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TOPIC REVIEW & CLINICAL GUIDELINES

The role of neuropathology in the management of progressive glioblastoma

A systematic review and evidence-based clinical practice guideline

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Abstract
Question 1. What are the most important diagnostic considerations in reporting progressive glioblastoma?
Target population These recommendations apply to adults with progressive glioblastoma
Recommendations *Level III* For patients who undergo biopsy or neurosurgical resection at the time of radiologic or clinical progression, it is recommended that the pathologist report the presence and extent of progressive neoplasm as well as the presence and extent of necrosis within the pathologic material examined.
Furthermore, to ensure the proper interpretation of progressive glioblastoma, it is recommended that the pathologist take into account the patient's previous diagnosis and treatment, as well as the current clinical and neuroimaging features that have led to a second biopsy or resection.

Question 2. What techniques and ancillary studies are most useful in separating malignant progression from treatment effect?
Target population These recommendations apply to adults with progressive glioblastoma
Recommendations *Level III* In the setting of prior radiation and chemotherapy, it is recommended to adhere to strict histologic criteria for microvascular proliferation and necrosis in order to establish a diagnosis of a glioblastoma. Immunohistochemistry and genetic studies are selectively recommended for distinguishing neoplastic cells from atypical reactive cells in progressive glioblastoma.

Keywords Adult · Glioblastoma · Progressive · Recurrent · Radiation therapy · Chemotherapy

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Neuropathology rationale

Primary malignant brain tumors (malignant glioma, anaplastic astrocytoma and glioblastoma) carry a nearly uniformly dismal outcome. The median survival of patients with newly diagnosed glioblastoma has improved slightly in the last decade and may approach 15–18 months [1, 2]. Nearly all patients will succumb to disease within 5 years, Because of the dismal prognosis, patients with these tumors are treated early in the course of the disease with aggressive multimodality regimens including surgical resection, radiotherapy and chemotherapy [3–5]. Despite this, nearly all malignant gliomas will eventually recur and progress.

The definitive diagnosis of progressive GBM in this setting not always straightforward. The development of new symptoms or neuroimaging evidence of a newly formed contrast-enhancing component on MRI may be due to progressive tumor, may be related to the effects of

therapy, such as those noted in pseudo-progression and radiation necrosis, or may represent a combination of the two. While neuroimaging techniques have evolved substantially, their ability to discriminate progressive GBMs from the effects of therapy is still imperfect [6–8]. Depending on the clinical setting, patients may require biopsy or surgical resection for symptomatic relief or for tumor sampling to guide further therapy. The neuropathologic classification and grading of progressive GBM based on the examination of tissue can be challenging. In order to ensure greatest accuracy, pathologic studies should be performed in a multidisciplinary setting, in conjunction with a patient's clinical history, the neurosurgical impression, and the neuroradiologic findings [9–11]. Knowledge of the patient's treatment status is an absolute necessity for the pathologist to interpret the pathologic findings. Therefore, strong lines of communication across clinical and diagnostic disciplines are recommended both for the most accurate appraisal of disease and to ensure that any diagnostic discrepancies are resolved prior to a initiating a new treatment regimen. The purpose here is to evaluate the current literature addressing the diagnosis of progressive GBM. The focus will be mostly on the effects of therapy on the pathology of the tissue examined and how this may impact classification and grading. The roles of immunohistochemistry and molecular diagnostic techniques in this setting will also be evaluated. This review will address the following questions:

1. What are the most important diagnostic considerations in reporting progressive glioblastoma?
2. What techniques and ancillary studies are most useful in separating malignant progression from treatment effect?

Neuropathology methodology

Neuropathology search strategy

The following electronic databases were searched from January 1990 through December 2011: MEDLINE®, and Embase®. A broad search strategy using a combination of subheadings and text words was employed. In brief, a search was executed for the neuropathologic changes associated with progressive glioblastoma, recurrent, or relapsing glioma or glioblastoma.

