Molecular pathology in adult gliomas: diagnostic, prognostic, and predictive markers

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Over the past 10 years, there has been an increasing use of molecular markers in the assessment and management of adult malignant gliomas. Some molecular signatures are used diagnostically to help pathologists classify tumours, whereas others are used to estimate prognosis for patients. Most crucial, however, are those markers that are used to predict response to certain therapies, thereby directing clinicians to a particular treatment while avoiding other potentially deleterious therapies. Recently, large-scale genome-wide surveys have been used to identify new biomarkers that have been rapidly developed as diagnostic and prognostic tools. Given these developments, the pace of discovery of new molecular assays will quicken to facilitate personalised medicine in the setting of malignant glioma.

Introduction

Primary tumours of the CNS account for about 2% of human malignancies. The most common CNS tumours in 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 that have improved survival and quality of life for patients to variable degrees. In this Review, we outline how new molecular diagnostic advances have also had a role in changing the management of patients with malignant gliomas over the past 20 years.

Until recently, treatment decisions regarding malignant gliomas began with establishing diagnosis by standard histopathology only. Histopathology has been a dynamic tool for nearly 100 years, providing knowledge about the biology and behaviour of many neoplasms. The 2007 WHO classification,1 the most recent consensus approach to CNS tumour diagnosis, divides the malignant gliomas of adults into astrocytic tumours—the most malignant of which is termed glioblastoma—and oligodendrogliomas and oligoastrocytomas. The data gained from histopathological examination of tumour tissue have been augmented, but not displaced, by molecular approaches to tissue diagnosis.

Knowledge about the molecular biology of cancer, including CNS tumours, continues to increase. Having a dynamic classification of tumours enables the integration of newly discovered markers to help determine prognosis and likelihood of therapeutic response. We focus on the markers that are used in the evaluation of adult malignant gliomas, specifically 1p/19q co-deletion, methylation of the O-6 methylguanine-DNA methyltransferase (MGMT) gene promoter, alterations in the epidermal growth factor receptor (EGFR) pathway, and isocitrate dehydrogenase 1 (IDH1) gene mutations. Molecular approaches are also used in the assessment of other CNS tumours of neuroepithelial origin, notably in paediatric tumours such as medulloblastoma,2 atypical teratoid/rhabdoid tumour,3 and potentially in pilocytic astrocytoma.4

1p/19q loss

Background

After empirical observations of favourable responses to chemotherapy in a high proportion of recurrent anaplastic oligodendrogliomas (a grade III tumour), Cairncross and colleagues1 documented that such oligodendrogliomas with loss of the short arm of chromosome 1 (1p) were preferentially chemo-sensitive when treated with a procarbazine, lomustine, and vincristine chemotherapy regimen. Moreover, patients whose tumours had co-deletions of 1p and the long arm of chromosome 19 (19q) had substantially improved survival times. These findings have been replicated many times over the past 10 years, and the correlations with therapeutic sensitivity have been extended to other drugs such as temozolomide5,6 and procedures such as radiotherapy.7 Such data suggest that this marker is more useful as an indicator of tumour vulnerability to a broad range of therapeutic options than as a specific predictor of chemo-sensitivity. As a result, assessment of 1p and 19q status has been widely implemented in the neuro-oncological management of patients with anaplastic oligodendroglioma.8

The frequency of 1p/19q co-deletion has been estimated to be 80–90% in grade II oligodendrogliomas and 50–70% in grade III oligodendrogliomas.9 Notably, however, although the 1p and 19q regions have been extensively mapped and many genes have been evaluated as candidate tumour suppressor genes, no tumorigenic genes have been definitively implicated. In most cases, deletions seem to represent complete chromosomal arm loss, which might be the result of an unbalanced centromeric translocation of 1p and 19q.10,11 Although the genes on 1p and 19q remain unidentified, many correlations have been made regarding 1p/19q loss. For example, tumours with 1p/19q co-deletion frequently show classic histology12,13 and commonly have IDH1 and IDH2 mutations.14 Anaplastic oligodendrogliomas with 1p/19q co-deletions preferentially have a proneural gene expression profile.15 This profile, which is partly characterised by expression of neuronal genes, is over-represented among low-grade gliomas and might predict therapeutic response in glioblastoma.16 However, 1p and...
19q loss correlates inversely with TP53 mutations, 10q deletions, and amplification of EGFR. Tumour location is also associated with 1p/19q co-deletions: low-grade oligodendroglia and anaplastic oligodendroglia in the frontal, parietal, and occipital lobes are likely to show 1p/19q loss than are tumours in the temporal lobe, insula, and diencephalon.

