Published in final edited form as:
Lancet Neurol. 2010 July ; 9(7): 717–726. doi:10.1016/S1474-4422(10)70105-8.

Molecular pathology in adult neuro-oncology: an update on diagnostic, prognostic and predictive markers

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Introduction

Primary tumours of the central nervous system (CNS) account for roughly two percent of human malignancies. The most common CNS tumours of adults are malignant gliomas. In general, malignant gliomas are not curable tumours, but their management has undergone change over the past two decades, with novel approaches to surgery, radiation and chemotherapy improving survival and quality of life for patients to variable degrees. The present review addresses how new molecular diagnostic advances have also played roles in changing the management of patients with malignant gliomas over the last twenty years.

Until recently, treatment decisions regarding malignant gliomas began with establishing diagnosis by standard histopathology only. Histopathology has been a dynamic tool for nearly a hundred years, providing knowledge about the biology and behaviour of many neoplasms. The 2007 WHO classification, the most recent consensus approach to CNS tumor diagnosis, divides the malignant gliomas of adults into astrocytic tumors—the most malignant of which is termed glioblastoma—as well as oligodendrogliomas and oligoastrocytomas.1 The data gained from histopathologic examination of tumour tissue has been augmented, but not supplanted, by molecular approaches to tissue diagnosis.

Knowledge about the molecular biology of cancer—including CNS tumours—continues to increase markedly. Having a dynamic classification of tumours allows the integration of new markers that are discovered to help determine prognosis and likelihood of therapeutic response. This review focuses on those markers that have found utility in the evaluation of adult malignant gliomas—specifically on 1p/19q co-deletion, methylation of the O_6 methylguanine methyltransferase (*MGMT*) gene promoter, evaluation of the epidermal growth factor receptor (EGFR) pathway and isocitrate dehydrogenase 1 (*IDH1*) gene mutations. The reader is also reminded that molecular approaches are also used in the assessment of other CNS tumours of neuroepithelial origin, notably in pediatric tumors such

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Conflicts of Interest
The authors have no conflicts of interest to declare.

as medulloblastoma,2 atypical teratoid rhabdoid tumour3 and potentially in pilocytic astrocyoma.4

1p 19q loss in glioma

Background

Following empiric observations of favourable responses to chemotherapy in a high proportion of recurrent anaplastic oligodendrogliomas (AO III), Cairncross et al documented that AO III's with loss of the short arm of chromosome 1 (1p) were preferentially chemosensitive when treated with a procarbazine-lomustine-vincristine (PCV) chemotherapy regimen. Moreover, they also found that patients whose tumours had co-deletions of 1p and the long arm of chromosome 19 (19q) had substantially improved survival times.5 These findings have been replicated many times over the past 10 years, and the correlations with therapeutic sensitivity have been extended to other agents such as temozolomide 6,7 and modalities like radiotherapy.8 Such data suggest the merit of this marker may not lie in specifically predicting chemosensitivity but rather in demonstrating tumour vulnerability to a broad range of therapeutic options. As a result, assessment of 1p and 19q status has been widely implemented in neuro-oncologic management of AO III patients.9

The prevalence of 1p/19q codeletion has been estimated at 80 to 90% of grade II oligodendrogliomas and 50 to 70% of grade III oligodendrogliomas, and around 70% overall.10 Notably, however, although the 1p and 19q regions have been extensively mapped and many genes evaluated as candidate tumour suppressor genes, no tumorigenic genes have been definitively implicated. In most cases, deletions appear to represent complete chromosomal arm loss, which may be the result of an unbalanced centromeric translocation of 1p and 19q.11,12 Although the responsible oncogenic genes on 1p and 19q remain unidentified, many correlations have been made regarding 1p/19q loss. For example, 1p/19q deleted tumours frequently show classic histology.13–15 and often have *IDH1* and *IDH2* mutations.16 1p/19q codeleted AO III preferentially display a proneural gene expression profile,17 This profile, which is partly characterized by expression of neuronal genes, is overrepresented among low grade gliomas and may predict for therapeutic response in glioblastoma.18 On the other hand, 1p and 19q loss correlates inversely with *TP53* mutations, 10q deletions and amplification of *EGFR*.13 Tumour location is also associated with 1p/ 19q co-deletions: low grade oligodendroglioma and AO III in the frontal, parietal and occipital lobes are more likely to show loss than tumours involving the temporal lobe, insula and diencephalon.19,20,21

1p/19q loss is also found in mixed glial tumours (oligoastrocytomas), albeit in a lower proportion than "pure" oligodendroglioma (20 to 30%).22 Eoli et al demonstrated that in mixed gliomas, 1p loss is associated with prolonged progression free survival. Conversely, tumours without 1p loss show shortened times to recurrence, are more frequently located in the temporal lobe and have radiological features suggesting malignancy.23 Deletions involving 1p and 19q are uncommon in glioblastoma, but in the small number identified seem to predict for shortened survival, possibly reflecting true genomic instability.15

Thus, over the past 10 years, the frequent evaluation of oligodendroglial tumours for 1p/ 19q status has shown that this molecular profile denotes a clinically distinct tumour type with progression, prognosis and treatment responses that are different from other gliomas.10,24 Although the mechanism whereby 1p/19q codeletion generates these phenomena remains unclear, the clear differences between 1p/19q-codeleted tumors and other oligodendrogliomas has made testing useful and commonplace.