Quality evaluation of diagnostic literature

A prospective classification scheme of the pertinent literature was used. Reports were designated Class I that studied the appropriate population with clearly stated inclusion criteria; included diagnostic entities within established histologic categories; compared the diagnostic criteria to survival over an interval that is meaningful for the disease studied; included consensus diagnosis; were blinded to outcome; and were statistically significant on a multivariate analysis of appropriate clinical and pathologic variables [12–14]. Reports were designated Class II that studied the appropriate population, compared the diagnostic criteria to survival over an interval that is meaningful for the disease studied, but fell short of Class I in one of the other criteria. Reports were designated Class III that had more than one shortcoming.

Literature reports that assessed variability between pathologists or that compared newer diagnostic techniques to more established ones were also categorized as Class I, II and III. Reports designated as Class I were required to: have an appropriate number of cases; include only specific tumor types; present an accepted standard for tumor identification against which the investigative assessment could be compared; be properly blinded and refereed; and provide data for calculation of sensitivity, specificity, positive and negative predictive value, accuracy, likelihood ratio of a positive and negative result, and κ. Those studies for which a κ correlation coefficient could be calculated and the value was greater than 0.6 were designated as Class I. Studies that had a κ value was between 0.41 and 0.6, even though all other criteria were met, were designated as Class II. Studies were also designated Class II if the study population was restricted or histologic confirmation was not present on all cases, even if the investigation was performed in blinded fashion and all parameters could be calculated. Those studies that had a κ of 0.4 or less were designated as Class III [15]. Class III designation was given to studies that were retrospective, to those that lumped together tumor grades or histologies, and to those that did not include histologic review. Class III data can be of value, but does not provide the same high degree of evidence as Class I and II data. Studies reported as randomized, controlled and/or prospective can vary significantly in quality and need to be reviewed in light of published recommendations for identification of items important in the valid reporting of clinical trials [16, 17]. Though there must be room for special considerations, Level I recommendations are generally based on Class I evidence, Level II recommendations are based on Class II evidence and Level III recommendations are based on Class III evidence [18].

Neuropathology scientific foundation

Diagnostic criteria of malignant gliomas

The pathologic diagnosis of malignant gliomas depends on the application of established histopathologic and

cytopathologic criteria to sampled tissue [19–23]. It relies heavily on examination of hematoxylin and eosin (H&E) stained slides, but may also incorporate results from ancillary immunohistochemical and genetic tests. A variety of schemes for classifying and grading gliomas have been employed and a review of this literature has been previously published [24]. For the current review of progressive glioblastoma, criteria of the World Health Organization classification will be used, since it represents a recent and updated international standard for classifying and grading [22].

Briefly, the WHO classification divides the diffuse gliomas into astrocytomas, oligodendrogliomas, and oligoastrocytomas and utilizes a 3-tiered grading system that ranges from grade II–IV [22, 25]. Infiltrative astrocytoma (A), WHO grade II is low in cellular density and shows little or no mitotic activity. Anaplastic astrocytoma (AA), WHO grade III, demonstrates higher cell density, more nuclear atypia and mitotic activity. Glioblastoma (GBM; WHO Grade IV) is distinguished from AA by abundant areas of microvascular proliferation or necrosis, with or without pseudopalisading. The WHO recognizes two grades of oligodendroglial neoplasms: oligodendroglioma (O), grade II and anaplastic oligodendrogliomas (AO), grade III [22]. Grade II tumors vary from low to moderate cellularity and can show occasional mitotic figures and cytological atypia, but marked mitotic activity, microvascular proliferation, or necrosis are diagnostic of grade III. Oligoastrocytomas contain distinct regions of oligodendroglial and astrocytic differentiation. The WHO criteria for distinguishing anaplastic oligoastrocytoma (AOA) WHO grade III from oligoastrocytoma (OA) grade II are not well defined, but "features of anaplasia" should be present [22]. This list includes nuclear atypia, cellular pleomorphism, high cellularity, high mitotic activity, and microvascular proliferation. The presence of necrosis with or without pseudopalisading, in a high grade gliomas with both oligodendroglial and astrocytic morphology qualifies it as a glioblastoma with oligodendroglioma component, WHO grade IV [26].