1p/19q loss is also found in mixed glial tumours (oligoastrocytomas), albeit in a lower proportion than in pure oligodendroglia (20–30%). Eoli and colleagues showed 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 that suggest malignancy. Deletions involving 1p and 19q are uncommon in glioblastomas, but, in the small number that have been identified, these deletions seem to predict shortened survival, possibly indicating true genomic instability.

Thus, over the past 10 years, the frequent evaluation of oligodendrogial 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 those for other gliomas. Although the mechanisms by which 1p/19q co-deletion generates these clinical differences remain unclear, the differences between tumours with 1p/19q co-deletion and other oligodendroglia has made testing a useful and common procedure.

Current laboratory testing

In general, most neuro-oncological institutions do 1p/19q testing on tumours with oligodendroglial morphology, for grade II or III and for pure oligodendroglia or mixed oligoastrocytomas. There are two main methods to assess the loss of 1p and 19q: PCR-based loss of heterozygosity (LOH) assays and fluorescence in situ hybridisation (FISH). LOH assays are based on the comparison of several polymorphic alleles in tumour DNA versus blood DNA as a negative control. Because not all alleles are informative, multiple loci need to be amplified for each chromosomal arm; additionally, the assay requires the availability of blood DNA, possibly delaying test results. Both these drawbacks limit the use of LOH assays in the study of 1p/19q status. FISH uses fluorescence-labelled probes to study chromosomes directly on tissue sections; preparation and assessment of tissue samples can be a time-consuming step but tissue architecture is preserved and this is therefore a common procedure in many pathology laboratories (figure 1). Until recently, results between LOH and FISH were similar. However, Snuderl and colleagues have shown that from copy number information revealed by FISH analysis, but not available from LOH studies, additional information can be obtained: FISH assays enable assessment of polysomy, which might predict reduced progression-free survival.

Other techniques might also affect testing in the near future. For example, array comparative genomic hybridisation, a technique that enables genome-wide differential labelling 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 might replace FISH as the preferred laboratory test.

Recommendations

Knowledge of 1p/19q status is now considered to be the standard of care when managing oligodendrogial tumours, and oncologists might choose particular therapeutic options in anticipation of the increased survival and progression-free survival times associated with this tumour. In this section, we discuss the use of 1p/19q testing in four current settings: anaplastic oligodendroglia, low-grade oligodendroglia, mixed oligoastrocytic tumours, and histological diagnosis.

The potential role of 1p/19q loss in aiding management decisions in the setting of anaplastic oligodendroglomia has been analysed in large studies. 1p/19q deletions were incorporated into two major therapeutic trials in patients with anaplastic oligodendroglia. Both confirmed the prognostic and possible predictive role of these biomarkers at initial therapy. However, whether 1p/19q co-deletion correlates with response to second-line chemotherapy is unclear. Interpretation of the later stages of treatment in such trials is confounded by the effects of salvage therapy being given to all treatment groups. A major question still being debated is whether radiotherapy can be delayed in patients whose anaplastic oligodendroglio have 1p/19q loss; although there is not yet a clear consensus, a recent survey of members of the Society of Neuro-Oncology has shown that many neuro-oncologists are following this practice in patients for whom the molecular genetic status of the tumour is known. To refine the role of testing, future therapeutic trials of grade III gliomas could recruit and randomly assign patients on the basis of 1p/19q co-deletion status rather than on histology.

For low-grade oligodendroglia, 1p/19q testing is also common. 1p/19q co-deletion predicts increased progression-free survival in temozolomide-treated patients with low-grade glioma, including radiotherapy-naïve tumours and tumours with 1p loss treated with temozolomide as monotherapy. In one retrospective series of patients with low-grade oligodendroglial tumours who were not treated after surgery with radiotherapy or chemotherapy until either follow-up ended or tumour recurred, 1p/19q co-deletion status did not confer any prognostic advantage in progression-free survival. Nonetheless, in asymptomatic patients who have low-grade oligodendroglia with 1p/19q loss, neuro-oncologists might argue that, because of the likely
slow growth and long survival, clinicians could opt for a “watchful waiting” approach in the expectation of a superior response to therapy in the future. However, in symptomatic patients with the same molecular signature, early therapy would probably be chosen because the tumour would be likely to respond to therapy and the symptoms ameliorated. At present, the way in which 1p/19q status could be used to manage patients with low-grade oligodendrogliomas is still being debated.

