimally enhancing tumors.

465. ADVANCES IN MANAGEMENT OF MALIGNANT MENINGITIS

M.C. Chamberlain; University of California, Norris Cancer Center, Los Angeles, California, USA

Neoplastic meningitis (NM) is a common problem in neuro-oncology, occurring in approximately 5% of all patients with cancer, and is the third most common site of central nervous system (CNS) metastases. NM is a disease affecting the entire neuraxis, and therefore clinical manifestations are pleomorphic, affecting the spine, cranial nerves, and cerebral hemispheres. Because of craniospinal disease involvement, staging and treatment needs encompass all cerebrospinal fluid (CSF) compartments. Treatment of NM utilizes involved-field radiotherapy of bulky or symptomatic disease sites and intra-CSF drug therapy. The inclusion of concomitant systemic therapy may benefit patients with NM and may obviate the need for intra-CSF chemotherapy. At present, intra-CSF drug therapy is confined to three chemotherapeutic agents (i.e., methotrexate, cytosine arabinoside, and thio-TEPA) administered by a variety of schedules either by intralumbar or intraventricular drug delivery. Although treatment of NM is palliative, with an expected median patient survival of 2 to 6 months, it often affords stabilization and protection from further neurologic deterioration in patients with NM.

466. ADVANCES IN MANAGEMENT OF BRAIN METASTASIS

R. Soffietti, R. Rudà, E. Laguzzi, and E. Trevisan; University of Torino, Neuroscience, Torino, Italy

The management of patients with brain metastases has improved over time, because of advances in technology and a better knowledge of prognostic factors, leading to a more accurate patient selection. In the diagnostic setting, MRI is the gold standard, and MRI diffusion/perfusion and MRS in certain circumstances improve the diagnostic accuracy. A tissue diagnosis is needed in patients with unknown primary tumor or with absent/controlled systemic disease when a long interval has elapsed since the initial cancer diagnosis or with active but treatable systemic disease when the radiologic appearance is atypical. Whole-body FdG PET is a sensitive tool to detect pulmonary foci as probable primary tumors in patients with biopsy-proven brain metastasis and a negative cancer history. Neurocognitive tests are a relatively sensitive measure of brain functioning, being important in predicting survival and monitoring treatment effects and tumor progression. The combination of surgical resection and WBRT is superior to WBRT alone for the treatment of single brain metastasis in patients with limited or absent systemic disease and good performance status. Radiosurgery yields results that are comparable to those reported after surgery, provided that the lesion's diameter does not exceed 3 to 3.5 cm. Radiosurgery combined with WBRT ("radiosurgical boost") is superior to WBRT alone in single but not in multiple brain metastases. Hypofractionated stereotactic radiotherapy can be an alternative to radiosurgery. Still controversial is the need for WBRT after surgery or radiosurgery: Local control on MRI is significantly better after the combined approach, but overall survival does not improve. Phase 3 studies (RTOG, EORTC, etc.) are ongoing, trying to identify which subgroups of patients are candidates for WBRT after local treatments. A new form of brachytherapy (Gliasite Radiation Therapy System) is now being investigated in patients who have undergone surgical resection. Novel radiation sensitizers (Motexafin gadolinium, RSR 13) have some activity in conjunction with WBRT. The response rate of brain metastases to chemotherapy is similar to that of the primary tumor and extracranial metastases. Promising results have been recently reported with temozolomide in melanoma, capecitabine in breast cancer, and gefitinib (ZD 1839), an EGFR tyrosine kinase inhibitor, in NSCLC. Chemotherapy may represent the initial treatment (with or without subsequent WBRT) in patients with multiple brain metastases from NSCLC and breast cancer who are asymptomatic, whereas WBRT remains the treatment of choice in symptomatic patients. Cognitive defects in long-surviving patients after WBRT are a real concern: Acetylcholinesterase inhibitors (donepezil, memantine) could be of some efficacy in improving cognitive functioning.