As a group, the diffuse gliomas are difficult to completely resect and are only partially responsive to therapy. Thus, the majority of these tumors will recur, with the time to recurrence depending on numerous factors, including tumor histology and grade, extent of resection, and the response of the tumor to therapy. A small subset of patients with diffuse gliomas, for a number of reasons, may not receive therapy following their initial diagnosis. When these tumors recur in the absence of intervening treatment, the pathologic diagnosis can be readily established using the WHO grading criteria, since there will be no effects of chemotherapy and radiation therapy. A previous publication has covered the role of neuropathology in establishing the diagnosis of low grade and malignant gliomas [24].

The majority of patients with GBM are treated with a regimen that includes radiotherapy and chemotherapy and the influence of this therapy on the neuropathologic diagnosis of progressive disease needs to be considered. First and foremost, the pathologist should be aware of the patient's previous diagnosis and the type of therapy that was administered. The pathologist should also be aware of the clinical setting and neuroimaging data that have led to the biopsy or resection for progression. This practice can reduce the possibility of misinterpretation of pathologic findings [10, 11]. It is good practice for the pathologist review the slides from the patient's prior biopsy or resection in conjunction with the slides from the recurrence. In this manner, the prior histologic classification and grading can be confirmed and interpreted in order to provide the most appropriate diagnosis for the recurrence. For example, in the setting of a biopsy of a recurrent neoplasm that is high grade and mitotically active, but without evidence of necrosis or vascular proliferation, the interpretation could be different if a patient was previously diagnosed with a GBM or with a grade II astrocytoma. If the patient was previously diagnosed with a GBM, the interpretation could be that the biopsy contains a high grade astrocytoma and most likely represents the under sampling of a recurrent GBM, as it would not be appropriate to diagnose AA in the setting of a prior diagnosis of GBM. If the previous diagnosis was a grade II astrocytoma, a reasonable interpretation would be that the tumor has progressed to a grade III astrocytoma. Thus, the proper interpretation of histologic features in the pathologic specimen should consider the patient's previous diagnosis and treatment, as well as the current clinical and neuroimaging features that prompted a second biopsy or resection.

Neuropathology of therapy effects

Radiation therapy has been a mainstay of brain tumor treatment and its side-effects on the brain have been well-documented [27]. The effects of radiation can be noted during the treatment period, which are considered to be "acute"; in the period immediately following treatment (up to 12 weeks), which is considered "early delayed" and also over a period of months to years following treatment, considered to be "late delayed" [28, 29]. The addition of chemotherapy to radiation, either concurrently or in the adjuvant setting may exacerbate the side effects of radiation.

Neuropathology of pseudoprogression

Due to results from recent clinical trials, a frequently used therapeutic regimen for GBM includes radiotherapy with concurrent temozolomide (TMZ) followed by adjuvant

TMZ [30]. In this treatment setting, a subset of patients (35–45 %) will have early neuroimaging evidence of progression on MRI, typically after the completion of radiotherapy and concurrent TMZ or during adjuvant TMZ treatment. Approximately half of these patients with early evidence of progression by neuro-imaging will indeed have biologically aggressive tumors that relentlessly advance by clinical and neuro-imaging measures [31, 32]. Others, representing between 35 and 50 % of those with a new area of enhancement by MRI on first follow-up, will show stabilization or regression of the MRI finding, and do not demonstrate neuroimaging or clinical progression during the following 6 months of adjuvant therapy. The latter outcome has been referred to as "pseudoprogression" since the lesion appears to be progressing by neuro-imaging criteria at first follow-up, yet the future clinical behavior suggests that the tumor has not truly progressed. The phenomenon of pseudoprogression was first recognized following treatment regimens that included radiation therapy alone. However, it is now recognized that the frequency of pseudoprogression is higher in the setting of concurrent radiation and chemotherapy [33]. For example, in one study, patients with malignant gliomas treated with radiation alone showed evidence of pseudoprogression in 9 % of cases [33]. In a similar patient population treated with radiation and TMZ, 21 % showed evidence of pseudoprogression [31]. Importantly, the finding of pseudoprogression seems to indicate a favorable response to therapy, since patients with pseudoprogression have longer survivals compared to those patients with no evidence of early progression [31].

