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Tumors of the Central Nervous System: Clinical Aspects, Molecular Mechanisms, Unanswered Questions, and Future Research Directions

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Abstract

Central nervous system tumors are the most common solid tumors in children. Many histological subtypes and biological variants exist. The 2007 Neurobiology of Disease in Children Symposium, held in conjunction with the 36th annual meeting of the Child Neurology Society, aimed to define current knowledge in the field and to develop specific aims for future clinical, translational, and fundamental science. Because of advances in structural and metabolic imaging, surgical technique, and combination therapies, the life expectancy of children with some of the most common tumors, such as cerebellar astrocytomas and medulloblastomas, has improved. Other common tumor types, including diffuse pontine gliomas and malignant embryonal tumors, still have a dismal prognosis. As novel therapies are identified for pediatric central nervous system tumors, long-term survival may be associated with considerable disability. A cooperative effort is crucial to early diagnosis and to translating basic research findings into safe, effective new treatments.

Clinical Aspects of Central Nervous System Tumors

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Accomplishments and Ongoing Challenges

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Dr Packer reviewed recent neurobiological advances in the diagnosis and treatment of childhood brain tumors and addressed implications for clinical management. Medulloblastoma is the most common malignant brain tumor in children, and much work over the past decades has focused upon it, with significant results. The average-risk medulloblastoma in the 1980s was associated with a 60% survival rate; today, the survival rate has risen to between 80% and 85%. This improvement can be partially attributed to an increase in the number of patients who undergo gross tumor resection.

Reclassification of this histologically heterogeneous tumor is an additional contributing factor. Patients with atypical teratoid/rhabdoid tumors, which account for 10% to 15% of infant embryonal tumors and carry a dismal prognosis, are no longer included under the medulloblastoma classification. This is a result of increased understanding of the disease, but it deceptively boosts medulloblastoma survival rates. Nodular/desmoplastic variant medulloblastoma has also been removed from the classical medulloblastoma classification. The 20% of patients who comprise the small subset with this tumor type enjoy a better prognosis. This is a histological stratification, but there may be a biological basis, as there is evidence this tumor arises from a different precursor cell, the granular cell precursor, instead of the 4th ventricle stem cell. Unfortunately, even as separation into risk groups has become a major tenet of treatment, a recent national study of more than 400 children showed only 80% are imaged appropriately. Accordingly, some children are placed in the wrong risk group.

With the increased survival rate, more children face posttreatment quality-of-life issues. Children who undergo radiation treatment for medulloblastoma experience a 10- to 30-point drop in intelligence quotient (IQ). This has led to treatment regimens designed to minimize or delay radiation treatments in infants as much as possible. According to two recent international studies, posterior fossa mutism syndrome — the delayed onset of mutism associated with hypotonia, cerebellar dysfunction, severe emotional lability, and supranuclear palsies — occurs in nearly 25% of patients after surgery, a much higher proportion than previously thought. Unfortunately, at least half of these children suffer permanent sequelae.

Biological advancements, such as the work of Dr Richard Gilbertson (St. Jude Children's Research Hospital) with *ERBB2*, will hopefully lead to more detailed and predictive stratification systems. Objective separation of risk groups will allow for variable treatment schedules, decreasing the amount of neurotoxic therapy given to patients with more favorable prognoses and intensifying therapy for those with poorer prognoses. According to preliminary data from a Children's Oncology Group study, the survival rate for high-risk medulloblastoma patients using a more intensive therapeutic regimen of chemotherapy with carboplatin, a radiation sensitizer, and post-radiation chemotherapy is 80%. However, using molecular markers to separate risk groups in real time poses difficulties. In the future, the key will be to use the biology of the tumor to inform studies that improve both survival and quality of life. Moving beyond stratification, molecular markers offer new biological targets for therapeutics that may be safer than conventional therapy. However, these tumors possess many escape mechanisms. One targeted therapeutic alone probably will not work and will have to be combined either with other targeted therapeutics or with conventional radiation and chemotherapy treatments.

Dr Packer briefly commented on other childhood tumors for which survival rates have not significantly improved. For low-grade glioma, unpublished data from the Children's Oncology Group shows the most important predictor of survival is degree of resection. However, because of location, some tumors remain unresectable. Chemotherapy for low-grade unresectable tumors has been used in management and can control tumor progression

in 60% to 70% of cases for 2 years to 3 years, but management has not changed in the past decade, and there is currently little biological data to support new therapeutic strategies. The role of chemotherapy in older patients needs to be defined; unfortunately, many older patients who should receive radiation are put on chemotherapy regimens instead. For brainstem glioma, there has been almost no progress. Tectal tumors, with a more benign prognosis, and cervicomedullary tumors, truly pilocytic astrocytomas, have been defined, but this has not changed management. Temozolomide, which has shown efficacy in adults, has yet to improve survival in pediatric trials. In ongoing studies of high-grade gliomas, molecular data objectively categorizes adult and pediatric tumors as two different diseases. It is hoped these data will lead to the discovery of effective therapeutic targets. Ependymoma is a significant problem that represents a larger patient population than previously thought. Degree of resection and the separation by histology from other tumor types are two factors that have changed prognosis. Many tumors infiltrate into important brain structures, making resection difficult. This causes a dilemma, as practitioners must weigh survival advantages of resection over quality-of-life issues. Recent data show good survival rates for patients who receive radiation, with at least excellent short-term quality of life. Studies currently being conducted will tremendously increase the biologic data on ependymomas, but as of yet biology has not been incorporated into management.

Advances and Limitations of Imaging

Gilbert Vezina, MD, Children's National Medical Center

Dr Vezina described the current state of imaging and the progress made in the field during the past 25 years. In the mid-1980s, computed tomography (CT) followed by magnetic resonance imaging (MRI) displaced pneumoencephalography and angiography as the standard of care. This was an important accomplishment, as the older modalities were cumbersome and often ambiguous. Today, there is ubiquitous access to high-field MRI, which can define tumors with respect to location, extent, and size and can even differentiate some well-localized and infiltrative tumors. With the use of additional modalities such as fluid-attenuated inversion recovery imaging and diffusion, tumor type can often be assessed, and brainstem gliomas are now diagnosed radiographically and treated without biopsy. While progress has been made, the field is still mostly confined to dimensional aspects of assessing tumors. The challenge is to better characterize and grade tumors, and to find new imaging biomarkers to predict tumor behavior. Given the interest in antiangiogenic agents, biomarkers that correlate with tumor vascularity are being sought. Because health practitioners are still confined to using tumor size to predict treatment response, it is imperative to find better markers to predict response. These advancements will come in the functional imaging realm, for which technology is rapidly advancing.

Magnetic resonance spectroscopy generates graphs of frequencies of resonance of hydrogen molecules attached to different molecules in the brain. Tumors show an elevation of choline and a decrease or absence of N-acetylaspartate, and, sometimes, creatine. A single-voxel technique is currently used. Because this method can only assess one area of the tumor, it is associated with large sampling errors and inconsistency. Multivoxel, or chemical shift, imaging is finally widely commercially available and addresses the problems of the single-voxel technique and allows radiologists to look at intratumoral heterogeneity and infiltration. Spectroscopy is very good at differentiating tumor recurrence from necrosis, and, with the advent of multivoxel imaging, deserves to be re-explored in multicenter trials.

Perfusion MRI measures the decrease in signal intensity after a bolus injection of gadolinium; this decrease is proportional to the density of capillaries within a single voxel. From this, cerebral blood volume can be measured, which has been shown to be

significantly greater in high-grade tumors in adults, and vascularity can be assessed. Perfusion MRI helps guide surgeons to more malignant areas of a tumor for biopsy and helps assess response to treatment with antiangiogenic drugs. However, this approach requires a large intravenous bolus infusion that is not easy to give to a child and contrast leaks out of tumors with leaky capillaries, creating measurement errors.

On the other hand, the propensity of tumor capillaries to leak can be used in permeability imaging, which measures the progressive increase (relaxation) in signal intensity over time to estimate the permeability of a tumor. Adult glial tumors show a good linear correlation between grade and permeability, and the Pediatric Brain Tumor Consortium is starting to use permeability imaging in brain tumor protocols. Importantly, this modality can be used to assess antiangiogenic agents because leakiness of capillaries tends to improve as tumors respond to these agents. Injection is by slow infusion rather than bolus, which children should better tolerate.

Another modality gaining commercial availability is diffusion tensor imaging, which can be used to reconstruct white matter tracts. Surgeons can use these reconstructions to assess locations of major tracts and plan resections. Diffusion imaging and white matter anisotropy can also be used to evaluate cognitive consequences of treatment in children. Khong and colleagues (2006) showed a nice linear correlation between fractional anisotropy and cognitive outcomes in pediatric leukemia survivors. Ideally, diffusion tensor imaging could be used to look at acute changes after treatment, and these results would guide future treatment plans. The Children's Oncology Group is working on a multicenter trial looking at this, and this is the first time the Children's Oncology Group has included a functional imaging question as part of a treatment protocol.

Apart from this one trial, no multicenter trials focus on functional imaging. However, several are assessing anatomical imaging and, in the Children's Oncology Group, there is good effort to share images across institutions. We are entering a stage where there is central review of all pediatric brain tumors. Studies in the late 1990s showed that suboptimal imaging leads to worse outcomes. Unfortunately, a new study started a few years ago indicates metastatic disease continues to be missed at the same rate and obtaining good quality studies continues to pose a challenge. Better education of radiologists and perhaps even rapid central review of imaging studies would help ensure accurate diagnoses.

Initial Management: Surgery

James Rutka, MD, PhD, FRCSC, FACS, FAAP, University of Toronto, Toronto, Ontario

Dr Rutka began by underscoring the importance of an early fundoscopic exam, which reveals papilledema and may shorten time to diagnosis. Dr Rutka then discussed the importance of neuroimaging advances to neurosurgery. Specifically, the increased utility of functional imaging has allowed surgeons to better manage tumor resections. Magnetoencephalography, which uses an electrical current converted into a magnetic signal detected by MRI, can map different areas of the cortex prior to surgery, and diffusion tensor imaging can define corticospinal tracts. These functional maps can be coupled with image-guided resection, which is like a GPS that allows surgeons to pinpoint tumor location within the brain and to predict, prior to surgery, which children are likely to do well and which may experience deficits. This knowledge gives neurosurgeons great confidence in describing expected outcomes to families. The ultimate in image guidance is intraoperative MRI, which is still going through developmental iterations. This allows the surgeon to see, intraoperatively, how much tumor remains. Endoscopes, currently useful for obtaining small biopsies used to plan treatment, may soon be useful in actually resecting tumors, thanks to better optics and more working channels.