468. NOA-03 MULTICENTER TRIAL OF HIGH-DOSE METHOTREXATE IN PRIMARY CNS LYMPHOMA: FINAL REPORT

U. Herrlinger,[1] W. Küker,[2] M. Uhl,[1] H.P. Blaicher,[3] H.-O. Karnath,[3] L. Kanz,[4] M. Bamberg,[5] and M. Weller[1]; Departments of [1]General Neurology, [2]Neuroradiology, [3]Cognitive Neurology, [4]Medical Oncology, and [5]Radiation Oncology, University of Tübingen, Tübingen, Germany

The German multicenter NOA-03 trial explored the value of primary high-dose methotrexate therapy (HD-MTX; 8 g/m^2) alone in patients with primary CNS lymphoma (PCNSL). Response data have already been published (Herrlinger et al., Ann. Neurol. 51, 247, 2002). The present report summarizes the long-term outcome and the development of late neurotoxic sequelae in the NOA-03 trial. Patients were evaluated for progression-free and overall survival. In patients surviving more than 12 months, the development of leukoencephalopathy on MRI was analyzed as a marker for late neurotoxicity. Six long-term survivors were tested for deficits in cognitive function and for quality of life using the EORTC-QLQ C30 and Brain 20 questionnaires. Thirty-seven patients were enrolled in the trial. Eleven patients achieved a complete remission (CR) with HD-MTX. Ten of these patients relapsed after a median time of 15 months (2–46 months), 4 of them with a systemic relapse. Thirty-three patients received second-line therapy. Two of 7 (28%) patients receiving PCV (procarbazine, lomustine, vincristine) and 12 of 20 patients (60%) receiving whole-brain radiotherapy (WBRT) achieved a CR upon second-line therapy. The median survival after the start of second-line therapy was 19 months. There was no significant difference between patients receiving WBRT (19 months) and PCV (12 months). The overall median survival was 25 months with a 2-year survival rate of 51% and a 3-year survival rate of 29%. Four years after the start of therapy, 34% of patients surviving more than 12 months had developed leukoencephalopathy. The rate of leukoencephalopathy was higher in patients treated with WBRT (58%) than in patients treated without WBRT (10%). Neuropsychological testing revealed mild to extensive attention deficits in all 6 patients tested (5 without WBRT, 1 with WBRT) and memory deficits in 4 of 6 patients. Two patients had normal, 3 had moderately restricted quality of life, and the only patient tested who had received WBRT had markedly restricted quality of life. High-dose MTX therapy with deferred radiotherapy had moderate efficacy. Radiotherapy was an effective second-line therapy but may induce a higher rate of late neurotoxicity. The final results of the NOA-03 trial illustrate the need for more effective first-line treatments.

469. TEMOZOLOMIDE AS PRIMARY CHEMOTHERAPY FOR LOW-GRADE OLIGODENDROGLIAL TUMORS (LGOT): PREDICTIVE IMPACT OF CHROMOSOME 1P LOSS ON RADIOGRAPHIC RESPONSE

K. Hoang-Xuan,[1] E. Crinière,[1] J. Lejeune,[1] F. Diakite,[1] S. Taillibert,[1] F. Laigle-Donadey,[1] L. Capelle,[2] A. Carpentier,[1] M. Sanson,[1] and J. Delattre[1]; Department of [1]Neurology & INSERM U495 and [2]Department of Neurosurgery, CHU Pitié-Salpêtrière, Paris; France

Temozolomide (TMZ) has recently been shown to be active as initial treatment for low-grade oligodendroglial tumors (LGOT). Preliminary results suggested that chromosome 1p loss may predict the response to TMZ in LGOT (Hoang-Xuan et al., J. Clin. Oncol. 22, 3133, 2004). The objectives of our study were to confirm in a larger series and with a longer follow-up (1) the efficacy of TMZ on LGOT and (2) the predictive value of 1p loss on the radiographic response. Patients suffering from WHO grade II oligodendroglioma and mixed glioma, with progressive disease on MRI, were eligible for the study. TMZ was delivered at the starting dose of 200 mg/m^2/day for 5 days, repeated monthly. Response was evaluated by MRI (T2–FLAIR weighted sequences). Deletion on 1p was searched by the LOH technique. From 1999 to 2004, 99 consecutive patients were included in the study (median age, 44 years; median Karnofsky score, 90). Median follow-up was 22 months (6–54), and the median number of TMZ cycles was 18 (6–26). The response rates were as follows: partial response (PR), 25%; minor response (MR), 14%; stable disease (SD), 52%; and progressive disease (PD), 9%, respectively. Grade 3–4 toxicity occurred in 6% of patients. The median PFS was 31 months (95% CI, 19 months–∞). Blood and tumor DNA pairs from 50 patients were available for LOH analysis. LOH 1p was present in 22 tumors (corresponding to 15 PR + MR, 7 SD, 0 PD) and absent in 28 tumors (corresponding to 4 PR + MR, 20 SD, 4 PD) ($P = 0.0001$). We confirm in a larger series that TMZ provides a substantial rate of objective response in LGOT and that LOH 1p is closely correlated with radiographic response.