It has been hypothesized that enhanced sensitivity to radiation by TMZ is greater in GBMs that don't express the enzyme MGMT (those with promoter methylation of *MGMT*) because they accumulate more DNA damage [34]. In an attempt to establish a relationship between MGMT status and pseudoprogression, Brandes et al. [32] followed a cohort of 103 patients diagnosed with GBM and treated with TMZ concurrent with radiotherapy followed by maintenance therapy. In this group, the MGMT promoter was methylated in 36 GBMs and unmethylated in 67. At the time of first clinical follow-up, MRI scan showed lesion enlargement in 50 of 103 patients, which was subsequently classified as pseudoprogression in 32 patients and early disease progression in 18 patients. Pseudoprogression was noted at a much higher rate in patients whose GBM contained a methylated MGMT promoter (91 %) than an unmethylated MGMT promoter ($P = 0.0002$). Moreover, in this study both MGMT status ($P = 0.001$) and pseudoprogression ($P = 0.045$) were associated with prolonged survival.

The underlying mechanisms and explanations for the early neuroimaging findings that mimic tumor progression are not clear. In one study that attempted to define the pathologic basis of such early neuroimaging progression, 51 % of the of 51 GBM patients treated with radiotherapy together with concurrent and adjuvant TMZ were found to have neuroimaging evidence of recurrence within 6 months [35]. Among the 15 patients in this group who underwent complete surgical resection of the radiologic recurrence, seven (47 %) were found to have only necrosis present within the tissue sent to pathology, without evidence of progressive malignant glioma. These results suggested that patients with pseudoprogression may have tumors that are more sensitive to the combination of radiotherapy and TMZ and show an exaggerated cytotoxic response that is represented as massive tumor necrosis on pathologic exam.

The pathologic finding of extensive necrosis in these cases of pseudoprogression, by itself, does not explain the growth of these tumors or the increased levels of enhancement noted by neuroimaging. It is most likely that a severe reactive response, including edema, by brain tissue adjacent to necrosis, is responsible for the apparent increased size. Enhanced permeability of nearby blood vessels may be responsible for increased enhancement noted on MRI, as both acute and subacute injury to blood vessels from radiation are thought to be associated with disruption of the blood–brain-barrier [28, 36]. These events appear to be reversible, since the neuro-imaging findings will often stabilize or regress and clinical symptoms often diminish, either by themselves or following treatment with corticosteroids.

Neuropathology of radiation necrosis

The effects of radiotherapy can be seen well after the subacute period following treatment, during which pseudoprogression occurs (i.e. "early delayed reaction") [37]. Late effects are most often associated with radiation necrosis and tend to occur over a period from 3 months to many years after treatment is completed (i.e. "late delayed reaction") [29]. MRI features associated with radiation necrosis may be indistinguishable from that of tumor progression, showing mass effect, edema and contrast-enhancement. Advanced neuroimaging may be helpful, yet not always definitive, in distinguishing tumor recurrence from radiation necrosis [6, 7].