Dr Rutka also discussed the current role of surgery in the treatment of different tumors. Surgical resection is definitive therapy for many tumors, such as some low-grade cerebral gliomas and cerebellar astrocytomas. For optic nerve gliomas confined to the optic nerve and causing visual failure and proptosis, surgery is curative, although it causes monocular blindness. For chiasmatic tumors, debulking of the mass can be accomplished before starting chemotherapy. For craniopharyngioma, the surgical plan depends very much on the exact location of the tumor; the trend is to achieve tumor resection that spares the hypothalamic-pituitary axis.

Brainstem tumors used to be surgically inaccessible. This is no longer true; midbrain, pontine, and cervicomedullary tumors can all be resected. However, surgical resection is not feasible for diffuse intrinsic pontine glioma, the most common brainstem tumor.

For other tumors, such as medulloblastoma and ependymoma, there is a role for surgery, albeit not to the same degree as in low-grade gliomas, and surgery is most likely coupled with either or both radiation and chemotherapy. For medulloblastoma, a Children's Oncology Group study concluded that degree of resection correlates with prognosis. Data also suggest the extent of resection is important in ependymoma; however, only 30% to 50% of these tumors can be completely resected. Prior to surgery for medulloblastoma or ependymoma, or whenever cerebrospinal fluid seeding is suspected, patients should have a preoperative MRI of the entire spine; although ependymomas rarely spread in the neuraxis via cerebrospinal fluid dissemination and much more commonly recur locally in the posterior fossa.

Looking toward the future, convection-enhanced delivery, already under development in adults, will soon be tested in children. Targeted toxins and genetic therapies are also in development, and neurosurgeons may soon use these strategies in parallel with resection to treat children. The challenge in neurosurgery remains balancing minimal morbidity with long-term quality of life.

Initial Management: Radiotherapy

Thomas E. Merchant, DO, PhD, St. Jude Children's Research Hospital, Memphis, TN

Dr Merchant discussed the use of radiotherapy to treat pediatric central nervous system tumors. In the past its use was restricted by age limitations, but it is now frontline therapy for nearly all these tumors in children. The current objective is to not only restrict the highest doses to the volume at risk but also to reduce or avoid dose to normal tissue. Current clinical trials are designed to reduce treatment-related side effects, either through dose reductions or modifications in treatment volumes. For example, treatment of children with medulloblastoma, who previously received high-dose craniospinal irradiation of 54 Gy, has been modified through the combined use of chemotherapy to reduce the radiation dose. Additionally, through the use of new techniques, we can further reduce radiation dose. For children under the age of 3 years, this concept has been advanced even further, as the use of chemotherapy and focused posterior fossa radiation has eliminated craniospinal irradiation. By taking advantage of the full complement of multimodality imaging to define the target volume, the area of treatment can be minimized. In 1997, the clinical target volume margin and planning target volume margin were 10 millimeters and 5 millimeters, respectively. These two measures are down to 4 millimeters and 3 millimeters.

For children with low-grade optic gliomas, the appropriate age at which to begin irradiation is controversial and the subject of ongoing investigation. Currently, this decision is institutionally based; however, visual outcomes are often improved in these patients when radiation is part of initial management. Additionally, recent data suggest a lack of cognitive

side effects of radiation compared with what was previously anticipated, indicating factors other than radiation contribute to poor functional outcomes. Many patients with low-grade gliomas have pre-existing endocrinopathies, and non-irradiated patients often have growth hormone deficiency or precocious puberty. When these conditions are not appropriately treated because of concerns that a tumor has not received definitive therapy, children miss the opportunity for normal growth and development. Dr Merchant argues for a reappraisal of the risks and benefits of radiation for this tumor in the modern era.

Although good data from the 2-dimensional era is lacking, by comparing anecdotal experience of that era with today's longitudinal data from the 3-dimensional era, we have a better understanding of side effects. Dose-related effects of radiation therapy are becoming more defined. For instance, dose-related side effects are known for the cochlea, the endocrine system, and some of the sensitive areas of the brain. Higher doses create higher hearing threshold levels in patients. The threshold for hearing loss in the high-frequency range is about 35 Gy of radiation administered over 6 weeks. Hearing loss in the intermediate and lower frequencies requires a much higher dose. Dose distribution to the hypothalamus can be correlated to clinical outcomes, including the need for hormone replacement therapy. However, it is the cognitive effects of radiation that parents and caregivers fear most, and there are great strides being made in this area. Radiation dose distributions to normal brain structures are being correlated with change in IQ, and models are being developed that will guide therapy to further minimize the effects of treatment. High-dose effects of radiation therapy are still very worrisome. About 10% of children unpredictably develop vasculopathy. Why do a minority of children develop vasculopathy, secondary tumors, necrosis, or subclinical abnormalities? What are the predisposing factors? These questions are currently being investigated.

With the advent of proton beam radiation therapy, parents want to know if their child could benefit from this new technology. Protons are more precise, which potentially allows for reduced side effects and increased doses to more malignant areas of a tumor. However, there is presently a lack of clinical data and experience in the pediatric population. The current delivery method relies on scatter beam technology, a rudimentary method of administration. The more advanced scanning beam technology should be commercially available by 2009. Dr Merchant believes that even in its primitive form there are advantages to proton beam therapy, and once it is administered as an intensity-modulated treatment (as currently done in standard radiotherapy) it will provide significant benefits for children. His group has done retrospective analysis to model the effects of radiation and the potential benefits of proton beam therapy. This model showed that a child treated with the old method of conventional posterior fossa irradiation would experience a decline in IQ, whereas a child treated with 3-dimensional conformal radiation would show some improvement and a child treated with intensity-modulated proton therapy would experience a potentially large comparative gain because of the reduced dose to normal tissue.

To effectively treat a patient's tumor, we cannot afford to cut corners on dose and volume for fear of side effects. However, risk-adapted therapy, proton beam radiation therapy, and modeling dose effects will allow us to properly assess and balance risks and benefits of radiation therapy.

Initial Management: Chemotherapy

Amar Gajjar, MD, St. Jude Children's Research Hospital, Memphis, TN

Dr Gajjar discussed the use of chemotherapy for the treatment of pediatric brain tumors. During the past 30 years, a number of agents have proved effective: vincristine, cyclophosphamide, cisplatin, carboplatin, lomustine, methotrexate, topotecan, and oral

etoposide. Each of these is commonly used in treating brain tumors and they have now been combined in treatment regimens. The first use of combination therapy was MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone), which was discovered at M.D. Anderson in the early 1970s. Since then a number of effective combinations have been found, although no new agents have been identified in the past decade. However, schedules have been altered by adding new adjuvant chemotherapy, modifying adjuvant chemotherapy, and giving chemotherapy as a radiosensitizer. Dose-intensified chemotherapy with autologous stem cell rescue is now commonly used, as well as, more recently, low-dose chemotherapy with chronic exposure, termed the metronomic schedule.

New therapeutic schedules seek to postpone radiation to protect infants from the damaging neurocognitive side effects associated with its use. Dr Patty Duffner published a landmark study on the use of a chemotherapy regimen of vincristine/cyclophosphamide/cisplatin/etoposide with multiple schedules and delayed radiation on 198 children less than 3 years old. The overall survival for the patients was 50%, with progression-free survival of 30% for the medulloblastoma patients prior to radiation therapy. After postponed radiation therapy, progression-free survival increased to 45%. So, chemotherapy can be effective in delaying radiation treatment for very young children. Recently, a German group published data using chemotherapy alone with high-dose systemic and intrathecal methotrexate to treat medulloblastoma. This group achieved progression-free survival in the 80% range and overall survival in the high 90% range without radiation. Although promising, this therapeutic regimen is still quite toxic to the developing nervous system. Two decades of work point to the presence of a small subset of medulloblastoma patients who can be cured without radiation. Further research needs to be focused on selecting this population of patients; desmoplastic histology seems to be a favorable feature. On the other end of the spectrum is atypical teratoid/rhabdoid tumor, which has now been separated from medulloblastoma. This group has a very dismal outcome, as no effective chemotherapy has been found.

In addition to delaying radiotherapy in infants, new chemotherapy strategies are also being used to reduce craniospinal irradiation in older children. Roger Packer recently published a study comparing lomustine/cisplatin/vincristine to cisplatin/vincristine/cyclophosphamide after reduced-dose craniospinal irradiation with weekly vincristine for standard risk, gross-totally resected, no evidence of metastatic disease medulloblastoma patients with an event-free survival at 5 years of about 80%. During this same period, Dr Gajjar and colleagues, in a multicenter trial, used a dose-intensified delivery of cyclophosphamide/cisplatin/vincristine along with reduced-dose craniospinal irradiation to shorten the total duration of treatment for standard-risk medulloblastoma patients from 15 months to about 7 months. With this dose-intensified schedule, the total dose of cisplatin and vincristine was decreased to reduce the neurotoxicity these children faced. The results of this study showed an 85% survival for average-risk patients. Very interesting data uncovered in this study showed a subgroup of patients with a mutation in the beta-catenin gene who had 100% survival. We are entering an era where it is possible, in prospective trials, to use molecular data to identify subgroups of patients and modify therapy for these individuals to deliver either less toxic or more effective therapy.