Current laboratory testing

In general, most neuro-oncological institutions perform 1p/19q testing on tumours with oligodendroglial morphology, whether grade II or III and whether “pure” oligodendroglioma or “mixed” oligoastrocytoma. Two major methods exist for assessing loss of 1p and 19q: polymerase chain reaction (PCR)-based loss of heterozygosity (LOH) assays and fluorescence in situ hybridization (FISH). LOH assays are based on comparing multiple polymorphic alleles in tumor DNA versus normal blood DNA. Because not all alleles are informative, it is necessary to amplify multiple loci for each chromosomal arm; in addition, the assay requires available normal DNA, possibly delaying test results. Both of these drawbacks limit the applicability of LOH assays to studying 1p/19q status. FISH uses fluorescence-labeled probes to study chromosomes directly on tissue sections, which can be a time-consuming undertaking but which preserves tissue architecture and is therefore a common procedure available in many pathology laboratories. Until recently, studies had shown roughly comparable results between LOH and FISH assays.14 However, Snuderl et al have now demonstrated that additional information can be gleaned from the copy number information also revealed by FISH analysis, but not available in LOH studies: FISH assays allow assessment of polysomy, which may predict for reduced progression free survival.25

Other techniques may also impact testing in the near future. For example, array comparative genomic hybridization, a technique resulting in genome-wide differential labeling of reference and tumour DNA, provides an estimate of copy number at all chromosomal loci examined on a microarray, and can thus provide a ready means for detecting losses and assessing polysomy. Once this technique becomes less expensive and more widely available, it may replace FISH as the preferred technique.

Recommendations

Knowledge of 1p/19q status is now considered standard of care when managing oligodendroglial tumours26 and oncologists more often choose particular therapeutic options in anticipation of the longer survival and progression free survival times associated with this lesion.9 The following section explores the use of 1p/19q testing in four current settings: AO III, lower grade oligodendroglioma, "mixed" oligoastrocytic tumours, and histological diagnosis.

Large studies have now analyzed the potential role of 1p/19q loss in aiding management decisions in the setting of AO III. 1p/19q deletions were incorporated into two major therapeutic trials in patients with AO III. Both confirmed the prognostic and possible predictive role of these biomarkers at initial therapy.27,28 However, it remains unclear whether 1p/19q codeletion correlates with response to second line chemotherapy.7,25 Interpretation of the later stages of treatment in such trials is confounded by the effects of salvage therapy being given to all treatment groups.28,29 The major question, still being debated by the field is whether one can delay radiotherapy in patients whose AO III harbor 1p/19q loss; while the answer is not yet clear, a recent survey has shown that many North American neuro-oncologists are following this practice in selected patients.9 To refine the role of testing, future therapeutic trials of grade III gliomas may wind up recruiting and randomizing based on 1p/19q codeletion status rather than histology30

For lower-grade oligodendrogliomas, 1p/19q testing is also commonplace. 1p/19q codeletion predicts for increased progression free survival in temozolomide treated low grade gliomas,15,31 including radiotherapy naïve tumours32 and when utilized as monotherapy in tumours with 1p loss.31,33 One retrospective series of "expectantly managed" low grade oligodendroglial tumours found that 1p/19q codeletion status did not confer any prognostic advantage in progression free survival.34 Nonetheless, in an

asymptomatic patient with a low grade oligodendroglioma harboring 1p/19q loss, a neuro-oncologist might argue that the likely slow growth and long survival allows for "watchful waiting" in the expectation of a superior response to therapy in the future. On the other hand, in a symptomatic patient with the same molecular signature, one would very likely opt for early therapy in the likelihood that the tumor will respond to therapy and patient symptoms will be ameliorated. At the present time, how 1p/19q status is used to manage patients with low grade oligodendrogliomas remains *sub judice*.

The diagnosis of "mixed" oligoastrocytic tumors is highly subjective, and varies from institution to institution, because of relatively lax diagnostic criteria. In the setting of "mixed" oligoastrocytic tumours, 1p/19q codeletions have been shown to have some influence on prognosis in some studies,23 but the data are probably not extensive or mature enough to affect therapeutic choices-- most notably because the entry criteria for oligoastrocytoma studies vary so much between institutions. Given this subjective diagnostic variability, the objective knowledge that an oligoastrocytoma has a genotype more like an astrocytoma versus more like an oligodendroglioma can provide some reassurance and guidance to clinicians.

Lastly, one must ask whether the 1p/19q codeletion signature should *define* a histologic class of tumour (i.e., oligodendroglioma) because of its prognostic and predictive value. Although it is tempting to do so, the issue remains unclear. Evidence has emerged that subgroups exist within 1p/19q codeleted tumours. These include tumours showing polysomy of chromosomes 1 and 19 with shorter progression free survival more akin to tumours without codeletion, but no decrease in overall survival or response to salvage chemotherapy.25 Moreover, Walker et al recently showed that a sample of tumours with oligodendroglial morphology lacking 1p/19q codeletions contained a small subgroup with a positive response to therapy, implying that other mechanisms contribute to the improved prognosis associated with this histologic entity.35 Such evidence reinforces the definition of oligodendroglioma in the World Health Organization Classification of Tumours of the CNS, as a histologically, clinically and genetically defined lesion.1