The incidence of radionecrosis depends on the type and dose of radiation administered as well as the type and timing of chemotherapy. In one large, retrospective study of 426 patients with gliomas diagnosed as GBM (317), anaplastic astrocytoma (88), astrocytoma (2), anaplastic oligodendroglioma (3) and oligodendroglioma (8), which were all treated with radiation therapy, 4.9 % of patients were documented to have radiation necrosis [38]. The mean time interval to radiation necrosis in this cohort was

11.6 months and ranged from 2 to 32 months. In this study, which was conducted before the era of concurrent and adjuvant TMZ, the frequency of radiation necrosis was determined to be much higher in patients who received a combination of radiotherapy and chemotherapy (9.3 %) than in those who received radiotherapy alone (1.3 %). The diagnosis of radionecrosis was made in 21 patients. In 18 of these, the diagnosis was based on the pathologic review of biopsied tissue, while the remaining 3 were diagnosed based on either MRS or PET imaging. Other studies of the frequency of radionecrosis have demonstrated a similar trend with rates generally ranging from 2 to 4 % in patients treated with radiation alone and from 5 to 21 % in the setting of combined chemotherapy and radiation therapy [27, 39–42].

The descriptions of the pathologic features of radiation necrosis have not always been complete in studies that document frequencies of recurrence and radionecrosis following therapy. However, it appears to be clinically relevant if pathologic examination of the resection specimen reveals progressive tumor together with the presence of radiation necrosis at the time of neuroimaging progression. The evidence supporting the discussion of reporting the extent of necrosis in progressive malignant glioma is provided in Table 1. In one investigation by Forsyth et al. [43], 51 patients with supratentorial gliomas, including grades I–IV astrocytomas, oligodendroglioma, oligoastrocytomas, were treated with radiotherapy and then later demonstrated clinical or radiographic evidence of disease progression. Stereotactic biopsy at the time of progression was performed to distinguish tumor recurrence from radiation necrosis. The biopsy material was examined for the presence and degree of radiation necrosis (rated as 0–100 %), amount of residual tumor (none, rare, moderate, plentiful) and histologic class and grade. Tumor recurrence was noted in 30 patients (59 %), radiation necrosis in three (6 %), and a mixture or

Table 1 Radionecrosis evidence table

Author (year)	Description of study	Data class	Conclusions
Ruben (2006) [38]	Retrospective review of adult patients (n = 426) with GBM treated with RT	III	Incidence of Radionecrosis: 4.9 % (21/426); Radionecrosis: RT alone (1.3 %) versus RT and Chemo (9.3 %); Mean time to radiation necrosis: 11.6 months (range 2–32 months)
Forsyth (1995) [43]	Retrospective study of (n = 51) patients with progressive (clinical and radiographic evidence) supratentorial glioma initially treated with RT and stereotactic biopsy at time of progression	III	Tumor Recurrence: 59 % (30/51); radiation necrosis 6 % (3/51); radionecrosis and recurrence: 33 % (17/51). Statistically significant ($P < 0.008$) difference in overall survival times between patients with tumor recurrence (0.83 years) and tumor recurrence and radionecrosis (1.86 years). Statistically significant factors associated with shorter survival times include proportion of residual tumor ($P = 0.024$), proportion of radionecrosis ($P < 0.001$) and histologic grade (III or IV) of tumor ($P = 0.005$)
Levin (2002) [42]	Prospective case series of 90 patients with untreated anaplastic glioma treated with fractionated RT and carboplatin followed by procarbazine, lomustine (CCNU), and vincristine (PCV) for 1 year or until progression; Biopsy or resection results of 56 % (50/90) of patients	III	Necrosis: 21 % (19/90); 23 % (21/90) mixed pattern of necrosis and tumor. Necrosis was a statistically significantly positive predictor of overall survival (hazard ratio: 0.6 (CI, (0.5–0.8) $P = 0.0002$)). Patients with treatment-induced necrosis survived significantly longer compared to patients without MRI or histologic evidence of necrosis (median survival; 108.5 months vs. 18-33 months; $P = 0.0002$)
Perry (2006) [29]	Retrospective expert opinion on the distinctive histologic features of therapy-related pathology in the CNS	III	Pathologic features associated with radiation therapy: vascular wall thickening with proteinaceous deposits and fibrosis; loss of viable luminal endothelial cells; vascular dilatation and intravascular thrombosis. Radiation necrosis appears as large infarct-like regions with "ghost cells" and few viable cells around edges. All cell types (glia, neurons, microglia and vascular cells) are necrotic, with no sparing of perivascular regions or non-dividing cells. More remotely, necrosis forms enlarged fields of eosinophilic material with dystrophic calcification, scattered lymphocytes and macrophages, and hemosiderin. Cytologic atypia can be seen in adjacent reactive brain, but few mitoses