The diagnosis of mixed oligoastrocytic tumours is highly subjective and varies between institutions because of relatively non-specific diagnostic criteria. In some studies of mixed oligoastrocytic tumours, 1p/19q co-deletions affected prognosis, but the data are probably not extensive 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 oligoastrocytomas have a genotype more similar to astrocytomas than to oligodendrogliomas can provide some reassurance and guidance for clinicians.

Lastly, one must ask whether the 1p/19q co-deletion signature should define a histological class of tumour (ie, oligodendrogliomas) because of its prognostic and predictive value. Although tempting to use this signature to classify a type of tumour, more information is needed. Evidence has emerged that there are subgroups within tumours with 1p/19q co-deletions. Polysomy of chromosomes 1 and 19 in these tumours is associated with short progression-free survival similar to tumours with no deletion. However, unlike tumours without deletion, such polysomy does not result in a decrease in overall survival or a decreased response to salvage chemotherapy. Moreover, Walker and colleagues have shown that a sample of tumours with oligodendrogial morphology without 1p/19q co-deletions contained a small subgroup with a positive response to therapy, implying that other mechanisms contribute to the improved prognosis associated with this histological feature. Such evidence reinforces the definition of oligodendroglioma in the WHO classification of tumours of the CNS, as a histologically, clinically, and genetically defined lesion.

**MGMT methylation status**

**Background**

Alkylating chemotherapeutic drugs have long been used in the treatment of patients with malignant gliomas. At present, nearly all patients with glioblastoma are treated with the orally administered alkylating drug temozolomide. This drug primarily methylates the O6 position of the nucleotide guanine, resulting in cell death. Cells typically have an inherent DNA repair mechanism that can counter the effects of temozolomide: the constitutively expressed DNA repair enzyme MGMT will irreversibly transfer a methyl group from the O6 position of the modified guanine to a cysteine residue of the MGMT protein, mitigating against the cytotoxic effects of chemotherapy. However, about half of glioblastomas have decreased concentrations of MGMT, which could make these tumours more susceptible to the effects of temozolomide. The primary mechanism by which MGMT expression is downregulated in glioblastomas seems to be methylation of the MGMT gene promoter, which is a common way of silencing gene expression in tumours. Thus, the expression level of MGMT and the methylation status of the MGMT gene promoter should have predictive value in patients with glioblastoma treated with alkylating drugs such as temozolomide. Indeed, results from several studies suggest such correlations in glioblastomas, paediatric glioblastomas, and low-grade gliomas. The methylation status of the MGMT promoter can also be a valid prognostic marker independent of temozolomide use in elderly patients with glioblastomas. Of the many studies, the most notable was that of Hegi and colleagues who studied MGMT gene promoter status in tumours from patients enrolled in a large trial that studied the role of concomitant temozolomide with radiation therapy versus radiation therapy alone. In 106 patients treated with temozolomide (46 with methylated tumours, 60 with unmethylated tumours), 46% of patients with MGMT-methylated tumours were alive at 2 years versus only...
23% of patients with tumours with unmethylated *MGMT*. Although assessment of overall survival was confounded by many patients having salvage therapy that included alkylating drugs, this trend for improved survival in *MGMT*-methylated tumours seemed to continue. These authors subsequently suggested that assays that determined *MGMT* methylation status, in addition to providing prognostic information, could be used as predictive tools to determine whether patients who have glioblastoma would benefit from treatment with temozolomide. However, in this study, the group with unmethylated *MGMT* also showed a survival benefit with temozolomide therapy that, although inferior to that in patients with *MGMT*-methylated tumours, nearly reached significance (p=0.06). An explanation for this finding remains unclear, and could, among other reasons, be because of different definitions of cut-off levels used when interpreting the assay for separating methylated and unmethylated groups. However, this beneficial response to temozolomide in *MGMT*- unmethylated tumours, as well as the current absence of a therapeutic alternative, suggests that treatment is unlikely to be withheld from patients on the basis of this test, particularly given the oral bioavailability and well tolerated nature of the drug.