470. PHASE 2 MULTICENTER STUDY OF DOSE-INTENSE TEMOZOLOMIDE IN PATIENTS WITH NEWLY DIAGNOSED PURE AND MIXED ANAPLASTIC OLIGODENDROGLIOMA

D. Peereboom,[1] C. Brewer,[1] D. Schiff,[2] P. Fisher,[3] S. Snodgrass,[4] R. Prayson,[1] G. Stevens,[1] M. Vogelbaum,[1] S. Panullo,[5] and G. Barnett[1]; [1]Brain Tumor Institute, Cleveland Clinic Foundation, Cleveland, Ohio; [2]Department of Neurology, University of Virginia, Charlottesville, Virginia; [3]Department of Neurology, Stanford University, Palo Alto, California; [4]Department of Neuro-Oncology, H. Lee Moffitt Cancer Center, Tampa, Florida; [5]Department of Neuro-Oncology, Columbia Medical College, New York, New York; USA

Standard initial therapy for patients with pure and mixed anaplastic oligodendrogliomas (AO/MAO) has included chemotherapy and radiation therapy. These gliomas have particular sensitivity to chemotherapy, which varies according to the molecular genetics of the tumor. Due to their chemo responsiveness, this trial has used a dose-intense regimen of temozolomide and reserved RT for patients with disease progression. Our objectives were to determine the progression-free survival, response rate, and quality of life (QOL) in patients with newly diagnosed AO/MAO treated with temozolomide every other week and to determine outcomes according to tumor cytogenetic status. Eligible patients had newly diagnosed AO/MAO with no prior chemotherapy or radiation therapy. All pathology had central review and tumor assay for 1p deletion using FISH (fluorescent in situ hybridization). Analysis was stratified by 1p status. Temozolomide was given 150 mg/m^2, days 1–7 and 15–21, every 28 days. Therapy was given for up to 8 cycles in the absence of progressive disease. Responses and QOL (FACT-BR and EORTC brain module) were measured every 8 weeks. To date, 41 patients have been enrolled from 7 centers. Four patients are too early to evaluate. Median age is 42 (range, 20–83); Karnofsky PS, 100 in 10 pts, 90 in 16 pts, 80 in 3 pts, and 70 in 7 pts. Histology: AO in 21 pts, MAO in 15 pts, and 1p deletion in 21 pts (15/21 with AO and 6/15 with MAO). Patients have received 0 to 8 cycles (median 8) of temozolomide (21 patients have completed all 8 cycles, 7 withdrew consent for toxicity or patient choice prior to completion, 5 withdrew for progressive disease prior to completion, 2 continue with stable disease in cycle 6, 1 withdrew prior to receiving treatment). Only 1 patient has required dose reduction for toxicity (20% reduction for thrombocytopenia). Nineteen patients remain free from progression with a median progression-free survival of 10 months (range, 2–25). The overall survival is 14 months (5–34). Of the 32 patients with response data available, 2 (AO with 1p loss) (6%) achieved complete remission, 17 (53%) have stable disease, and 13 (41%) had progression. Toxicity was as follows: Grade 3 and 4 toxicities have included 6 pts with grade 3/4 neutropenia, 3 with grade 3 thrombocytopenia, and 11 with grade 3 lymphopenia; grade 1 and 2 toxicities have included fatigue, nausea, vomiting and constipation. QOL data will be presented upon study completion. This trial supports the concept of using chemotherapy alone as initial therapy for patients with chemotherapy-responsive gliomas. The durability of responses is not yet known. Temozolomide offers a reasonable alternative to initial RT for patients with newly diagnosed AO/MAO. Toxicity in this trial has been manageable. Accrual continues.