MGMT in glioma

Background

Alkylating chemotherapeutic agents have long been used in the treatment of patients with malignant gliomas. At the present time, nearly all glioblastoma patients are treated with the orally administered alkylating agent temozolomide (TMZ).36 TMZ acts to methylate primarily the O_6 position of the nucleotide guanine, resulting in cell death.37 Cells, however, have an inherent DNA repair mechanism that can counter the effects of TMZ: the constitutively expressed DNA repair enzyme MGMT will irreversibly transfer a methyl group from the O_6 position of the modified guanine to a cysteine residue of the MGMT protein, mitigating against the cytotoxic effects of chemotherapy. However, this enzyme is present in finite quantities and approximately half of glioblastomas show decreased levels of MGMT protein, which could make these tumours more susceptible to the effects of TMZ. The primary mechanism whereby MGMT expression is downregulated in glioblastomas appears to be methylation of the *MGMT* gene promoter, which is a common way of silencing gene expression in tumours.37 Thus, the expression level of MGMT and the methylation status of the *MGMT* gene promoter should have predictive value in glioblastoma patients treated with alkylating agents such as TMZ.37,38 Indeed, a number of studies suggest such correlations in glioblastomas,39,40 paediatric glioblastoma41 and lower grade gliomas.42 *MGMT* promoter methylation status has also been shown to be a valid prognostic marker independent of TMZ use in elderly GBM patients.43 Of the many studies, the most notable was that of Hegi et al. who studied *MGMT* gene promoter status in

tumours from patients entered into a large trial to evaluate the role of concomitant TMZ with radiation therapy versus radiation therapy alone.36 In 106 TMZ-treated patients (46 with methylated tumours; 60 with unmethylated tumours), 46% of patients with *MGMT* methylated tumours were alive at 2 years versus only 22·7% of tumours with unmethylated *MGMT*. Assessment of overall survival was confounded by many patients having salvage therapy that included alkylating agents, but this trend appeared to continue. These authors subsequently suggested that assays examining MGMT methylation status, in addition to providing prognostic information, may have utility as predictive tools to determine treatment with TMZ in glioblastoma.44 However, the group with unmethylated *MGMT* in this study also showed a survival benefit with TMZ therapy that, while inferior to that in *MGMT* methylated tumours, nearly reached significance (p=0·06).36 An explanation for this remains unclear, and could, among other reasons, reflect definitions of cut-off levels for separating methylated and unmethylated groups.45,46 However, this beneficial response to TMZ in *MGMT* unmethylated tumours, as well the current lack of a therapeutic alternative, suggests that treatment is unlikely to be withheld from patients on the basis of this test, especially given the oral bioavailability and well tolerated nature of the drug.

Notably, *MGMT* promoter methylation patterns may change in the time between primary diagnosis and recurrence, and change more frequently in tumours that initially show *MGMT* methylation.47 In this regard, the prognostic information conveyed by knowledge of the *MGMT* methylation status appears to apply only to primary tumours and not to recurrent tumours.45,47

Recently, the utility of *MGMT* methylation status in anaplastic (i.e. WHO grade III) gliomas was evaluated in the NAO-04 trial29 and the EORTC 26951 trial.48 Both trials found *MGMT* methylation status conferred prognostic benefit but did not specifically predict for response to therapy with alkylating agents. Interestingly *MGMT* promoter methylation has recently been re-examined in a cohort of patients with GBM treated with radiotherapy only. 49 In this study, *MGMT* methylation appeared to predict for response to radiation therapy.49 Thus in the context of anaplastic glioma and possibly in GBM, the prognostic information conveyed by testing may not solely be attributable to *MGMT* promoter methylation enhancing the effect of therapy with alkylating agents, but may instead indicate a prognostically favorable molecular phenotype.45,49

The attention that surrounded the introduction of TMZ as the standard of care for glioblastomas patients in 2005, and with response to TMZ being associated with *MGMT* promoter methylation status, generated interest on the part of neuro-oncologists and patients to have MGMT testing performed. Although it was not going to alter therapy, many testing centers experienced pressure, generated largely by patients being informed by the internet, to set up the assay nonetheless.

More recently, it has been reported that patients whose glioblastomas have *MGMT* gene promoter methylation have a greater likelihood of demonstrating radiographic "pseudoprogression" when treated with TMZ than those whose tumours lack such methylation.45,50 "Pseudoprogression" is a phenomenon in which the neuroimaging appears to worsen following TMZ, but then improves with continued therapy. Thus, another role for *MGMT* promoter methylation testing could be evaluation of the likelihood of "pseudoprogression".50

Current laboratory testing

Laboratory testing of *MGMT* status entails the use of methylation specific PCR, in which extracted DNA is treated with sodium bisulfite, resulting in substitution of unmethylated cytosine for uracil. Primers specific for methylated and unmethylated CpG rich areas of the