Methylation patterns of the *MGMT* promoter might change in the time between primary diagnosis and recurrence, and might change more frequently in tumours that initially show *MGMT* methylation. The prognostic information conveyed by knowledge of the *MGMT* methylation status therefore seems to apply only to primary tumours and not to recurrent tumours.

Recently, the use of *MGMT* methylation status in anaplastic (ie, WHO grade III) gliomas was evaluated in the Neuro-Oncology Working Group of the German Cancer Society NOA-04 trial and the European Organisation for Research and Treatment of Cancer (EORTC) 26951 trial. In both trials, *MGMT* methylation status conferred prognostic benefit but did not specifically predict response to therapy with alkylating drugs. *MGMT* promoter methylation has been recently re-examined in a cohort of patients with glioblastomas treated with radiotherapy only. In this study, *MGMT* methylation seemed to predict response to radiation therapy. Thus, in anaplastic gliomas and possibly in glioblastomas, the prognostic benefit conveyed by a positive result might not solely be attributable to *MGMT* promoter methylation enhancing the effect of therapy with alkylating drugs, but might instead indicate a prognostically favourable molecular phenotype.

The introduction in 2005 of temozolomide as the standard of care for patients with glioblastoma, and the response to temozolomide being associated with *MGMT* promoter methylation status, generated interest in *MGMT* methylation status testing for both neuro-oncologists and patients. Although the information provided by the assay was not going to alter therapy, many testing centres experienced pressure, generated mostly by patients being informed by the internet, to set up the assay nonetheless.

Recently, patients whose glioblastomas have methylated of the *MGMT* gene promoter have been reported to have a greater likelihood of a radiographic pseudoprogression when treated with temozolomide than do patients whose tumours do not have such methylation. During pseudoprogression, the neuroimaging profile seems to worsen after temozolomide, but then improves with continued therapy. Thus, another role for testing for *MGMT* promoter methylation could be evaluation of the likelihood of pseudoprogression.

**Current laboratory testing**

Laboratory testing of *MGMT* status entails the use of methylation-specific PCR, in which extracted DNA is treated with sodium bisulphite, resulting in substitution of unmethylated cytosine for uracil. Primers specific for methylated and unmethylated CpG-rich areas of the *MGMT* gene promoter are then used to amplify the modified DNA sequences. The assay can be done on paraffin-embedded tissue, but is dependent on tissue quantity and quality. Additional factors such as adequacy of bisulphite treatment, primer specificity, and PCR conditions further affect the usefulness of the assay. Both methylated and unmethylated sequences are often found in glioblastoma tissue, attributed to either inclusion of non-neoplastic tissue or tumour heterogeneity. Other bisulphite-based techniques, including quantitative methylation-specific PCR, bisulphite sequencing, methylation-sensitive single strand conformation polymorphism analysis, and mass spectrophotometer-based quantitative analysis, are available, some of which have been used to assess *MGMT* methylation status. Non-bisulphite-based semi-quantitative techniques, such as pyrosequencing and methylation-specific-multiplex ligand-dependent probe amplification (MS-MLPA), have also emerged.

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

**Recommendations**

Given that temozolomide and radiation therapy remain the standard of care for all patients with glioblastoma, regardless of *MGMT* promoter methylation status, upfront diagnostic testing is not necessary. Nonetheless,
the methylation status of the MGMT promoter 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 therapies on patients who are enrolled in clinical trials, and must be included in all glioblastoma trials.

As mentioned earlier, MGMT promoter methylation status can be useful in the differential diagnosis of true tumour progression versus pseudoprogression seen on imaging. In this case, for a tumour with MGMT promoter methylation, pseudoprogression might be more likely than true progression and thus therapy with temozolomide is often continued despite the worse appearance on neuroimaging. Conversely, a worsening appearance on imaging in a patient whose glioblastoma does not have MGMT promoter methylation might prompt consideration to change therapy.50,57