Work in high-grade gliomas has recently focused on temozolomide, which has generated great excitement in the adult field. However, data published by Dr Gajjar as well as a recently concluded Children's Oncology Group study have failed to replicate the success seen in adult high-grade gliomas. Importantly, data from adult studies have shown that temozolomide only benefits patients with methylated methylguanine-DNA-methyltransferase (*MGMT*). Current work is focused on determining whether this is a subgroup in the pediatric population that may benefit from temozolomide. Resistance to

alkylating agents is an area of intense research, and two agents that may combat this resistance, 06-benzylguanine and poly(ADP-ribose)polymerase inhibitors, are being incorporated into pediatric clinical trials. Lastly, there is great enthusiasm in the field of molecularly targeted therapies; tyrosine kinase inhibitors, RAS inhibitors, protein kinase C inhibitors, and mammalian target of rapamycin inhibitors have all gone through phase 1 and 2 preclinical trials. Importantly, we must be sensitive to toxicities in the pediatric population, as some of the targeted pathways are crucial for growth and normal development.

Preventing Late Effects

Bartlett Moore, PhD, MD Anderson Cancer Center, Houston, TX

Dr Moore summarized the current understanding off neurocognitive late effects. Survival has increased with recent advances in brain tumor treatment, shifting emphasis to looking at long-term neurocognitive and quality-of-life outcomes. Dr Moore described a scale that balances medical success versus late effects from riskier surgery and more intensive chemotherapy and radiotherapy that increase neurocognitive sequelae. Late effects include generalized declines in IQ, attention, memory, visual spatial functioning, executive function, and processing speed. This can manifest as extreme difficulties in school achievement, career attainment, and quality of life. Language, however, is usually not affected. Unfortunately, these late effects tend to worsen with time. At the center of these late effects is white matter development. The primary damage from cranial radiation is to the vascular endothelial cells, which leads to demyelination. Chemotherapy also damages white matter. White matter is very important for the speed and coordination of cognitive processing and integrating different brain centers. In quantifying these late effects, reaction time serves as a surrogate marker, as it is correlated with distractibility and performance intelligence. Studies have shown children who receive cranial radiation and intrathecal chemotherapy have slower reaction times to a simple reaction time task.

To prevent these late effects, one option is to delay treatment. Chemotherapy can be used to delay radiation in young children with no effect on survival but a great effect on neurocognitive outcome; children whose radiation is delayed until they are at least 3 years old have a much better neurocognitive outcome than children who are irradiated initially. The white matter damage from brain cancer treatment is similar to that seen in traumatic brain injury, and this field can be looked to for treatment options. Repetition is used in traumatic brain injury and has been applied post-therapeutically to make different cognitive strategies second nature to a child. This has shown some success in trials, although whether this applies to real-world gains is still unknown. Coupled with training, pharmacological intervention (ie, methylphenidate) may prove useful, and there are some promising results in adult studies using donepezil. In addition to post-therapy treatment, prophylactic therapy is also possible. In prophylactic cognitive training, children are taught, prior to treatment, the same type of strategies as in post-therapeutic rehabilitation. This is a very intensive program, requiring 40 hours of sessions over a 6-week period. The economics of this are not very practical, because there are not good indicators of whether a particular child is actually going to require this treatment. Prophylactic therapy needs to be targeted to those who are more high risk.

The prediction of late effects is not only important for planning prophylactic cognitive training, but also for preparing risk-adjusted therapy. Dr Ray Mulhern reviewed a large cohort of studies on late effects and found risk factors include patient age, location and type of surgery, presence of hydrocephalus, dose of radiation and chemotherapy, and some other perioperative events. Highly significant was patient age, with children less than 4 years old

having IQs almost 2 standard deviations below average. Interestingly, nonmedical demographic factors such as sex influenced outcomes, with evidence showing girls are more susceptible to neurocognitive decline than boys. Genetic polymorphisms for methotrexate metabolism, DNA repair genes, and other such genes could also influence late effects and could be analyzed for their predictive value. Finally, measuring white matter integrity acutely, whether it is with white matter fractional anisotropy or functional MRI, would help detect children at risk for neurocognitive sequelae. Ideally, if you can identify the total risk in a patient, you can adapt prophylactic therapy and disease treatment to achieve a balance between medical efficacy and toxicity.

Question-and-Answer Session 1

Dr Packer: Dr Rutka, why do children have posterior fossa mutism syndrome and why is it more prevalent in this day and age?

Dr Rutka: The neuro-oncologists are demanding greater resections to improve survival, and I think that comes at a cost. So, more retraction on the cerebellar peduncles, deeper extractions of tumors, longer surgical times, more cerebellar edema after surgery, all equate with cerebellar mutism, and we don't know the anatomical pathways that are involved. But the incidence is about 15% to 20%.

Dr Maria: Along the lines of the mutism question, have there been any animal modeling studies in non-human primates? Does resection of a big part of the cerebellum without there being a tumor produce a syndrome?

Dr Rutka: I am not aware of any animal modeling. You could look at some of the symptoms and signs that would be similar between animal species and humans. What I think would be very interesting, and maybe Dr Vezina could speak to this, is what would tractography look like? What would more molecular imaging of pathways after surgery look like to try to get a handle on the mechanism of why these children have a very profound ataxic syndrome?

Dr Vezina: We are actually starting to do tractography on some of these patients. I don't have the answer now, but maybe in a few years we will know.

Dr Gajjar: What we are doing in a series of about six patients who had mutism is functional imaging post-operatively and then when they recover their speech we are trying to figure out what are the areas of the cortex which are getting stimulated when they recover. All of them are also getting diffusion tensor imaging.

Dr Packer: A lot of us at different centers are looking at different ways to try to assess what are the tracts involved and the difficulty is doing this in retrospect, and I think some of this is going to have to be done prospectively.

Dr Maria: I have one more question for all of you, which has to do with diffuse pontine glioma. We have already heard this morning that tumors that structurally look the same as diffuse pontine gliomas may actually, with better imaging, be very different from one another, and it is probably the case that there are important molecular differences between them also. With our being able to do a lot more with small bits of tumor in terms of molecular analyses, is it time for us to be revisiting the surgical sampling of diffuse pontine gliomas?

Dr Rutka: I think now I would be willing, as a neurosurgeon, to look at sampling the pons by stereotactic means because our systems are getting better; they are associated with less

morbidity than they used to be. I think it could be done safely enough to sample. I think it is an extremely important question because we have made no inroads on the survival of this very poor-prognosis patient population. As a surgeon, I think it is safe, I think our techniques have improved. I would be willing to look at it in the context of some kind of trial, where the genetics are being studied carefully by groups that are very interested in this question.

Dr Packer: First, there is a paper recently published in the *Journal of Neurosurgery* by the group at Necker, 21 consecutive patients with a relatively low morbidity in one group. Now whether that is going to be translatable to multiple different centers, where people don't do 21 over 5 or 6 years but do one every 2 years, is going to be a real issue. The second is an ethical issue, and it is a significant one. Can you put a patient through a surgery without any direct benefit for the patient? That is a very difficult issue with morbidity. The way we are trying to get around that in the Pediatric Brain Tumor Consortium, and I don't know if it is going to fly, is to base some therapy on whatever the molecular results of the tests show, whether you go to an epidermal growth factor receptor or a different kind of drug. It is a real stretch, because there is not clear data that taking biology and stratifying is going to make a difference for the brainstem gliomas, and whether that study is going to see the light of day, we will know in the next 6 months. There is a tremendous amount of interest. Whether it is ethical and allowable with the way the institutional review boards go at this time is going to be interesting. Also, the issue of sampling error is going to be interesting, and I am absolutely convinced, even though maybe in your hands, Dr Rutka, and a few other surgeons, the morbidity is low, I did live through the era where surgeons did do biopsies and this was not a uniform finding.

Audience Member: I didn't hear any real discussion of the cyberknife in the discussion of radiation, or the gamma knife.

Dr Merchant: Gamma knife and cyberknife is radiation therapy for neurosurgeons. For gamma knife, there is not a lot of data using high-dose single-fraction radiation in the treatment of children's brain tumors. It has been tried as a treatment in relapse, but the reason we don't see large series on it is that surgery has become better, so for recurrent tumors, often those children will go on to resection when they go on to salvage therapy. Then in many of the tumors where radiosurgery might be of interest, either it is in a critical area adjacent to the brainstem, where high-dose single-fraction radiation is dangerous, or the surgeons are able to resect the tumor. You cannot do radiosurgery without a target, so that is a very important point to understand. Cyberknife is robotic radiosurgery, compared to gamma knife. Gamma knife is a single treatment; cyberknife could be done multiple times, 1, 2, 3, 4, 5 times. There is no data on that for the use of that type of radiosurgery in children. It is a very conformal treatment. It has a lot of advantages, but because it cannot be used for continuous course of therapy, you will not see a lot of data on that for a long period of time. I would be very cautious about applying radiosurgery in children; one of the things that we see are children who are inappropriately treated with radiosurgery who then burn a bridge on their ability to receive external beam radiation therapy in an appropriate manner, because normal tissue tolerances have been exceeded. So there is not a lot of data; there are some very specific indications, it is something we have tried to study in the cooperative groups, but every time we put it into a protocol, we can't get enough centers to participate.

Audience Member: I believe in one of your slides you mentioned something about precaution about radiation therapy in persons with neurofibromatosis. I know Dr Gutmann is going to talk about neurofibromatosis this afternoon and I wondered if you or somebody else would give some substance to that last line on that slide.

Dr Packer: There is a paper that was recently published showing a higher incidence of secondary brain tumors in those patients who had neurofibromatosis compared to a historical group that did not have neurofibromatosis. That is actually about the first study that ever showed that. The other issue is the higher incidence of vasculopathy in neurofibromatosis patients, so there is now actually data to support that comment.

Dr Merchant: The vasculopathy concern is a real one. We actually, in our own series, don't see it more in neurofibromatosis type 1 patients over those without neurofibromatosis type 1.

Audience Member: The cerebellar mutism, some of them get better, some of them don't. Is there anything you can do in the meantime to improve the chances of them getting better?

Dr Moore: What I have seen is the deficits in language tend to persist, and it is primarily in naming abilities and expressive speech, and so I think working with a good speech therapist is the initial thing that I would do.

Dr Packer: There was an abstract put out last year about using a drug intervention that may speed improvement that has not been clarified. I think the answer is that we really don't know, and I think we are still scratching the surface to determine not only the speech deficits, but the neurocognitive deficits these kids have. So I don't have any specific recommendation except good speech and academic support.