MGMT gene promoter are then utilized to amplify the modified DNA sequences. The assay can be performed on paraffin-embedded tissue, but is dependent on tissue quantity and quality.51 Additional factors such as adequacy of bisulfite treatment, primer specificity and PCR conditions further input into determining the usefulness of the assay.51,52 Not infrequently, both methylated and unmethylated sequences are found in glioblastoma tissue, attributed to either inclusion of non-neoplastic tissue or tumour heterogeneity. Other bisulfite-based techniques including quantitative MSP, bisulfite sequencing, methylation-sensitive single strand conformation polymorphism analysis and mass spectrophotometer-based quantitative analysis are available,26 some of which have been utilized to assess *MGMT* methylation status.51,53 Non-bisulfite- based semi-quantitative techniques have also emerged, such as methylation specific-multiplex ligand-dependent probe amplification (MS-MLPA).54

Given that promoter methylation typically silences gene transcription and protein synthesis, assessment of protein expression directly in cells via immunohistochemistry, a widely available technique, would seem a logical way to assess *MGMT* status. However, immunohistochemical MGMT expression has been found to be heterogeneous both within tumours and within small regions of tumour.55 Reports have attempted to stratify staining intensity to identify methylated tumours,55 but the significance of these indices of staining intensities is currently not clear. Moreover, discordant results have been reported between genetic and protein assessments of MGMT status.56 At the present time, immunohistochemistry is not recommended for evaluating MGMT status in glioblastomas.

Recommendations

Given that TMZ and radiation therapy remain the standard of care for all glioblastoma patients, regardless of *MGMT* promoter methylation status, upfront diagnostic testing for MGMT is not necessary. Nonetheless, *MGMT* promoter methylation status provides prognostic information that many patients and oncologists will wish to have. Moreover, knowledge of *MGMT* promoter methylation status will be important for ascertaining the effects of novel therapy on patients who are enrolled in clinical trials, and must be included in all glioblastoma trials.

As mentioned above *MGMT* promoter methylation status can be useful in the differential diagnosis of true tumour progression versus "pseudoprogression" seen on imaging. In this scenario, for a tumour with *MGMT* promoter methylation, "pseudoprogression" appears more likely and thus therapy with TMZ is often continued despite the "worse" appearance of the neuroimaging. Conversely, a worsening appearance on imaging, in a patient whose glioblastoma lacks MGMT promoter methylation, might prompt consideration to change therapy.50,57

Epidermal growth factor receptor (EGFR) pathway testing

Background

Traditional chemotherapeutic approaches non-specifically target dividing cells through a variety of mechanisms. Molecularly targeted therapies, on the other hand, aim for specific inhibition of aberrant or amplified proteins that directly drive tumour cell growth. Recent successful examples have included imatinab to inhibit the tyrosine kinase fusion protein BCR-ABL in chronic myeloid leukaemia, as well as the C-KIT oncogene in gastrointestinal stromal tumours,58,59 and trastuzamab in the treatment of HER2-positive breast carcinoma.60

A number of growth factors and their receptors are upregulated in malignant gliomas, particularly in glioblastomas. For example, upregulation of epidermal growth factor receptor

(EGFR)-mediated signaling is present in around 30% of gliomas[61,62] and 60% of glioblastoma,[63] with overexpression in glioblastomas generally driven by *EGFR* gene amplification.[64,65] In addition, about half of EGFRoverexpressing glioblastomas feature genetically mutant EGFR molecules, the most frequent of which is EGFRvIII, which constitutively activates the EGFR-PI3K pathway due to deletion of the ligand binding domain.[66] Additional missense mutations have been identified in the exons encoding extracellular EGFR domains.[67,68] Moreover, other described activating mutations in exons encoding extracellular EGFR have been shown *in vitro* to drive oncogenesis and can be inhibited by small molecule tyrosine kinase inhibitors.[68]

The prognostic utility of *EGFR* amplification is not clear, with a number of studies producing contradictory findings.[69] *EGFR* amplification may be prognostically related to patient age[70] and EGFRvIII expression may identify a subgroup of tumours with more aggressive behaviour.[71]

The presence of EGFR overexpression and *EGFR* mutation in glioblastomas raises the possibility that EGFR-targeted agents could be used to treat those patients with glioblastomas that feature these aberrations-- a similar situation to that of some non-small cell lung carcinomas that harbour activating *EGFR* mutations and that show remarkable responses to the EGFR inhibitors erlotinib and gefitinib. However, this optimistic scenario is clouded in the setting of glioblastoma for a number of biological reasons. Many glioblastomas feature dysregulation of signaling cascades downstream of EGFR, such as that mediated by the *PTEN* tumour suppressor gene and involving the PI3K pathway and AKT. This phenomenon may contribute to loss of reactivity to the down regulation of EGFR.[72] In addition, other growth factors such as PDGF also exercise their effect by modulation of these pathways. Finally, many glioblastoma cells may feature activation of multiple growth factor pathways, suggesting the need to use cocktails of molecular targeting agents in glioblastoma patients.[73]

The results in the literature examining EGFR targeting agents looked promising initially. Two studies published in 2005 sought to clarify if assessment of *EGFR* status was useful in aiding decision making to use the small molecule kinase inhibitors gefitinib and erlotinib. Mellinghoff et al showed that co-expression of PTEN and EGFRvIII was associated with increased sensitivity to erlotinib, whereas tumours that lacked PTEN expression failed to respond to erlotinib.[74] Haas-Kogan et al. suggested that glioblastomas with diffuse EGFR immunopositivity that lacked p-AKT stained were likely to show a clinical response to erlotinib.[75] Both studies implied that those tumours that respond to small molecule kinase inhibitors overexpress EGFR or express EGFRvIII; and have an intact PTEN-AKT pathway. A curious feature of these two studies is that, although the immunohistochemical assays measured similar molecules, they did not necessarily represent the same biological endpoints. More problematically, subsequent trials did not show major responses or survival benefits in glioblastoma patients treated with these agents.[76,77] Further studies have since evaluated erlotinib used concurrently with TMZ and radiotherapy.[78,79] These trials have produced mixed results, one showing neither overall benefit nor identifying a vulnerable subgroup of tumours that might respond to therapy;[78] the other suggesting a role for this regimen in patients with tumours showing *MGMT* promoter methylation and intact *PTEN*.[79]