471. TEMOZOLOMIDE (TMZ) WITH O6-BENZYLGUANINE (BG) FOR TMZ-RESISTANT MALIGNANT GLIOMA: A PHASE 2 TRIAL

A. Desjardins, J. Quinn, D. Reardon, J. Rich, S. Sathornsumetee, J. Vredenburgh, A. Walker, S. Tourt-Uhlig, D. Bigner, and H. Friedman; Duke University Medical Center, Durham, North Carolina, USA

TMZ is a methylating agent with confirmed activity in recurrent and newly diagnosed malignant glioma. One of the major mechanisms of resistance to TMZ is determined by the DNA repair protein O6-alkylguanine-DNA alkyltransferase (AGT), which removes the methylation damage from O6 position of guanine. BG inhibits AGT by acting as an AGT substrate. We performed a phase 2 study of TMZ combined with BG in patients with TMZ-resistant malignant glioma. Eligibility included adult patients with malignant gliomas resistant to TMZ. Patients were divided in two strata, one for glioblastoma multiforme (GBM) and the second for anaplastic astrocytoma or oligodendroglioma (AA/AO). Each patient was treated with 1-h BG infusion at 120 mg/m^2, followed by a single dose of TMZ at 472 mg/m^2, and thereafter a 48-h continuous BG infusion at 30 mg/m^2/day. Responses were assessed by functional and imaging criteria after two cycles (8 weeks). Patients were treated for one year or until disease progression or unacceptable toxicity. The primary end points of this study were to define the role of BG in restoring TMZ sensitivity in patients with TMZ-resistant malignant glioma and to determine the activity and toxicity of TMZ plus BG. To date, 62 patients with recurrent malignant gliomas (GBM = 34, AA/AO =28) have been enrolled, for a planned accrual of 64 patients. Fifty-five patients are assessable for response. Five patients showed a partial response (AA/AO n = 4, GBM n = 1), 17 showed a stable disease, and 33 patients progressed at the 6-week evaluation. Toxicities are limited to hematologic toxicity and included neutropenia (36 grade 4, no grade 3), leukopenia (one grade 4), thrombocytopenia (8 grade 4), anemia (one grade 3, one grade 4) and one case of febrile neutropenia. Toxicities are limited to hematologic events. Activity is seen in TMZ refractory AA/AO and warrants further study. Trivial activity is seen in TMZ refractory GBM, and the reasons may include suboptimal TMZ schedule, alternative mechanisms of resistance, and AGT mutations.

472. A PHASE 2 STUDY OF TEMOZOLOMIDE (TMZ) AND THE FARNESYLTRANSFERASE INHIBITOR (FTI) LONAFARNIB (SARAZARTM, SCH66336) IN RECURRENT GLIOBLASTOMA

M. Gilbert,[1] K. Hess,[1] V. Liu,[1] M. Groves,[1] V. Puduvalli,[1] V. Levin,[1] C. Conrad,[1] H. Colman,[1] S. Hsu,[1] M.M. Sugrue,[2] and W.K.A. Yung[1]; [1]Neuro-oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [2]Schering-Plough Research Institute, Kennilworth, New Jersey; USA

Farnesylation is an essential step in the post-translational modification of several proteins that play a role in cell proliferation and growth, including Ras, RhoB, centromere binding proteins (CENP-E/CENP-F), lamin B, and protein tyrosine phosphatase (PTP). Inhibition of farnesylation may inhibit tumor cell proliferation. Preclinical testing demonstrates antiproliferative effects of FTIs in a variety of tumor cell lines and xenograft models. Additional laboratory data from animal models demonstrated enhanced efficacy of combining lonafarnib with TMZ compared with TMZ alone. Our objective was to determine the efficacy as measured by response rate and 6-month progression-free survival rate of the combination of TMZ with lonafarnib. Eligibility criteria were as follows: histologically proven GBM, unequivocal evidence of tumor relapse or progression after radiation therapy, up to 2 prior chemotherapy regimens for recurrent disease, ability to provide informed consent, and KPS of 60. Patients may not be on cytochrome P450-inducing anticonvulsants. The treatment plan was as follows: TMZ was administered at 150 to 200 mg/m^2 days 1–5 and lonafarnib at 150 mg BID on days 8–28 of a 28-day cycle. Response was evaluated every 2 cycles. Standard response criteria established by MacDonald were used. Twenty-three patients were accrued to the study. Average age was 53 years and median KPS was 90. Male to female ratio was 15:8. Overall, 10 patients demonstrated stable disease (including 3 minor responses), and 3 patients had a partial response. The 6-month PFS rate was 13%, median PFS was 16 weeks, and overall survival from study entry was 38 weeks. Treatment was well tolerated. One patient had grade 3 granulocytopenia, thrombocytopenia, and anemia, one patient had grade 4 granulocytopenia, and one patient had grade 4 thrombocytopenia. One patient developed grade 3 diarrhea. The combination of TMZ with lonafarnib using a conventional TMZ dosing schedule was well tolerated, but these data do not support improved efficacy over TMZ alone. Recent reports suggest that the "dose-density" of TMZ can be safely increased, and this may enhance activity. Additionally, modifying the dose and schedule of the lonafarnib may improve efficacy when combined with the dose-intensified TMZ regimen. A phase 1 study is under way to optimize the TMZ-lonafarnib combination.