Dr Allen: Radiobiology for neurologists — treating a medulloblastoma, we know that we have to kill every last cell or we lose the battle, but with a low-grade tumor, we often see residual tumor for the lifetime of the patient. Can you perceive any difference in either the delivery of radiation therapy or give us some insight as to how it works, where you don't necessarily have to kill every last cell, but alter the growth rate of a tumor that isn't really dividing very quickly? What is actually occurring in low-grade tumors?

Dr Merchant: I think in low-grade tumors you also have to kill every last cell, that the tumor remains after treatment and continues to enhance, is part of the response process, and one of the things that we haven't done very well is to track imaging responses of low-grade glioma after radiation therapy. But what you will find is a diminution of enhancement that really takes maybe 2 or 3 years. You will find shrinkage or maybe the tumor does go away over a very extended period of time, but with large bulky tumors that are infiltrated with normal tissues, I wouldn't expect to see much of a change over time. So I think with low-grade glioma, you have to kill every cell as well. Those patients tend to have very late recurrences. Despite our claim that there is good survivorship, those are the ones who are prone to late recurrence.

Dr Packer: The only other way to look at this is that in some of our diseases we are trying to turn these into low-grade static diseases where you have to use metronomic or other therapies to turn something from an acute to a chronic disease.

Dr Allen: The real issue is do we have any evidence, either biological or histological, of a treatment effect in a child who is 5 years out from radiation therapy and still has residual disease?

Dr Packer: We don't.

Molecular Mechanisms in Central Nervous System Tumors

Moderator: Ian Pollack, MD, University of Pittsburgh, Pittsburgh, PA

Stem Cell Hypothesis in Pediatric Central Nervous System Tumors

Robert Wechsler-Reya, PhD, Duke University Medical Center, Durham, NC

Dr Wechsler-Reya focused on new developments surrounding the stem cell hypothesis, which in relation to pediatric central nervous system tumors is still in its early stages, with many unanswered questions. It is centrally important to treatment, because to be effective and completely eradicate tumors, future cancer drugs need to target cancer stem cells as well as the bulk of the tumor. In this new field, it is important to understand the terminology. A stem cell is a normal cell that can self-renew and give rise to all the different cell types in a given tissue, while a progenitor is a normal cell that can proliferate but can only give rise to a limited number of cell types. The cell of origin is the normal cell type from which a tumor can arise. For even a subtype of medulloblastoma, it can vary depending on the oncogenic mutation and location, and may be a stem cell or a progenitor. A cancer stem cell, by comparison, is not a normal cell; it is a tumor cell that has the ability to maintain the growth of a tumor in vivo and, perhaps, regrow the tumor after therapy. There is no reason to assume that a cancer stem cell has any properties in common with a multipotent stem cell, though in some cases it may.

Determining the cell of origin for medulloblastoma has always been an area of controversy. Historically, two candidates have been considered, a multipotent stem cell that resides on the floor of the fourth ventricle, and the granule neuron precursor cell, a restricted progenitor population that starts as a multipotent progenitor in the ventricular zone, migrates to the rhombic lip, streams around the outside of the cerebellum, and then proliferates and gives rise only to postmitotic granule neurons. There is evidence to support claims for each of these populations: a subpopulation of medulloblastoma tumor cells express markers associated with multipotent stem cells and a small set can form self-renewing neurospheres (a hallmark of neural stem cells), whereas medulloblastomas display predominantly neuronal morphology, express neuronal markers, and are frequently found on the surface of the cerebellum, the same location as granule neuron precursor cells. This evidence is not substantiated, and there is no good method to determine the cell of origin from an established tumor. However, it is possible to use a mouse model to experimentally create a tumor in a particular cell type and in this way prove the cell type is susceptible to transformation.

PTCH1 is a tumor suppressor gene that is an antagonist to the Sonic hedgehog signaling pathway. A mutation in patched is found in about 15% of medulloblastomas. Mice with heterozygous *PTCH1* mutations develop tumors that resemble desmoplastic medulloblastomas. However, these mice cannot be used to study the cell of origin because the mutations are present on every cell. Dr Wechsler-Reya discussed his creation of conditional patched knockouts, in which the *PTCH1* gene can be targeted in specific cell populations, in particular, granule neuron precursor cells or multipotent stem cells. With conditional knockouts of *PTCH1* in granule neuron precursor cells, most cells undergo transient aberrant proliferation, but not tumorigenesis at first. However, in every animal there is a cohort of cells unable to exit the cell cycle that create tumors located on the cerebellar surface, suggesting that granule neuron precursor cells can serve as the cell of origin for medulloblastoma.

When the ventricular zone stem cells in the conditional knockout for *PTCH1* are targeted, aside from an increase in the number of overall neural stem cells, the cerebellum has tumors that resemble the granule neuron precursor knockouts' but occur at an earlier time. There is much less differentiation accompanying the aberrant proliferation, suggesting that the consequence of the same oncogenic mutation may differ depending on the stage at which

that mutation occurs. This data suggests that both granule neuron precursor cells and ventricular floor stem cells can be the cell of origin of certain subtypes of medulloblastoma.

Recently, it has been accepted that tumors are heterogeneous mixes of cells, containing cancer stem cells as well as more differentiated cell types. Long-term tumor growth hinges upon the small subpopulation of cancer stem cells that retain the ability to self-renew, and, perhaps, are more resistant to conventional therapies. Evidence of the existence of cancer stem cells is largely based on subpopulations of tumors displaying stem cell markers, notably CD133. Peter Dierks showed that only these CD133+ cells are able to recapitulate a tumor upon transplantation. Additionally, Jeremy Rich has shown that this CD133 population of tumor cells promotes angiogenesis and is more radioresistant than CD133− cells.

Dr Wechsler-Reya sought this CD133 subpopulation in his mouse model of medulloblastoma, and, indeed, a small subpopulation was found. Interestingly, the CD133+ population of cells was incapable of propagating the tumor upon transplantation, whereas the CD133− population could, indicating the CD133 “stemlike” population was not responsible for tumorigenesis in this tumor. Moving away from multipotent stem markers, progenitor markers such as Math1 and CD15 were tested, and small subpopulations of Math1+/CD15+ cells were found that always recapitulated a tumor upon transplantation. This data throws caution to the use of CD133 as a marker for all cancer stem cells, as it appears a different, more progenitor-like subpopulation are the true cancer stem cells for Dr Wechsler-Reya's mouse model of medulloblastoma.

In conclusion, though still in its infancy, the study of the stem cell hypothesis in pediatric brain tumors is essential. Future cancer drugs, to be effective, will have to not only target the tumor mass but also the small population of cells that are capable of regrowing the tumor and are more resistant to conventional therapies. Understanding the biology of these tumors and gaining insight into their origins and the cells that maintain them will help us move toward more effective and less toxic therapies.

Molecular Mechanisms in Embryonal Tumors

Richard Gilbertson, MD, PhD, St. Jude Children's Research Hospital, Memphis, TN

The World Health Organization classification system for embryonal tumors has historically been based on morphology. From this, we have the archetypal medulloblastoma, as well as various bizarre forms such as medulloepithelioma, ependymoblastoma, atypical teratoid/rhabdoid tumors, and supratentorial primitive neuroectodermal tumors. However, what these morphological differences signify about the biology of these tumors is not well-understood. Dr Gilbertson focused on the study of these tumors as separate molecular entities. A number of molecular alterations have been discovered, such as the infamous iso17q, gains of 1q, *MYC* amplification, *ERBB2*, mutations in beta-catenin, and deletions of 22q. While exactly what each of these alterations signifies remains unknown, we are beginning to study them as biomarkers. For example, *MYC* amplification is reported mainly in large cell anaplastic tumors, *ERBB2* prognosticates poor survival, mutations in beta-catenin are found in classic morphological forms of medulloblastoma and portend a good prognosis, and 22q deletions are a feature of the poor prognosis atypical teratoid/rhabdoid tumors.

Dr Gilbertson focused the rest of his discussion on medulloblastoma, which comprises 20% of all pediatric brain tumors and about which the most is known. This heterogeneous group of tumors with variable morphology is currently treated as one tumor type. Using a variety of advanced genomic techniques, Dr Gilbertson and colleagues have provided a more global view of this disease group. After looking at 30,000 probes in each of 50 medulloblastomas,

tumors clustered into five distinct subgroups, with one group dominated by signature genes of the beta-catenin pathway and another group by signatures of the Sonic hedgehog pathway. This suggests that two different pathways drive two separate disease processes and is clinically significant because these two subgroups may need to be treated differently. Drs David Ellison and Steve Clifford looked at a prospective population of medulloblastoma patients and found those with mutations of the beta-catenin pathway had a better prognosis.

Why are these diseases different and what is it that makes them segregate into different molecular populations? Before answering this, Dr Gilbertson attacked the popular dogma that tumor cells represent a totally aberrant process. Comparing the genomic profile of a medulloblastoma to an age-matched control does show a bizarre and disorganized collection of genes. However, if compared to an earlier, normal stage of development, for instance when the cerebellum is full of granule neuron precursor cells, the expression signature is very similar. At this normal stage of development, granule neuron precursor cells are acutely dependent on the Sonic hedgehog pathway, so it is not surprising that mutations in this pathway can result in malignant transformation of these cells. Dr Wechsler-Reya and others have provided good evidence that medulloblastomas containing mutations in *PTCH1* originate from granule neuron precursor cells. Normally, PTCH1 binds Sonic hedgehog to release the suppression of Smoothened. Mutations in *PTCH1* lead to unbridled activation of Smoothened and transformation. With this knowledge, Tom Curran showed, in a mouse model, an inhibitor of Smoothened cured mice with *PTCH1* mutation medulloblastomas.

In looking toward other subgroups, Dr Gilbertson, echoing work from Dr Wechsler-Reya and others, tested Wnt pathway mutations on granule neuron precursor cells. Both Gilbertson and others showed that this population of cells was not susceptible to transformation through mutations in beta-catenin. This suggests there is another population in the cerebellum that is susceptible to beta-catenin mutations, and the reason we see completely different morphologies, molecular markers, and prognoses in patients is because their tumors have distinct cells of origin which, not surprisingly, accrue different mutations during tumorigenesis. We are entering a stagewhere these patients can be triaged into specific subgroups, and as drugs come along we can begin to target these subgroups and no longer treat all medulloblastoma patients as one large indistinct group.