At the present time, therefore, assessment of the EGFR signaling pathway in planning therapy is not clinically indicated, since current standard therapies are not directed specifically at this pathway. Nonetheless, the paradigm illustrated by the EGFR-non-small cell lung cancer story and hinted at in the EGFR-glioblastoma papers from 2005 is a critical one that is likely to surface repeatedly over the next decade. As new molecularly targeted agents reach clinical trials, alone and in combination, these will be tried in the setting of

glioblastoma and it is highly likely that use of these novel agents will be guided by molecular testing of the respective signaling pathways, either at a DNA or a protein level. New methods should enable inquiry along multiple signaling pathways in single tumors on a routine basis. For example, at the Massachusetts General Hospital, a full range of mutational analyses are now carried out in standard paraffin-embedded samples using the Applied Biosystems SNaPshot system, incorporating multiplex PCR, single base extension and capillary electrophoresis. Such prospective and timely analysis already allows patient tailored therapy based on genotyping of known oncogenes and tumour suppressor genes.[80,81]

Current laboratory testing

In glioblastomas, amplification of *EGFR* occurs largely as extrachromosomal, double minute fragments. This makes confirmation of major copy number gain by FISH practical and accessible to many pathology laboratories. Moreover, FISH assays maintain *in situ* information, avoiding false negative assays due to intratumoural heterogeneity.[82,83] Other techniques such as quantitative-PCR and RT-PCR also identify *EGFR* amplification;[84] in addition, these and other molecular techniques can identify mutations in *EGFR*.[84] Immunohistochemical assessment for EGFR expression is widely available, but of less clear value (see below). EGFR expression can be found in lower grade astrocytomas and other malignant gliomas. Moreover, scoring of EGFR immunopositivity can be variable[85] and there may be discrepancies between *EGFR* amplification as determined by FISH and increased EGFR expression as determined using immunohistochemistry.[86,87] Scoring of EGFR immunopositivity is also dependent on the antigenic specificity of the individual antibody clone used. Notably, however, antibodies specific for the novel antigenic epitope generated from the exonic deletion event in EGFRvIII permit the specific detection of cells expressing this variant protein.[88]

Recommendations

In the future, as new agents are developed to target EGFR in glioblastoma, *EGFR* amplification and mutation status could become an important variable to be assessed in patients involved in clinical trials of such agents. At the present time, EGFR testing has found utility in adult neuro-oncology in two situations. These involve the pathological characterization of glioblastomas in two settings: the diagnosis of small cell glioblastoma and the differential diagnosis of the edge of a glioblastoma versus an anaplastic astrocytoma.

Some glioblastomas may be composed of densely packed monotonous, small cells that may not stain strongly for the common glial immunohistochemical marker GFAP, resulting in diagnostic confusion with high grade oligodendroglial tumours. *EGFR* gene amplification is quite common in such "small cell" glioblastomas, being found in about 71% in one series.[89] Thus, in such diagnostically challenging cases, the presence of *EGFR* gene amplification favors a diagnosis of small cell glioblastoma while 1p/19q loss would favor a diagnosis of anaplastic oligodendroglioma.

Another vexing scenario for the neuropathologist is the small biopsy that samples the edge of a glioblastoma. Although the biopsy does not demonstrate histological features sufficient for the diagnosis, the clinical and neuroimaging features may suggest glioblastoma. In such cases, the finding of *EGFR* gene amplification in the infiltrating tumor cells strongly suggests the presence of a tumor that is likely to act along the lines of a glioblastoma.[90] In essence, in this latter scenario, *EGFR* gene copy number status is being used as a surrogate for grade in estimating prognosis. *EGFR* copy number status can be readily determined by FISH in both of these diagnostically challenging scenarios.[85–87] Thus, in these two settings, we recommend diagnostic assessment of *EGFR* copy number using FISH.[26] *EGFR*

gene amplification implies, in both situations, that the tumor at hand will follow a course more similar to that of a glioblastoma.