**EGFR pathway alterations**

*Background*

Traditional chemotherapeutic approaches non-specifically target dividing cells through various mechanisms. Molecularly targeted therapies, however, specifically inhibit aberrant or amplified proteins that directly drive tumour cell growth. Recent successful examples have included imatinib to inhibit the tyrosine kinase fusion protein BCR-ABL in chronic myeloid leukaemia, and the C-KIT oncogene in gastrointestinal stromal tumours,58,59 as well as trastuzumab in the treatment of HER2 (ERBB2)-positive breast carcinoma.60

Several growth factors and their receptors are upregulated in malignant gliomas, particularly in glioblastomas. For example, there is upregulation of EGFR-mediated signalling in about 30% of gliomas62–64 and 60% of glioblastomas,65 with overexpression in glioblastomas generally driven by EGFR gene amplification.66,67 Additionally, in about half of glioblastomas that overexpress EGFR there is also expression of mutant forms of EGFR molecules, the most frequent of which is EGFR variant III (EGFRvIII); this variant does not have the ligand-binding domain and therefore constitutively activates the EGFR-phosphoinositide 3-kinase pathway.68 Additional missense mutations have been identified in the exons that encode extracellular EGFR domains.69–71 Moreover, other activating mutations in exons encoding extracellular EGFR have been shown to drive oncogenesis in vitro and can be inhibited by small-molecule tyrosine kinase inhibitors.68

The prognostic use of EGFR amplification is not clear, with several studies producing contradictory findings.69 EGFR amplification might be prognostically associated with the age of the patient,70 and EGFRvIII expression might enable identification of a subgroup of tumours with more aggressive behaviour.71

The presence of EGFR overexpression and EGFR mutations in glioblastomas raises the possibility that EGFR-targeted drugs could be used to treat patients who have glioblastomas with these aberrations—a similar situation to that of some non-small-cell lung carcinomas that have activating EGFR mutations and that show remarkable responses to the EGFR inhibitors erlotinib and gefitinib. However, this potential treatment approach might be less successful in the setting of glioblastoma for several biological reasons. The EGFR activates several downstream pathways that might act in parallel to drive oncogenesis. Many glioblastomas have dysregulation of signalling cascades downstream of the EGFR, such as that mediated by the PTEN tumour suppressor gene and those that involve the phosphoinositide 3-kinase pathway and AKT. The activity of these pathways might contribute to reduced effectiveness of the downregulation of the EGFR.72 Additionally, other growth factors such as platelet-derived growth factor (PDGF) also exercise their effect by modulation of these pathways. Finally, many glioblastoma cells can have activation of several growth factor pathways, suggesting that a range of molecular targeting drugs is needed in patients with glioblastoma.73

The results of studies that have investigated drugs that target EGFR looked initially promising. Two studies published in 2005 sought to clarify whether assessment of EGFR status was useful in aiding decision making to use the small-molecule kinase inhibitors gefitinib and erlotinib.74,75 Mellinghoff and colleagues74 showed that co-expression of PTEN and EGFRvIII was associated with increased sensitivity to erlotinib, whereas tumours without PTEN expression did not respond to erlotinib. Haas-Kogan and colleagues76 suggested that glioblastomas with diffuse EGFR immunopositivity that did not have phosphorylated protein kinase B/AKT immunostaining were likely to show a clinical response to erlotinib. Results from both studies implied that the tumours that respond to small-molecule kinase inhibitors overexpress EGFR or express EGFRvIII, and have an intact PTEN-AKT pathway. Although the immunohistochemical assays of these two studies 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 patients with glioblastoma treated with these drugs.76,77 Further studies have since assessed erlotinib used concurrently with temozolomide and radiotherapy.78,79 These trials have produced mixed results: one showed no overall benefit and did not help to identify a subgroup of tumours that might respond to therapy;78 the other suggested that this regimen might be useful for patients with tumours with MGMT promoter methylation and intact PTEN.79 Therefore, assessment of the EGFR signalling pathway when planning therapy is not clinically indicated because current standard therapies are not specifically directed at this pathway. Nonetheless, the targeted therapy approach provided by the EGFR and non-small-cell lung cancer case and the EGFR and glioblastoma papers from 2005 is important and is likely to be of use over the next decade. As new molecularly targeted drugs reach clinical trials,
alone and in combination, these will be studied in the setting of glioblastoma and it is likely that use of these novel drugs will be guided by molecular testing of the respective signalling pathways, either at a DNA or protein level. New methods should enable study into multiple signalling pathways in single tumours on a routine basis. For example, at the Massachusetts General Hospital, MA, USA, several mutational analyses are now carried out in standard paraffin-embedded samples by use of the SNaPshot system (Applied Biosystems, Life Technologies; Carlsbad, CA, USA), incorporating multiplex PCR, single-base extension, and capillary electrophoresis. Such prospective and timely analysis enables patient-tailored therapy based on genotyping of known oncogenes and tumour suppressor genes.80,81