Animal Models for Mechanistic and Preclinical Studies: Xenografts

C. David James, PhD, University of California, San Francisco, San Francisco, CA

Dr James discussed xenograft models for preclinical studies. The xenograft approach, using human tumor cells in an animal model, dates back to the 1960s. The major advantages of this approach are the tumor cells are human cells, a wide variety of cell lines are available with a long history and a strong baseline of drug response data, and the immune-deficient host mice are cheap and easily available for studies requiring large numbers. However, this approach is far from perfect, with numerous disadvantages. The hosts, for example, are immune-deficient, bringing into question studies that involve invoking the immune response. Also, the established tumor cell lines have been growing in vitro for years and have acquired characteristics not necessarily representative of the tumors from which they were derived. Fortunately, in recent years a number of improvements to the xenograft approach have been made.

Xenografts have conventionally been studied after subcutaneous implantation of tumor cells. Dr James argues this is mostly irrelevant for brain tumor research, and there is, thankfully, decreasing use of this technique. Instead, researchers are using an orthotopic model system, in which tumor cells are injected into the brain, creating a clinically more relevant tumor environment. To address the problem of the tumor cell source having been subjected to

years of in vitro propagation in plastic and the resulting loss of heterogeneity, recent papers have included a panel of tumor sources to determine the consistency of response in a particular investigation. Dr James has established a panel of 24 different human glioblastoma xenografts that have been investigated and characterized; this allows responses to therapeutics of one glioblastoma to be compared with any number of the others in the panel. Additionally, instead of propagating tumor cell lines in vitro with growth conditions far removed from the original tumor environment, patient-derived tumor cells can be propagated in vivo as subcutaneous growths in immune-deficient mice.

Another improvement in xenografts is the use of two optical imaging modalities, fluorescence and bioluminescence, to longitudinally and noninvasively monitor tumor growth. Fluorescence imaging requires an emission source of visible and near infrared light. While administering an exogenous agent for imaging is unnecessary, the introduction of an optical recorder into the tumor cells is required, modifying the cells away from their natural state. The fluorescence response is prone to attenuation and is only useful for mouse models, as distances in rat models are prohibitive. Additionally, background auto-fluorescence results in greatly compromised sensitivity, with a roughly 16:1 signal-to-background ratio. In comparison, bioluminescence offers an almost 100,000:1 signal-to-background ratio and is not limited by as steep an attenuation curve, proving it to be useful in both mouse and rat models. A luciferase modified glioblastoma cell line was used in a study investigating the effects of erlotinib and an epidermal growth factor receptor kinase inhibitor. The untreated control group showed a rapid increase in luminescence, while the treatment group showed decreasing luminescence that was sustained for the period of treatment. However, once treatment was terminated, luminescence increased. The same cohort of mice was followed for survival, with the treatment group enjoying a greatly extended survival, corresponding with the luminescence effect.

With this longitudinal imaging technique, new phenomena are being discovered that were impossible to observe from survival studies or studies that required animal sacrifice. For instance, studies using bioluminescence have shown there is a greater effect from the first dose of administration of a therapeutic agent compared with the second dose in multidose regimens, suggesting resistance develops after the initial treatment. Xenograft bioluminescence models, therefore, can be used to study mechanisms by which tumors acquire resistance.

Dr James has incorporated the previously mentioned advancements into his xenograft model. This model uses patient-derived tumors, modified in culture for optical imaging, and propagated subcutaneously in mice. When needed for experimentation, the tumors are harvested and injected intracranially into mice and monitored longitudinally with optical imaging, as well as with conventional survival analysis and harvesting of brain tissue. Orthotopic tumors grown in this system produce invasive growths, with tumor cells even migrating across the corpus collosum, representative of normal glioblastoma behavior in humans. In contrast, when cell lines are used in xenograft models, their tendency is to produce well-circumscribed growths, with a loss of the invasive nature of the cancer.

Much of this work to date has been done on adult glioblastoma, but it has translated well into use in pediatric models. In looking at other types of pediatric brain tumors, atypical teratoid/rhabdoid tumors and some of the other more malignant tumors have proved amenable to the xenograft approach. However, this model can also be useful in low-grade tumors, as luminescence in grade II astrocytoma investigations was found to be nearly constant for extended periods of time, and therapeutics can be tested for their ability to decrease this signal. The xenograft approach, therefore, will prove useful in investigating new questions in a wide array of pediatric brain tumor types.

Implications for Translational Research

Ian Pollack, MD, University of Pittsburgh, PA

Dr Pollack explored the recent molecular findings in pediatric central nervous system tumors and their translational applications. Any given patient's prognosis is extremely unpredictable; what is the best treatment for a given patient and which patients should be subjected to potentially higher risk therapies? As the molecular signatures of these tumors are more fully characterized, more accurate stratification of patient risk groups is possible.

Dr Pollack's interest in looking at pediatric glioma markers began a decade ago based on data from adult high-grade gliomas showing the status of some markers, such as p53, correlated with transition from grade II to grade III to grade IV. Biological material from 150 pediatric tumors was obtained and analyzed with a variety of genetic techniques. The choice to further explore p53 proved fortuitous, as a very significant association between overexpression and poor outcome was found. Since then, a wealth of information on other markers has been discovered. Importantly, this molecular data provides evidence that pediatric and adult gliomas are distinct diseases. Epidermal growth factor receptor is overexpressed in both adult and pediatric malignant gliomas, however the mechanism of overexpression is different; it is often due to amplification of the *EGFR* gene in adults, which is rare in pediatrics. *PTEN* mutation, common in adult tumors, is exceedingly rare in pediatric tumors. Additionally, prognostic implications of markers vary, as chromosome 1p deletions, favorable in adult tumors, have no bearing on risk in pediatrics. These differences reinforce the idea that treatment regimens tailored to adult diseases may not necessarily be effective therapy for pediatrics.

From a classification standpoint, biological markers are already being used to stratify therapy for certain types of pediatric brain tumors. In the infant tumor protocols within the Children's Oncology Group, loss of chromosome 22 or mutations in the *INI1* gene are used to identify and distinguish atypical teratoid/rhabdoid tumors from infant supratentorial primitive neuroectodermal tumors. These two molecularly distinct tumors then receive different treatment. In the current older patient medulloblastoma studies, analysis of neurotrophins, a positive prognostic factor, and *ERBB2*, a negative prognostic factor, are being analyzed prospectively to determine whether it is possible to influence therapy through real-time analysis of tumor material.

An important prognostic indicator, directly tied to therapy, was found when MGMT status was analyzed. MGMT is a proximal resistance mechanism to alkylating agents such as lomustine and temozolomide. Tumors with unmethylated promoters overexpress MGMT and carry a much worse prognosis than those that do not. Two of the most recent high-grade glioma studies used alkylators as part of the treatment regimen, but the importance of MGMT status was not known at the time of the trials. Upon retrospective analysis, all of the patients with tumors that overexpressed MGMT had a much worse prognosis and died within 2 years. In response to this, new studies being conducted by the Pediatric Oncology Group and the Pediatric Brain Tumor Consortium are treating tumors that overexpress MGMT with O6-benzylguanine, which depletes MGMT. This is just one example of how the molecular information being gathered can translate into therapeutic strategies.

In addition to providing potential therapeutic targets, understanding the molecular biology of tumors provides the rationale for prioritizing drugs for clinical trials. There are far too few patients to test all of the potential therapeutics being developed, and the development of relevant animal and in vitro models is a necessity. As new drugs based on molecular targets come out, the genotype-dependent impact on response needs to be followed and correlated. A decade ago, Dr Pollack was studying UCN-01, a protein kinase C inhibitor. The agent was

effective against U87 and A172 cell lines. However, as more cell lines were tested, this response was not maintained, and it was found that p53 pathway alterations were a common feature in all nonresponding cell lines. The optimal use of therapeutics depends upon an understanding of tumor genotype. Currently, a Pediatric Brain Tumor Consortium study testing lapatinib and an epidermal growth factor receptor inhibitor is obtaining tissue specimens during treatment to correlate with clinical response.

Dr Pollack closed by discussing an additional implication of the use of more directed molecularly targeted therapies. Multiple signaling pathways are involved in pediatric tumors. Many agents effectively block their target, but the downstream effect of this blockade is reduced due to parallel signaling cascades. Blocking just one target will likely prove ineffective, as multiple pathways may be deregulated. As we move forward, combinatorial approaches, as well as other strategies such as immunological receptor targeting, will be very important. One study that assessed the combination of an epidermal growth factor receptor inhibitor coupled with Raf kinase inhibitors or heat shock protein inhibitors showed a synergistic potentiation of cytotoxicity correlated with synergistic inhibition of downstream signaling phosphorylation. Importantly, in combinatorial studies there is a shifting of the dose response curve to the left in glioma cells but not in normal cells. There are some challenges with this, as it will require multiple companies to collaborate in study development and patients may experience enhanced toxicities as targeted therapeutics are combined. In conclusion, optimal patient-tailored therapy is a work in progress.

Question-and-Answer Session 2

Audience Member: The histologic slides that you showed of the mouse in which you deleted *PTCH1* in the granule cell precursors looked very much like the human disease Lhermitte-Duclos syndrome, which is part of Cowden disease, which is a *PTEN* mutation, except they don't develop medulloblastomas. They do develop breast cancers and other things, and I am wondering whether there is something, maybe a *PTEN* mutation that could stop that particular thing from going further.

Dr Wechsler-Reya: That is a really interesting observation. I think the features of that disordered cerebellum you are referring to are the nodules distributed through the cerebellum prior to full formation of a tumor. What is really interesting is the tight association between developing granule cell precursors and Purkinje cells, and maybe some of the other cell types, so that ectopic cells of one type draw in the cells of the other type to create a sort of microarchitecture. So, in Lhermitte-Duclos, my impression is that there is a primary defect in the Purkinje cells and that may lead to ectopic Purkinje cells that draw in the granule cells in a sort of obverse of, I think, what we are seeing, which is ectopic granule cells clustering around Purkinje cells. But it is a really interesting observation, and, separate from that, I think it would be worth looking at PTEN status and PTEN/AKT pathway in those tumors, because there is certainly evidence for that playing a role in tumorigenesis.