IDH testing

Background

In a recent genome-wide survey, mutations in isocitrate dehydrogenase 1 (*IDH1*) were identified at high frequency in younger patients with secondary glioblastoma91 and subsequently in lower-grade diffuse gliomas.16,92 The somatic mutations identified at codon 132 were present in 12% of all glioblastomas and appeared to confer a prognostic advantage, even after adjustment for age.91 A follow-up study showed improved outcome for *IDH1* and *IDH2* mutated tumours, with median overall survival 31 months versus 15 months for glioblastoma lacking mutations and 65 versus 20 months for anaplastic astrocytoma.16 How these alterations cause oncogenesis is uncertain, although recently the role that metabolic pathways may have in contributing to oncogenic activation has been examined.93,94

Potential applications

The role of *IDH* mutations as prognostic indicators is still being defined. From a *diagnostic* point of view, however, it is interesting that *IDH1* and *IDH2* mutations occur rarely in primary glioblastomas (0·05%), but at higher frequencies in secondary glioblastoma (84·6%) and lower grade gliomas (including diffuse astrocytomas (83·3%), anaplastic astrocytoma (69·2%), oligodendroglioma (80·4%), anaplastic oligodendrogliomas (86·1%) and oligoastrocytomas(100%)).16 This is of diagnostic interest since lower grade, infiltrating gliomas may prove diagnostically challenging, especially on small biopsies in which the differential diagnosis includes reactive gliosis. The presence of mutant IDH1/2 clearly has potential to identify infiltrating glial tumours and aid in this clinically essential differential diagnosis.95 One study has already demonstrated this using a PCR-based assay, showing mutations in the tumors but not in a series of reactive conditions.96 Following the development of an antibody to mutant R132H IDH1 protein95 which could discriminate single infiltrating cells expressing wild type and mutant IDH1,97 it was demonstrated that immunohistochemistry utilizing an antibody specific for the mutant R132H IDH1 protein could successfully identify infiltrating cells of low grade infiltrating glioma in 9 of 21 cases, while not staining at all in 20 cases of gliosis. Moreover, when combined with p53 immunhistochemistry, 14 of 21 tumours could be positively identified and distinguished from gliosis.98 Thus, the detection of *IDH* mutation, whether by DNA sequencing or by simple immunohistochemistry will prove a powerful addition to the neuropathological armamentarium in evaluating small, diagnostically challenging biopsies.

Concluding remarks

In this review, we have endeavored to cover those markers that have achieved a place in current diagnostic adult neuro-oncology. In doing so we have avoided discussion of those markers that are sometimes requested by clinicians and patients based on more limited data; while such markers may assume a place in the diagnostic armamentarium in the future, the current literature does not support their routine use at the present time. Moreover, we have covered preferred technical approaches to evaluating certain markers, but it is important to realize that the field of molecular diagnostics is moving rapidly, and the advent of technologies such as next-generation sequencing may “disrupt” our current thinking about preferred approaches.

The IDH story discussed above also demonstrates the rapidity with which molecular assays are being brought into common use. For instance, over the past 15 years, the diagnosis of the

rare pediatric atypical teratoid/rhabdoid tumor transitioned from using FISH for chromosome 22q loss, to *INI1* gene sequencing, to INI1 immunohistochemistry. The IDH1 story in gliomas, however, has progressed over one year, from initial description of the mutations to implementation of a practical immunohistochemical assay.

This progress is likely to quicken even more. The Cancer Genome Atlas was initiated by the National Cancer Institute of the National Institutes of Health in 2006 to accelerate our molecular understanding of cancer. This is to be achieved by large scale integrative genomic and epigenomic analyses at multiple levels, studying gene copy number, mutation and expression. The first tumor to be studied was glioblastoma.99 Other large-scale projects have pursued similar approaches.91 These systematic examinations of the glioblastoma genome have already provided a wealth of information, reiterating and elaborating upon prior findings, and creating avenues for new insights. Also, second generation sequencing technology is becoming increasingly accessible for researchers and clinicians in the high throughput analysis of cancers.100 Coupled with improved bioinformatic tools and filtering algorithms, new sequencing technology could prove extremely useful in elucidating genetic alterations such as gene fusion events and aberrant RNA-editing.

TCGA and similar projects will quickly further understanding of the molecular basis of neoplasia. This progress in turn portends rapid expansion in the development of molecular assays, with the potential to improve our ability to predict tumour behaviour in individual patients. These exciting developments are the first steps toward personalized medicine in the setting of malignant glioma.

Search Strategy and Research Criteria

References for this review were identified by a series of Pubmed searches from January 1998 to January 2010. Search terms included “glioma”, "astrocytoma," "oligodendroglioma”, “oligoastrocytoma”, “mixed glioma”, “glioblastoma”, “1p" ,"19q", “MGMT”, “IDH1” and “EGFR”. Articles from the authors’ own files were also identified and utilized. Only papers published in English were reviewed.