GBM glioblastoma, *RT* radiotherapy, *CI* confidence interval

radionecrosis and recurrence in 17 (33 %). The median survival times after biopsy were 0.83 year for patients with tumor recurrence and 1.86 years for patients with both tumor recurrence and radionecrosis ($P = 0.008$). Moreover, shorter survivals were also associated with a high proportion of residual tumor ($P = 0.024$); a low proportion of radionecrosis ($P < 0.001$); and a histologic grade of III or IV ($P = 0.005$). Thus, the presence and extent of both radiation necrosis and progressive tumor were significantly associated with survival.

Similarly, Levin et al. [42] evaluated the long-term efficacy and safety of accelerated fractionated radiotherapy combined with carboplatin for patients with previously untreated anaplastic gliomas, including those with astrocytic, oligodendroglial or oligoastrocytoma differentiation. Ninety patients received radiation and carboplatin, followed by procarbazine, lomustine (CCNU), and vincristine (PCV) for 1 year or until progression. For those 50 patients who underwent biopsy or resection at the time of clinical or radiologic progression, lesions were classified pathologically as having: (1) no evidence of necrosis; (2) a mixed appearance of tumor and necrosis; or (3) necrosis only. Treatment-induced necrosis was documented in 19 (21 %) patients, while 21 (23 %) had a mixed pattern of necrosis and tumor. Multivariate analysis showed that a younger age ($P = 0.026$), high Karnofsky performance score (KPS; $P = 0.009$), and necrosis ($P = 0.0002$) were predictive of a better survival. On this therapeutic regimen, patients with treatment-induced necrosis survived significantly longer than patients who did not demonstrate MRI or histologic evidence of necrosis (median survival, 106 months vs. 18–33 months)($P = 0.0002$). Interestingly, those anaplastic gliomas that had an oligodendroglioma component had a slightly higher frequency of necrosis than anaplastic astrocytomas (see Table 1).

Specific histologic findings in radiation necrosis

At the time of radiologic or clinical evidence of recurrence following radiation and chemotherapy, there is often a combination of radiation necrosis and progressive glioma in biopsy and resection specimens. A number of pathologic changes in neoplastic tissue and adjacent reactive brain tissue have been associated with prior therapy [29, 44–48]. The evidence supporting the specificity of pathologic changes in progressive malignant gliomas is provided in evidence Table 1. One of the most prominent therapy-related changes is seen in the blood vessels of affected regions. Following radiation therapy, vessels show mural thickening, often dramatic, with deposition of brightly eosinophilic proteinaceous material within their walls. Vascular thickening may be due to deposition of either fibrinous material as a part of fibrinoid necrosis, or composed of hyaline material in more chronic examples. In this setting, there is often a nearly complete loss of viable endothelial cells on the internal lumen. Other vascular changes include vascular dilatation, intravascular thrombosis, exudates of fibrinous material in the immediate perivascular region and the development of vascular malformative lesions. The specific type of necrosis noted following radiation ("radiation necrosis") is distinct from that of spontaneous tumor necrosis of progressive GBM, which may be of the pseudopalisading or coagulative type. Radiation necrosis is typically composed of large infarct-like regions that show low cellular density of dying "ghost cells" within the necrotic regions and only small numbers of dying and viable cells around its edges. Importantly, the necrosis seems to non-discriminatively involve all cell types across it path, including glia, neurons, microglia and vascular cells, with no sparing of perivascular regions or non-dividing cells. In its advanced stages, the necrosis takes the form of an enlarged coagulum of eosinophilic material with zones of dystrophic calcification, scant number of individual and aggregated lymphocytes and macrophages, and evidence of recent and remote microhemorrhages (hemosiderin). Brain tissue surrounding radiation necrosis is highly reactive, showing significant gliosis and perivascular inflammatory cells, as well as stromal and glial cytologic atypia and frankly bizarre cells. Despite cytologic atypia, mitotic figures are generally difficult to find in reactive changes following radiation. In some instances, morphologic features of post-therapy reactive changes mimic progressive gliomas and may require ancillary tests to make the distinction.