Audience Member: Neurologists often run into patients with aplastic or dysplastic cerebellum. Is there anything to learn from these patients with aplasia of the cerebellum in terms of their biologic features and how it may relate to organogenesis and perhaps to tumorigenesis?

Dr Gilbertson: Because they are generally rare phenomena, there haven't been much molecular studies done specifically in the cerebellum, making it difficult to answer that question. Although I would like to pick up on the Lhermitte-Duclos issue. Suzy Baker made a model of Lhermitte-Duclos by deleting *PTEN* within a granule neuron precursor. In these

models, one of the critical issues to remember is the morphology is largely a feature of migration, and so you can have a profound effect on how the cerebellum develops simply by messing up the migration of those particular populations, which may relate to the relationship of cells to atrophic effect. But that doesn't mean they are particularly modeling a specific feature. So by having an impact on a number of different molecular pathways, you can have a similar morphology at the end of the day, which may reflect an underlying defect of migration rather than a common sharing of molecular elements as the basis of those diseases.

Dr Allen: Dr Pollack, the *MGMT* methylation status, is that a tumor-specific phenomenon or is that a constitutional phenomenon, that is to say something you could measure in a patient's blood or nontumor tissue, or does the tumor acquire that in the process of its transformative events?

Dr Pollack: There is a two-part answer to that. The MGMT expression status is something that is acquired, so MGMT expression status varies throughout the body in different tissues and in different states of disease. White blood cells, for example, normally express MGMT, and interpreting the MGMT assays often can be challenging, because if you have normal white blood cells you will see MGMT staining. Brain cells normally don't express MGMT, so any expression is abnormal and is an acquired phenomenon. Tumors with previously low MGMT expression can become high MGMT expressors as they get exposed to alkylating agents. The issue of measuring that in the blood would be challenging, since it is expressed in normal cells and methylation status can vary widely, although some groups have talked about using methylated DNA as a surrogate for tumor behavior. Methylated *MGMT* is a favorable prognostic factor, so I am not sure that is necessarily going to be applicable for that, but people are trying to use surrogate markers in the blood to assess disease status.

Dr Allen: The molecular story unfolding for medulloblastoma in the various hypotheses of the correlation of the histologic types. How does this apply to pineoblastoma and supratentorial primitive neuroectodermal tumors?

Dr Wechsler-Reya: What we know about supratentorial primitive neuroectodermal tumors — actually, Scott Pomeroy's data is probably the first impetus for people to begin to look carefully at these and say, “These are molecularly distinct entities even though they look similar to a pathologist.” I don't know whether anyone would yet venture to guess at the cell of origin, for example, for a supratentorial primitive neuroectodermal tumor, or to go out on a limb about the molecular lesions that are important in those and whether they are homogeneous or heterogeneous. But, as a general comment, I would say that for any given region of the brain, ignoring the possibility that precursors are migrating from one region of the brain to the other, there are candidate progenitors or stem cells that could give rise to the tumors. I think it is really worthwhile, as we have begun to do for the cerebellum, to look at those regions and say, “What are the stem cells and what are the progenitors, and how do those normal cells resemble the tumor cells?” That can give clues as to where the tumors are coming from.

Dr Gilbertson: This began with Rubenstein, Rork, and others who argued whether or not these tumors are related to some common submatrix stem cell. The critical issue is, why do you want to know the answer? The reason we want to know the answer is because of therapeutics, so what is emerging in astrocytomas from David Gutmann and others, as well as our work in ependymomas, suggests that with a distinct cell of origin, the reason these cells develop into tumors that have distinct pathway mutations is because they inherently depend upon different pathways during development. The implications of that is if we understand, as Dr Wechsler-Reya has alluded to, the distinct cells of origin, and what their

biology is, that should help us guide therapeutics for those patients at the end of the day. I think various approaches that are being used should actually lead, hopefully, to those kinds of discoveries soon.

Controversies & Unanswered Questions

Moderator: David H. Gutmann, MD, PhD, Washington University, St. Louis, MO

Low-Grade Gliomas

David H. Gutmann, MD, PhD, Washington University, St. Louis, MO

Dr Gutmann spoke about low-grade gliomas: grade I pilocytic astrocytomas, noted for their hair-like histological appearance, and grade II fibrillary astrocytomas, a more cellular tumor type. Researching the molecular pathogenesis of these low-grade tumors has included studying inherited cancer syndromes in which patients develop low-grade pilocytic astrocytomas, such as Li-Fraumeni syndrome and neurofibromatosis type 1. Dr Gutmann focused on the use of the differentiated tumor type and microenvironment effects on tumorigenesis, and how these can be used to find new targets for therapeutic drug design.

Using wild-type and neurofibromin-deficient astrocytes, it was found that many of the proteins that were deregulated by a mutation in *NF1* were in the ribosome biogenesis pathway and, thus, were involved in protein translation. Loss of *NF1* leads to high levels of activation of the mammalian target of rapamycin pathway and associated proteins, S6 kinase and ribosomal S6. This observation was tested in a mouse neurofibromatosis type 1 optic glioma model, and neurofibromatosis type 1-associated pilocytic astrocytomas again demonstrated high levels of mammalian target of rapamycin pathway activation. A low dose of rapamycin was used in vitro to block the growth of neurofibromin-deficient astrocytes without affecting wild-type cell proliferation. These promising findings have led to preclinical tests, and rapamycin is currently being considered as a drug for human neurofibromatosis type 1 tumors.

To find additional therapeutic targets, it is important to understand how the mammalian target of rapamycin pathway regulates protein translation and ribosome biogenesis. This appears to be by regulating a protein essential for ribosome transport out of the nucleolus. In addition, pathway-associated proteins that may be involved in growth or motility are also potential targets, and research is under way to identify any candidates.

Another important factor in tumorigenesis is the microenvironment. Evidence for its significance comes from work aimed at creating a mouse model of neurofibromatosis type 1 optic gliomas. Mice in which *NF1* was selectively inactivated in astrocytes of the brain and spinal cord failed to develop tumors. However, when every cell in the body contained one mutated *NF1* allele and one wild-type and the astrocytes in the brain and spinal cord lacked *NF1*, the mice developed typical low-grade optic gliomas. So, the *NF1* heterozygous brain environment may contribute to tumorigenesis. It was found that microglia infiltrated the mouse optic gliomas, which is consistent with observations of human pilocytic astrocytomas. Further investigation suggests that microglia may actually promote the growth of *NF1*-deficient astrocytes via specific growth factors.

To find the effects of *NF1* heterozygous microglia, minocycline, an antibiotic used to inactivate microglia, was used to ablate them in a cohort of neurofibromatosis type 1 tumor-bearing mice. Ablating the microglia resulted in a significant reduction of tumor cell proliferation. Then, using co-culture and supernatant studies, it was shown that these microglia produce paracrine factors, one of which is believed to be a member of the

hyaluronidase family, meningioma-expressed antigen 5. This factor was shown to not only support *NF1*-deficient astrocyte growth but also other tumors. Such paracrine tumorigenic factors may provide additional treatment targets.

Dr Gutmann concluded that an integrated approach to low-grade glioma therapy that not only targets tumor cells but also the microenvironment and progenitor cells may prove to be the most complete and effective.

Aggressive Infantile Embryonal Tumors

Tobey MacDonald, MD, Children's National Medical Center, Washington, DC

Dr MacDonald spoke about aggressive infantile embryonic tumors. They remain rare (100 to 200 are diagnosed per year in the United States) and thus not much is known about their biology or the most effective therapies. The diagnosis and classification of these tumors remains controversial and outcomes of infants remain poor. Although we are continually gaining knowledge about the molecular genetics of brain tumors, validated therapeutic targets are still lacking.

Patients with medulloblastoma typically present with increased intracranial pressure and hydrocephalus. However, infants may instead present with increasing head circumference, ataxia, dysmetria, and even hemiparesis from cerebellar invasion. Signs and symptoms associated with atypical teratoid/rhabdoid tumor and supratentorial primitive neuroectodermal tumor vary depending on their location. Atypical teratoid/rhabdoid tumors appear mainly in children under the age of 3 years and approximately half are in the posterior fossa.

Progress has been made in the treatment of nonmetastatic medulloblastoma patients with intensive therapy such as high-dose methotrexate or high-dose chemotherapy with stem cell rescue, but there has been markedly less success in the treatment of metastatic tumors and atypical teratoid/rhabdoid tumors. A collection of tissue samples to explore the genomic and proteomic characteristics of these tumors is necessary to help identify new therapies. Identified targets must first be systematically validated in preclinical models and then tested clinically. Dr MacDonald asked all neurosurgeons, neurologists, and oncologists to place a concerted focus on the collaborative collection of tumor tissue, whether frozen or in paraffin sections, or of primary cells.

Cutting-Edge Research in Ependymomas

Thomas E. Merchant, DO, PhD, St. Jude Children's Research Hospital, Memphis, TN

Dr Merchant discussed pediatric ependymomas. According to data from the Central Brain Tumor Registry of the United States, 1200 cases of ependymoma are diagnosed yearly, and 300 of these are in children under 19. The extent of disease at presentation, the degree of resection, and tumor grade are all prognostic factors for ependymoma outcome. These tumors can occur throughout the central nervous system, and the best way to assess their grade remains controversial.

Treatment of ependymomas includes surgical resection of the tumor. The extent of resection has been shown to significantly affect 5-year event-free survival, and surgical treatments have improved with advances in neuroimaging and navigation and as the idea of performing a second surgery to achieve gross total resection for tumors that are difficult to remove, such as those that surround the brainstem, has caught on. The risks of an aggressive surgical approach include neurologic morbidity if cranial nerves or motor pathways are affected as

well as basilar invagination or cervical subluxation. Additionally, damage of perforating vessels during surgery may increase the risk of necrosis from radiation therapy.