473. PHASE 2 TRIAL OF VINCRISTINE, NIMUSTINE (ACNU), CARBOPLATIN, AND INTERFERON-BETA WITH RADIATION THERAPY (VAC-FERON-R) FOR NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

T.A. Aoki,[1] J.A. Takahashi,[2] T.U. Ueba,[2] K.N. Nozaki,[2] N.O. Oya,[3] M.H. Hiraoka,[3] A.N. Nakajima,[4] K.M. Matsui,[5] T.F. Fukui,[5] M.I. Ishikawa,[1] and N.H. Hashimoto[2]; [1]Neurosurgery, Osaka; [2]Neurosurgery, Kyoto; [3]Radiation Oncology, Kyoto; [4]Pathology, Kyoto; [5]Kyoto University Hospital, Biostatics, Kyoto; and the Kyoto Neuro-Oncology Group (KNOG): Kyoto University, Kitano Hospital, Shimizu Hospital, Kyoto National Hospital, Himeji National Hospital, Tenri Yorozu Hospital, Takeda Hospital, Osaka Red Cross Hospital, Kishiwada City Hospital, Fukui Red Cross Hospital, Kurasiki Central Hospital, National Cardio-Vascular Center, Saiseikai Noe Hospital, Kizugawa Hospital, Hikone City Hospital, Shizuoka Preferectual Hospital, Saisekai Nakatsu Hospital, Hamamatsu Rousai Hospital, Tsukaguchi Preferectual Hospital, Kobe Metal Hospital, Nagahama City Hospital, Takatsuki Red Cross Hospital, Kobe Central City Hospital; Japan

Novel combination chemotherapies have been needed for patients with glioblastoma multiforme, because single drugs have not been sufficiently effective. Based on previous published reports, we select four drugs (vincristine, ACNU, carboplatin, and interferon-beta). This phase 2 study was performed to determine the safety, tolerability, and efficacy for this therapy, after a dose setting by our feasibility study. This multicenter phase 2 trial enrolled 97 patients (intent-to-treat population). After central histologic review, 97 patients were confirmed to have had a GBM. ACNU (60 mg/

m^2), carboplatin (110 mg/m^2), vincristine (0.6 mg/m^2) and interferon-beta (300 million/body) were administered in day 1, concomitant with fractionated radiotherapy (63 Gy total dose; 1.8 Gy × 5 days for 7 cycles) and vincristine (0.6 mg/m^2) and interferon-beta (300 million/body) in day 7 and 15, interferon-beta (300 million/body) three times for a week during the radiation course. Two months after the radiation, ACNU (60 mg/m^2), carboplatin (110 mg/m^2), vincristine (0.6 mg/m^2) and interferon-beta (300 million/body) were administered in day 1 and vincristine (0.6 mg/m^2) and interferon-beta (300 million/body) in day 7 and 15, every 58 days. The primary end points were safety and tolerability, and the secondary end points were time to progression and overall survival. Nonhematologic toxicities were rare and mild to moderate in severity. During the radiation treatment phase, grade 3 toxicities in neurocytopenia, or thrombocytopenia, or both were observed in 23% of patients. Grade 4 hematological toxicities were not observed. During adjuvant maintenance chemotherapy after radiation, 7% of all cycles were associated with grade 3 toxicities in neurocytopenia or thrombocytopenia. None of the cycles were with grade 4 toxicities. Time to progression (TTP) was 11 months, and median survival was 16 months. VAC-feron-R is safe and well tolerated. This regimen of concomitant chemo-radiation therapy followed by adjuvant maintenance chemotherapy may prolong the survival of patients with GBM. Further investigation is warranted.

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