In contrast radiation necrosis, the necrosis associated with progressive GBM is typically noted in the setting of a high tumor cell density, often with pseudopalisading of cells around variably-sized foci of necrosis. Necrosis often spares the perivascular regions of the neoplasm and the vascular structures themselves, leaving islands of viable tumor within the enlarging regions of necrosis. In contrast to the vascular changes of radiation necrosis, progressive GBMs will show endothelial hypertrophy and hyperplasia, often in the form of complex and multilayered proliferative vascular structures (glomeruloid bodies). Unlike the reactive stromal cells and reactive astrocytes of gliosis, which tend to have abundant cell cytoplasm and a low level of mitosis, the neoplastic cells associated with recurrence have high nuclear-to-cytoplasmic ratios and mitotic figures are more frequent.

Although there are differences in the histologic features classic for radiation necrosis and progressive GBM, there are cases in which the pathologic features are not straightforward. The findings of cytologic atypia, necrosis and vascular pathology can be seen in both radiation

necrosis and in progressive malignant gliomas. In order to use necrosis as a pathologic feature for grading a glioma, it is critical that it is spontaneous tumor necrosis, such as is seen in pseudopalisading necrosis. Similarly, if vascular changes are used to grade infiltrative gliomas, these changes must represent microvascular hyperplasia with multilayering of endothelial cells [22, 24, 29].

Frozen section

The purpose of a frozen section diagnosis is to guide the neurosurgeon at the time of the operation, to ensure that diagnostic tissue has been obtained, and to give the most accurate intra-operative diagnostic interpretation. Expert opinion suggests that the histologic interpretation of a progressive GBM by the pathologist should be performed in the context of previous pathology findings, clinical history, radiographic features, and neurosurgical findings [9, 10]. This is especially true in the case of a previously treated malignant glioma, since the history of previous therapy will often be necessary for the interpretation of the pathologic findings at the time of frozen section. The diagnostic accuracy of frozen section in malignant gliomas has been previously covered [24]. There are no evidence-based recommendations on the diagnostic accuracy of frozen sections specific to the diagnosis of progressive GBM. However, certain points are worth reiterating.

First, frozen sections are not an optimal technique for detecting cytologic and histologic features that are relied upon for proper classification of a progressive gliomas [25]. For example, the features of oligodendrogliomas, including perinuclear halos, delicate chromatin pattern, and nuclear regularity, are not as evident in frozen tissue. In most instances, the distinction between oligodendroglial and astrocytic differentiation at frozen section will not be critical and the diagnosis of "recurrent infiltrating glial neoplasm" together with a general degree of histologic differentiation (well-, moderately, or poorly differentiated) or histologic grade is sufficient for guiding intra-operative management. Definitive classification and grading of a progressive malignant glioma is most accurate following examination of all tissue submitted for permanent sections, as tissue examined at frozen section may not represent the entire disease process [49].