Dr Merchant is currently studying the use of chemotherapy prior to a second surgery to help improve the degree of resection. Chemotherapy has also been used to delay radiation therapy in children under the age of 3 years, but recent data show that this negatively affects overall survival. A newer approach of focusing radiation on the postoperative tumor bed and on the small clinical target volume margin rather than the gross tumor volume allows the irradiation of even small children, with improved sparing of normal tissues. A phase 2 clinical study of ependymomas conducted at St. Jude's and published in 2004 found that 5-year event-free survival was 86%, an improvement over previously reported figures. Gross total resection remains a major prognostic factor; however, children who undergo additional surgeries have poorer outcomes. Tumor grade also remains a significant factor, with children with anaplastic tumors having worse outcomes than those with differentiated tumors, even with gross total resection and an increased radiation dose. Tumor location and patient age appear not to be significant factors, but girls tend to have better outcomes than boys. This clinical trial also considered functional outcomes and showed that IQ and other measures of cognitive function remained in the normal range for most patients, including those less than 3 years old at the time of treatment. It is possible to predict functional outcomes based on radiation dose, and this suggests that minimizing radiation to normal tissue may further improve functional outcomes.

Endocrine function was also studied as part of this trial; it correlated positively with cognitive outcome and was related to radiation dose. Baseline endocrine testing also showed that a subset of patients had growth hormone deficiency prior to radiation therapy, and aggressive intervention may be necessary for these patients. Hydrocephalus and ventricle size were also predictive of growth hormone deficiency and endocrine dysfunction.

Dr Merchant also reported that his institution has had success with irradiating the cervical spinal cord and brainstem with higher radiation doses without significant complications. He stated that as more is known about the biology of these tumors, markers may arise to predict the need for radiation or adjunct therapy for specific tumors. The goals for improving ependymoma outcomes remain continual improvements in macroscopic and microscopic resection, improved radiation targeting, and improved detection of central nervous system dissemination to find both local and distant metastases.

Pontine & Spinal Cord Gliomas: *Neur* axis of Evil

Bernard L. Maria, MD, MBA, Medical University of South Carolina, Charleston, SC

Dr Maria spoke about the challenges of managing brainstem and spinal cord tumors in children. He first touched on the importance of developing a mechanism for tissue collection to improve molecular characterization and the identification of therapy targets for these tumors. The major tasks in this area include identifying the cells of origin of these tumors (which may differ based on tumor location) and studying them in the context of their cellular environment, expanding preclinical testing and the use of appropriate animal models or human cell xenografts, and, lastly, focusing potential therapies on the biological characteristics of individual tumors.

Brainstem tumors account for 10% to 20% of pediatric central nervous system tumors. There are approximately 300 cases of pontine glioma per year, of which three-fourths are the diffuse infiltrative type. The focal types are midbrain, pontine, or medullary, and neurological presentation of these tumors varies with location. The focal lesions tend to represent low-grade tumors, whereas the diffuse pontine gliomas are high-grade.

Several dose and protocol variations have been tested for the treatment of diffuse pontine glioma, but the standard protocol remains single daily radiation fractions of 54 Gy and an unsupported role for chemotherapy and immunotherapy. Trials that use a biologic therapy based on the status and characteristics of the tumor are in the planning stages (Table 1). This again highlights the need for banking and studying human tumor tissue.

Glioma studies

One method of preclinical modeling of brainstem glioma uses the U87 malignant human glioma cell line engrafted into the brainstem of nude rats. This cell line forms well-circumscribed lesions that lack the invasive characteristics observed in children with this disease, but spectroscopic analysis of cerebrally implanted U87 suggests these tumors metabolically resemble those seen in human patients. Another benefit of this approach is the ability to transfect tumor cells with luciferase so they can be easily imaged with bioluminescence as they grow. It is hoped that in the future a similar orthotopic xenograft approach advanced by Dr David James from the University of California at San Francisco can be taken, with primary tissue collected from human tumors that can be used to test various therapeutic agents.

As seen elsewhere in this journal issue, CD133+ stemlike glioma cells can be identified in human diffuse pontine gliomas, and it is likely they contribute to radiation and chemotherapy resistance. Dr Maria also discussed intramedullary and spinal cord tumors in children. These are relatively rare tumors with a great histological diversity that can disseminate readily throughout the cord despite benign histology. The outcome associated with these tumors is not favorable, as two-thirds of spinal cord tumors in the largest reported series were high-grade gliomas. Dr Maria presented a preclinical model of stereotactically engrafting C6 rat glioma cells into rat spinal cords. These engrafted cells appear to readily invade white matter tracts of the cord and replicate patterns of invasion of Sherer structures observed in human malignant gliomas. Immunohistological staining of rat spinal cord sections that were engrafted with these cells shows the presence of nestin+ cells, some of which appear to be progenitor cells arising from the pericentral canalor from the bone marrow.

Hyaluronan, a proteoglycan that surrounds most cells and interacts with the cellular CD44 receptor, has been shown to play both a structural role in cells and an instructive role in promoting the malignant properties of tumor cells. Oligomer fragments of hyaluronan that do not interact with the receptor have shown some neuroprotective effects. Dr Maria is exploring the use of 2500-dalton hyaluronan oligomers that may displace hyaluronan, limiting its interaction with CD44, and thus limiting the activation of signaling pathways involved in drug resistance and anti-apoptotic behaviors. In a preclinical rat spinal cord glioma model, a single treatment with hyaluronan oligomer showed antitumor activity against C6 rat glioma cells and an isolated population of breast cancer resistance protein/CD133+ progenitor cells, which typically show greater treatment resistance. Dr Maria emphasized that the translation of any promising treatment to clinical use depends on effective delivery methods. He again highlighted the need for a collaborative, standardized mechanism of collecting and banking tumor tissue to identify additional potential therapy targets.

Novel Delivery Strategies

Russell Lonser, MD, National Institutes of Health (NIH), Bethesda, MD

Dr Lonser discussed the current applications of convection-enhanced therapy. Many potential therapies have been identified over the past several years; however, their

translation from in vitro success to clinical use has been limited in part by available drug delivery techniques, including systemic, intraventricular, or intrathecal application or the use of drug-infused polymers. Systemic delivery is limited by the blood-brain barrier and systemic toxicity, and the drug distribution is non-targeted. Intraventricular or intrathecal administration bypasses the blood-brain barrier by infusing the treatment directly into the central nervous system, but it is a diffusion-driven system and penetration is limited to millimeters, with an exponential drop in penetrating concentration. It is also nontargeted and thus holds the potential for significant toxicity. Lastly, polymer wafers infused with drugs that diffuse through the surrounding aqueous environment are a newer approach, but are also diffusion-dependent, and penetration is only 1 mm to 2 mm, with an exponential concentration decline.

Instead of diffusion, bulk flow or convection-enhanced delivery uses a continuous pressure pump to create a hydrostatic pressure potential that drives the therapeutic agent through the interstitium of the central nervous system. It has the benefits of bypassing the blood-brain barrier, and molecules to which the barrier is impermeable cannot pass out of the central nervous system after infusion; it can directly target gray or white matter areas; and it infuses areas in predictable and homogenous ways related to the interstitial space of an area. In mini-pig studies, infusion of C14-albumin in the spinal cord showed that high concentrations can be infused to very localized regions.

Another limitation of some treatments that show success in animal models but fail to translate into human clinical use is perfusion of a therapeutic volume. Convection-enhanced delivery has been shown in large animal brains to provide holohemispheric and complete brainstem infusions. Real-time imaging of convection-enhanced delivery is an important aspect of evaluating a drug's efficacy by ensuring that it reaches the treatment target. One method of doing this is via surrogate image tracers, which can be mixed with the treatment and imaged on CT or MRI. Tracer substances must be developed that have no effect on the treatment, are safe, are readily produced, and can be imaged in high-resolution. Two compounds Dr Lonser has explored for MRI are gadolinium-diethylnetriamine penta-acetic acid and gadolinium bound to albumin. Autoradiography experiments of these compounds coinfused with a variety of molecular substances show a less than 15% (or < 1 mm radius) difference in drug and tracer distribution.

After the success of these preclinical trials, this approach was tested clinically in a 3-year-old patient with diffuse pontine glioma, a universally fatal tumor for which radiation and chemotherapy are only palliative. Interleukin-13 bound to *Pseudomonas* toxin was chosen as the treatment to infuse, as it is taken up by glioma cells that contain an interleukin-13 receptor, causing selective glioma cell death. This treatment, mixed with gadolinium-diethylnetriamine penta-acetic acid, was initially tested in the nonhuman primate brainstem to confirm its safety both functionally and histologically. Additional in vitro studies confirmed the gadolinium was not neurotoxic. Before convection-enhanced delivery of interleukin-13 bound to *Pseudomonas* toxin, the patient had left facial weakness, bilateral VIth nerve palsy, and asymmetric gait ataxia, findings typical of diffuse pontine glioma. Interleukin-13 bound to *Pseudomonas* toxin was infused at 0.5 μL/minute to 5 μL/minute and sequential MRIs were taken throughout the process. Infusion continued until imaging suggested that the whole region was infused. A greater volume than expected was necessary, potentially because of vasogenic edema that increased the interstitial volume. Posttreatment imaging showed no evidence of toxicity and stable tumor size for 3½ months postoperatively, although the patient developed subsequent disease progression and expired. This clinical trial suggests that this method of treatment infusion is safe, even with neurological dysfunction or edema, and can be used to test drug efficacy.

Question-and-Answer Session 3

Audience Member: I have two questions. First, we saw several slides that showed an animal model of tumors and they were non-immune animals, and my question is, what role does immunity play in tumor treatment? The other question is for Dr Gutmann, who mentioned that neurofibromatosis type 1 tumors can disappear, and we know that the tubers described in the heart of patients with tuberous sclerosis also disappear. What is known about this process of spontaneous tumor regression?