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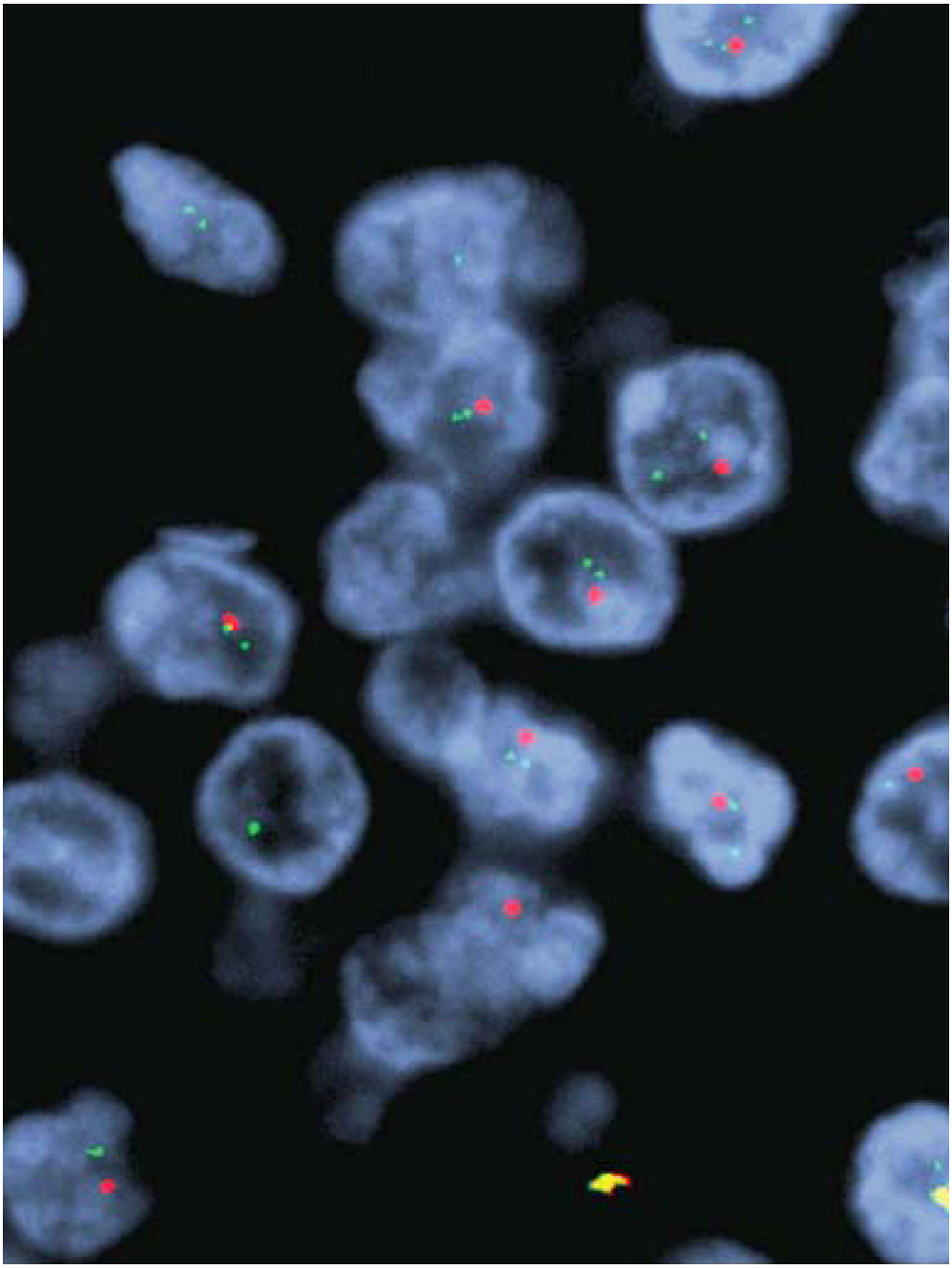

Figure 1.