Current laboratory testing

In glioblastomas, amplification of EGFR mostly occurs as double-minutes, that is, small fragments of extrachromosomal DNA. Confirmation of major copy number gain by use of FISH is therefore practical and accessible to many pathology laboratories. Moreover, FISH assays maintain tissue architecture and thus provide information about genetic heterogeneity within a tissue section, decreasing the likelihood of false negative assays.82,83 Other techniques such as quantitative PCR and RT-PCR can also be used to identify EGFR amplification,84 additionally, these and other molecular techniques can be used to identify mutations in EGFR.84 Immunochemical assessment for EGFR expression is widely available, but of less clear value (see below). EGFR expression can be found in low-grade astrocytomas and other malignant gliomas. Moreover, scoring of EGFR immunopositivity can be variable85 and there might be discrepancies between EGFR amplification as determined with FISH and increased EGFR expression as determined with immunochemistry.86,87 Scoring of EGFR immunopositivity is also dependent on the antigenic specificity of the antibody used. Notably, however, antibodies specific for the novel antigenic epitope generated from the exonic deletion event in EGFRvIII enable the specific detection of cells that express this variant protein.88

Recommendations

In the future, as new drugs are developed to target EGFR in glioblastomas, EGFR amplification and mutation status could become an important variable to be assessed in patients involved in clinical trials of such drugs. At present, EGFR testing is used in adult neuro-oncology in two situations involving the pathological characterisation of glioblastomas: the diagnosis of small-cell glioblastoma and the differential diagnosis of the edge of a glioblastoma versus an anaplastic astrocytoma.

Some glioblastomas can be composed of densely packed, monotonous small cells that might not stain strongly for the common glial immunohistochemical marker glial fibrillary acidic protein (GFAP), resulting in diagnostic confusion with high-grade oligodendroglial tumours. EGFR gene amplification is quite common in such small-cell glioblastomas—found in 71% in one series.89 Thus, in such diagnostically challenging cases, the presence of EGFR gene amplification favours a diagnosis of small-cell glioblastoma, whereas 1p/19q loss would favour a diagnosis of anaplastic oligodendroglioma.

Another difficult scenario for neuropathologists is caused by small biopsy samples of the edge of a glioblastoma. Although the biopsy sample might not show histological features sufficient for diagnosis, the clinical and neuroimaging features could suggest glioblastoma. In such cases, the finding of EGFR gene amplification in the infiltrating tumour cells strongly suggests the presence of a tumour that is likely to act in a similar way to a glioblastoma.90 Therefore, EGFR gene copy number status can be used as a surrogate for histological grade in estimating prognosis. EGFR copy number status can be readily determined by use of FISH in both of these diagnostically challenging scenarios.85–87 Thus, in these two settings, we recommend diagnostic assessment of EGFR copy number by use of FISH.86 EGFR gene amplification implies, in both situations, that the tumour will follow a course more similar to that of a glioblastoma.
**IDH mutations**

**Background**

In a recent genome-wide survey, mutations in \textit{IDH1} were identified at high frequency in younger patients with secondary glioblastoma (median age ~45 years vs ~60 years for primary glioblastomas)\textsuperscript{91} and subsequently in low-grade diffuse gliomas.\textsuperscript{91,92} The somatic mutations identified at codon 132 were present in 18 of 149 (12%) glioblastomas and seemed to confer a prognostic advantage, even after adjustment for age.\textsuperscript{91} In a follow-up study, there was improved outcome for patients with tumours with \textit{IDH1} and \textit{IDH2} mutations, with a median overall survival of 31 months versus 15 months for patients with glioblastomas without these mutations, and 65 months versus 20 months for patients with anaplastic astrocytomas.\textsuperscript{91} How these alterations cause oncogenesis is uncertain, although the role that metabolic pathways might have in contributing to oncogenic activation has been recently examined. It is possible that \textit{IDH1} mutations lead to increased formation of R(\textendash)2-hydroxyglutarate, which might be directly oncogenic, or that the reduced catalytic ability of IDH might lead to increased levels of the transcription factor hypoxia-inducible factor-\textalpha, which is known to facilitate tumour growth.\textsuperscript{91,92}