Dr Gutmann: I think it gets back to a theme that I think everybody is developing here, which is that some of these pediatric cancers or pediatric tumors may be really on the verge of mildly abnormal developmental processes, and it is entirely possible that in the case of the optic gliomas and the tubers, they are still responsive to environmental signals that dictate their growth, and in the absence of those developmental signals, the tumor may either stop growing, or in some situations may even shrink. That certainly is a thought that has been posited for the optic gliomas. In the case of the neurofibromas and the peripheral nerve tumors, there has been some thought that you have a locally contained tumor microcosm that has a limited number of growth factors that are produced by fibroblasts and perineural cells and mast cells, and after awhile those may be used up, they may not be continually produced, and we certainly notice that patients with neurofibroma have their tumors grow for periods of time, stop growing, and then feel as if they are mostly composed of extracellular matrix and very few tumor cells.

Dr Maria: David James, could you address the question on the importance of immunity, because I think you pointed out that if one was looking at immune therapies that you would have to be careful about using a nude mouse in terms of modeling, obviously, but can you address the question of whether eliminating the immune system in modeling is a potential problem?

Dr James: I guess my opinion is it is dependent on the nature of the therapeutic that is used. I think for the small molecule inhibitors, the targeted kinase inhibitors, that the lack of immune function is not a major issue in assessing efficacy.

Dr Gutmann: I think you could also make the argument that in some tumors the innate immune system cells that are present in the brain either positively or negatively regulate tumor growth, and so there may actually be a role for macrophage-like cells in either the continued growth or even regression of brain tumors.

Dr James: Yeah, I think that is an interesting idea and it is an idea that can be investigated experimentally, such as with a model system that you described earlier today.

Dr Packer: A question to Dr Maria but then to the entire group. You postulated a different way to attack these tumors by antagonizing hyaluronan. Can you expand a little bit on how these drugs are going to be delivered, what the effects of these kinds of drugs may be on normal cells, or other mechanisms, and whether these things, even if given selectively, within the brainstem, are going to stay there? This seems to be such a common mechanism for cells.

Dr Maria: So you are asking about issues of diffusibility and delivery, which are obviously key issues to any new agent. These oligomers are not hyaluronidases, but rather they are shorter fragments of the native hyaluronic acid polymer that keep it from its normal interaction on the cell surface. So far all of the work that we have done has been intratumoral work, direct installation, single injection, measuring tumor volumes, invasiveness, and also potentiation of radiation and chemotherapy in that animal model.

Systemic routes may or may not be effective. Whether delivering oligomers via the use of hyaluronidases in the skin that would generate essentially a native oligomer that would circulate systemically and potentially radio and chemosensitize an intracranial or intraspinal tumor needs to be addressed. We haven't looked at intrathecal or intranasal delivery, but these are 2500 daltons in size, so we have concerns that it wouldn't necessarily get there systemically. One might have to use things like convection-enhanced delivery described by Dr Lonser. We expect the oligomers to be highly diffusible in the central nervous system, which is a positive and a negative depending on the treatment paradigm and tumor type.

Dr Allen: A question perhaps to Dr Gutmann, or anyone else who wants to field it. The problem we have with the low-grade fibrillary astrocytomas, certainly in adults, and I guess in pediatrics in the thalamus and the brainstem, is its genetic instability and tendency to transform. And yet we have another tumor, the pilocytic, which doesn't seem to do that. It can grow and make cysts, but it is biologically distinct and really it is a reportable case that transforms. So, I guess David, knowing as much as you do about pilocytic astrocytomas, what do you think is different about a grade I versus a grade II that makes the biology totally different?

Dr Gutmann: That is a fantastic question, and I think there is a lot of work going on, not only in my lab, in collaboration with Tobey MacDonald, but in other places, to begin to look at the differences in the genetics between the grade I and the grade II astrocytomas. I think, at least at this point, I have to profess ignorance, I don't think we know enough about what the molecular differences are, about what the cell of origin is, I think we just simply don't know at this point.

Executive Summary

Abhijit Guha, MD, MSc, FRCSC, University of Toronto, Toronto, Ontario

Dr Guha presented a closing talk summarizing the major points of the day and including some remaining questions for the Future Directions discussion. The first session focused on the clinical aspects of central nervous system tumors. As outcomes have somewhat improved in low-grade tumors, medulloblastoma and some embryonal tumors, quality of life remains an important focus. This includes not only decreasing treatment toxicity but also improving posttreatment care. Another major clinical focus is developing improved biological understanding of pediatric brain tumors and translating this understanding into practical management strategies. However, pediatric brain tumors remain relatively rare, so recruiting enough patients to successfully test potential treatments is a major barrier. Expanding beyond North America to potentially include the large Chinese and Indian patient populations as well as European patients, whether via biological samples or in clinical trials, was suggested.

Improvements in imaging have resulted in improved anatomic localization and staging of tumors. Imaging has also successfully been used as a surgical aid. However, imaging of infiltrative tumors remains limited. Functional and biological imaging has great promise in improving this as well as helping to define tumor biology and possible toxicity or late effects of treatment. In terms of radiotherapy and chemotherapy, there have been steady improvements in focusing radiation protocols to the tumor and decreasing radiation to normal surrounding structures. Advances in chemotherapy have been mainly through combinations and dosage changes. The side effects of radiation and chemotherapy on neurocognitive and neuropsychological functions as well as central nervous system biology must still be better understood and addressed. It is agreed that delaying radiation in the

developing brain if possible reduces these side effects but may worsen disease outcome. Dr Guha suggested that quality-of-life studies should be built into every clinical trial.

Another area of great interest is the origin of pediatric brain tumors, particularly the question of cancer stem cells, which are capable of repopulating an entire tumor. Unmasking the origin and development of specific tumors may provide more promising therapeutic approaches. Identifying the different phenotypes that comprise both child and adult brain tumors may lend information about their radioresistance, angiogenesis, metabolism, and apoptosis, which can be used to optimize therapy. Low-grade gliomas were identified as an area of pediatric brain tumors where very little is known. There is a need for better preclinical models of these low-grade tumors as well as a central patient registry. These research priorities were similar for the brainstem and spinal cord gliomas, ependymomas, and embryonal tumors.

In the field of preclinical models, Dr Guha concluded that the best model depends on the question being asked, but that each one has limitations and the stromal cells, immune cells, microenvironment, and epigenetic factors must all be accounted for in each model. Finally, in the field of molecular-targeted therapies, identifying effective combinations of therapies, working with industry to develop these therapies, moving therapies used for adults into the pediatric realm, and validating treatment targets remain major challenges.

Future Directions

Moderator: Jane Fountain, PhD, National Institute of Neurological Disorders and Stroke Program Director, National Cancer Institute (Cancer Therapy Evaluation Program)

Panel Discussion

Jeffrey C. Allen, MD, New York University Medical Center, New York, NY

Susan Blaney, MD, Texas Children's Cancer Center, Houston, TX

Nalin Gupta, MD, PhD, University of California San Francisco, San Francisco, CA

Scott Pomeroy, MD, PhD, Children's Hospital, Boston, MA

Michael D. Taylor, MD, PhD, Hospital for Sick Children, Toronto, Canada

The panel discussion focused on addressing the questions and challenges highlighted in Dr Guha's executive summary. Dr Jane Fountain of the National Institute of Neurological Disorders and Stroke also asked that panelists attempt to identify the top priorities in pediatric central nervous system tumors that the community and NIH should focus on. The panel suggested the following topics as important areas for future research:

Clinical Management

- Care must be taken to maximize posttreatment quality of life for patients by continuing to reduce treatment toxicity and improving posttreatment care and monitoring of cognitive, neurological, and endocrine functions.
- Imaging modalities must be improved in their ability to image infiltrative tumors.
- Organizations such as the Children's Oncology Group allow for improved care by allowing every cancer center to provide online information, instantly updated, possibly contributing to the latest standard of care throughout this country and many others.

- Better education of primary-care practitioners in the identification and management of central nervous system tumors in children is a priority in improving treatment and functional outcomes.

Translational Research

- A new method is necessary to identify and validate treatment targets in patients and more predictive preclinical studies must be done to evaluate targets before embarking on clinical trials.
- Because of the great number of potential therapies, a new testing paradigm is necessary to test the efficacy of multiple agents rapidly in phase 2 trials.
- It was suggested that the National Institute of Neurological Disorders and Stroke organize a meeting of the best neurobiologists from the leading pediatric neuro-oncology centers to develop a new paradigm for translating neurobiologic advances from what we understand about brain development into therapeutic approaches for children with pediatric brain tumors.
- Additional multiple-principal investigator grants that pair basic scientists with clinical researchers to work on therapeutic development must be developed.
- Mechanisms of effective therapeutic delivery and validation of reaching the target must also be further investigated.
- A method of collaborative specimen collection of pediatric central nervous system tumor tissue is necessary to improve molecular characterization of tumors and to continue to identify additional targets.

Clinical Trials

- The struggle to recruit enough patients for clinical trials was highlighted as a major concern. Expanding trials outside North America was suggested as a possible way to do significant clinical trials in a timelier manner.
- Because potential therapies and delivery mechanisms carry great cost, the appropriate manner of collaboration with industry should be considered. A method of drawing pharmaceutical companies to the field of pediatric central nervous system tumors is also necessary.
- A gap was identified in funding and research focused on juvenile pilocytic astrocytomas and other low-grade gliomas.

The panel's overall message was to continue to emphasize collaboration both between fields and across borders to improve care for children with central nervous system tumors as significantly and rapidly as possible. The roles of education for physicians and recruitment of young investigators to the field were also stressed as areas of major importance.

Table 1

Compounds Tested for Treatment of Diffuse Pontine Glioma

Primary Trials
tipifarnib (PBTC-014)
capecitabine RDT (PBTC-021)
vandetanib (SJBG07)
Recurrence Trials
06-benzylguanine and temozolomide (PBTC-015)
bevacizumab and irinotecan (PBTC-022)
In Design
bevacizumab, MGMT-directed temozolomide, and EGFR-directed erlotinib

Abbreviations: EGFR = epidermal growth factor receptor; MGMT = methylguanine-DNAmethyltransferase; PBTC = Pediatric Brain Tumor Consortium; RDT = rapidly disintegrating tablets; SJBG = St. Jude's Brainstem Glioma.