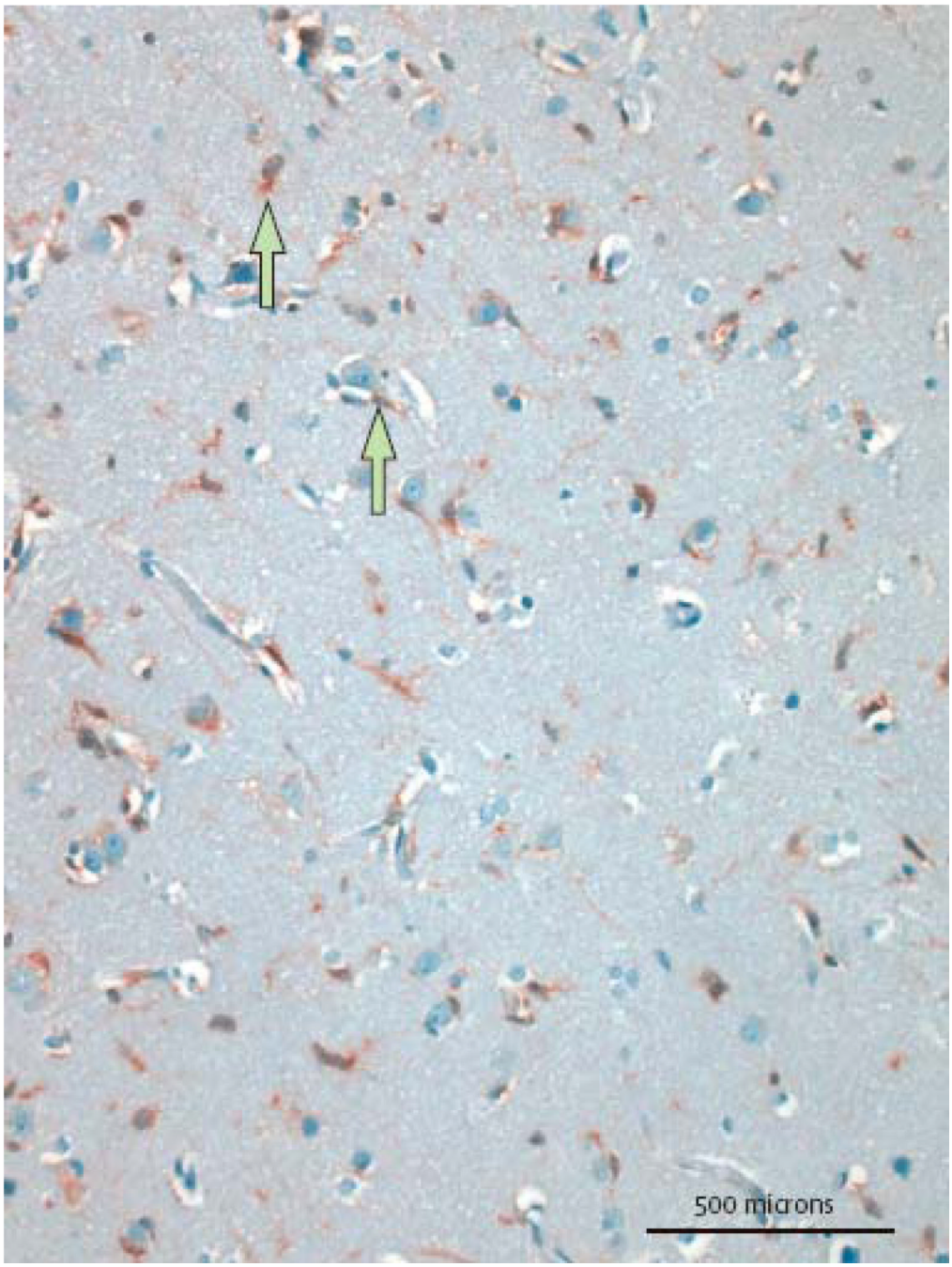

Figure 2.

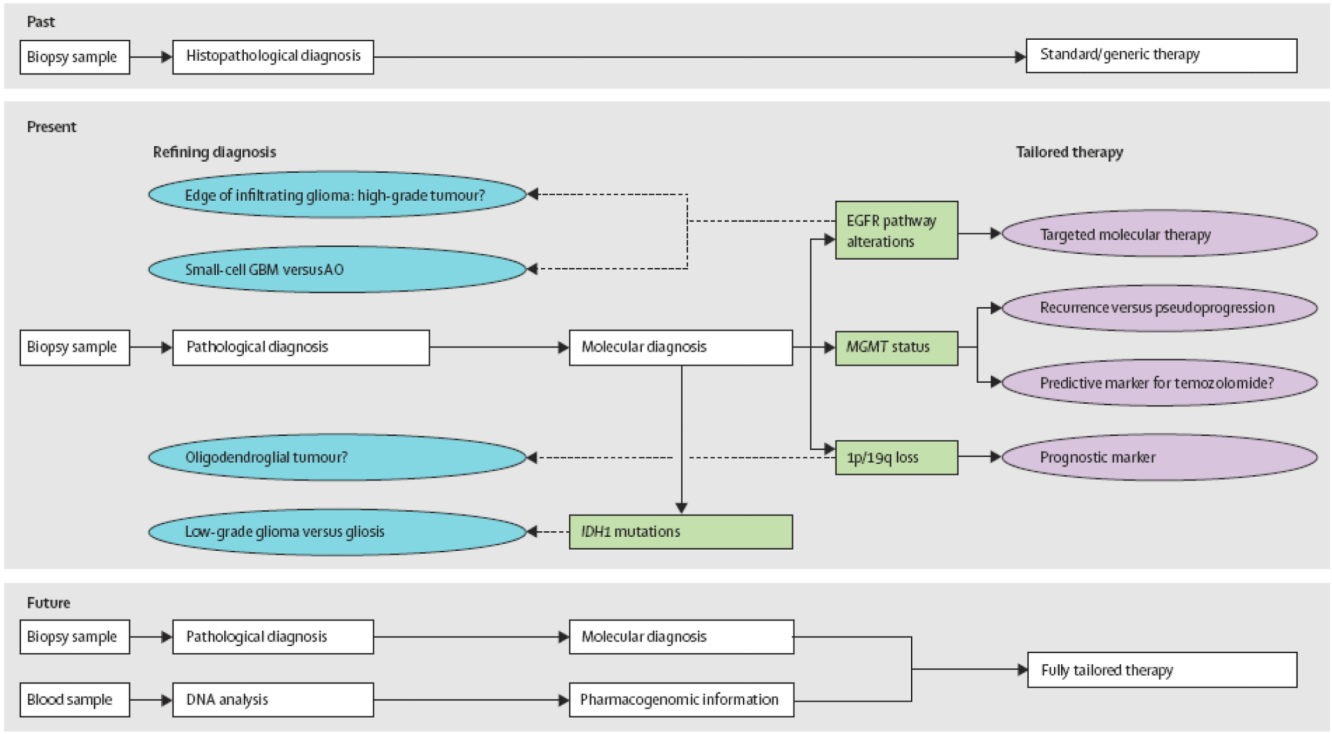

Figure 3.

Table 1

Recommendations for Molecular Markers in Malignant Gliomas

	Known Biologic Mechanism	Laboratory Testing Ready	Research Testing for Clinical Trials	Clinical Testing for Patients
1p19q LOH	−	+	+	+
MGMT	+	−	+	+/−
EGFR pathway assessment	+	+/−	+	−
IDH mutation testing	+/−	+	+?	+

EGFR, epidermal growth factor receptor; LOH, loss of heterozygosity; MGMT, O_6-Methylguanine DNA methyltransferase; IDH, isocitrate dehydrogenase