**Potential uses**

The role of \textit{IDH} mutations as prognostic indicators is still being defined. From a diagnostic point of view, however, it is interesting that \textit{IDH1} and \textit{IDH2} mutations occur rarely in primary glioblastomas (0·05%), but at higher frequencies in secondary glioblastomas (84·6%) and low-grade gliomas (including diffuse astrocytomas [83·3%], anaplastic astrocytomas [69·2%], oligodendrogliomas [80·4%], anaplastic oligodendrogliomas [86·1%], and oligoastrocytomas [100%]).\textsuperscript{16} This is of interest because low-grade, infiltrating gliomas might prove diagnostically challenging, particularly with small biopsies in which the differential diagnosis includes reactive gliosis. The presence of mutant \textit{IDH1} and \textit{IDH2} has potential to help identify infiltrating glial tumours and to help with this clinically important differential diagnosis.\textsuperscript{91} One study\textsuperscript{96} has already indicated this by use of a PCR-based assay, showing mutations in the tumours but not in a series of reactive conditions such as radiation change, infarct, and viral infection. After the development of an antibody to mutant Arg132His IDH1 protein,\textsuperscript{95} which could discriminate single infiltrating cells expressing wild-type and mutant IDH1 (figure 2),\textsuperscript{97} immunohistochemistry with an antibody specific for the mutant protein successfully identified infiltrating cells of
low-grade infiltrating glioma in nine of 21 cases, but did not stain tumour cells in any of the 20 cases of gliosis. Moreover, when combined with p53 immunohistochemistry, 14 of 21 tumours were positively identified and distinguished from gliosis. 9 Thus, the detection of IDH mutations, whether by use of DNA sequencing or by simple immunohistochemistry, could prove to be a powerful addition to the neuropathological armamentarium in evaluating small, diagnostically challenging biopsies.

Conclusions
In this Review, we have outlined the markers that are currently used in diagnostic adult neuro-oncology (figure 3, table). We have not discussed the markers that are sometimes requested by clinicians and patients on the basis of less established data; although such markers might assume a place in the diagnostic armamentarium in the future, the current evidence does not support their routine use at present. Moreover, we have reviewed preferred technical approaches to studying certain markers, but the area of molecular diagnostics is moving rapidly, and the advent of technologies such as next-generation sequencing will modify our current thinking about preferred approaches.

The history of IDH testing discussed earlier also indicates the rapidity with which molecular assays are being brought into common use. For example, over the past 15 years, the techniques used for the diagnosis of the rare paediatric atypical teratoid/rhabdoid tumour have changed from use of FISH for chromosome 22q loss to SMARCB1 (INI1) gene sequencing toINI1 immunohistochemistry. The IDH1 story in gliomas, however, has progressed over 1 year, from initial description of the mutations to implementation of a practical immunohistochemical assay.

This progress is likely to be more rapid in the future. The Cancer Genome Atlas was initiated by the US National Cancer Institute of the National Institutes of Health in 2006 to accelerate the molecular understanding of cancer. Large-scale integrative genomic and epigenomic analyses at several levels, studying gene copy number, mutation, and expression, will help advance this knowledge. The first tumour to be studied was glioblastoma. 10 Other large-scale projects have pursued similar approaches. 11 These systematic studies of the glioblastoma genome have already provided a wealth of information, reinforcing and expanding on earlier findings, and creating avenues for new insights. Furthermore, next-generation sequencing technology is becoming increasingly accessible for researchers and clinicians in the high-throughput analysis of cancers. 12 Together with improved bioinformatic tools and filtering algorithms, new sequencing technology could prove very useful in elucidating genetic alterations such as gene fusion events and aberrant RNA editing.

The Cancer Genome Atlas and similar projects will rapidly improve understanding of the molecular basis of neoplasia. This progress, in turn, will lead to a 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 towards personalised medicine in the setting of malignant glioma.

Conflicts of interest
We have no conflicts of interest.

References


Review