In addition to the diagnostic limitations of frozen section, the process of freezing tissue introduces artifacts that remain in permanent sections and can limit their interpretation. Most notably, nuclei appear more hyperchromatic and atypical in previously frozen tissue; perinuclear halos of oligodendroglioma are not as evident; and the overall cytologic resolution is lower. Therefore, it is recommended that a portion of sampled tumor tissue be reserved for permanent sections without freezing. If it is not clear at the time of frozen section whether additional tissue will be available for permanent sections, it is prudent to freeze only a portion of the tissue submitted for frozen section [50].

Cytologic preparations

Neurosurgically sampled tissue is often examined cytologically following touch (imprint) or smear preparations. Cytological preparations can be examined quickly and reliably at the time of procedure in order to assess specimen adequacy and to establish a diagnosis. These techniques are diagnostically useful because they maintain high cellular detail not present in frozen sections [51, 52]. In particular, the presence of glial processes emerging from neoplastic cells is more evident on smear preparations than frozen sections. Additionally, nuclear features in cytologic preparations show more detail and can assist in establishing the diagnosis of a neoplasm. Finally, macrophages and other inflammatory cells are often best appreciated on cytologic preparations and these findings can be highly valuable in some cases for excluding a neoplastic diagnosis. While the use of cytologic preparations is a mainstay of neuropathologic diagnosis, there is no objective evidence-based literature that assesses its diagnostic utility specifically for progressive malignant gliomas.

Cell proliferation

While a variety of techniques have been utilized to assess cell proliferation in gliomas, the most reliable and technically feasible method for most pathology laboratories is the Ki-67/MIB-1 antibody [53, 54]. This antibody identifies an antigen present in the nuclei of cells in the G1, S, G2 and M phases of the cell cycle, but is not expressed in the resting phase, G0. The results are usually expressed as a percentage of positive staining tumor cell nuclei. The Ki67/MIB-1 labeling index is not a component of the WHO grading scheme for glial neoplasms [20, 55]. However, many investigations have demonstrated a significant positive correlation between Ki-67/MIB-1 indices and histologic grade. Evidence for the utility of Ki-67/MIB-1 in the diagnosis of primary malignant gliomas has been previously covered (see Table 2) [56].

The determination of a labeling index is not warranted as a routine part of the evaluation of all progressive malignant gliomas, due to limitations associated with tumor heterogeneity and sampling, as well as differences in staining methodology, index determination, and the degree of inter-observer variability. In certain instances, it may have utility in distinguishing between progressive gliomas and reactive gliosis associated with radiation necrosis. In

Table 2 Cell proliferation

Author (year)	Description of study	Data class	Conclusions
Colodner (2005) [57]	Retrospective review of biopsy or autopsy results of patients with brisk reactive gliosis (Pediatric patients 36 % (21/59))	III	No proliferation based on Ki-67/MIB was noted in 49.2 % (29/59) of reactive conditions; average proliferation rate of those with proliferations was 0.9 %; progressive multifocal leukoencephalopathy proliferation: average Ki-67/MIB-1 labeling index: 5.8 %. Therefore, proliferation can be seen in reactive conditions, but is generally low
Deininger[a] (2000) [59]	Retrospective comparative study and post hoc subgroup analyses of 41 patients with initial histologically diagnosed GBM and tumor regrowth treated with either radiochemotherapy (n = 16), or irradiation (n = 18), or no treatment (n = 7). Thirty-nine biopsy results and 2 autopsy results	III	Radiation therapy led to a statistically significant decrease in MIB-1 in the recurrence compared to the primary GBM (decrease, 0.944; $P = 0.0015$)

[a] Categorized positive cells as 0 (no staining), 1 (single positive cells representing less than 2 %), 2 (positively labeled cells up to 10 %), 3 (up to 50 %) and 4 (more than 50 %)

the setting of a biopsy showing a small population of cytologically atypical glial cells together with necrosis and other reactive and inflammatory changes, a high MIB-1 proliferation index within this atypical glial population could favor a progressive malignant glioma. The evidence supporting the discussion of Ki-67/MIB-1 in progressive malignant gliomas is provided in Table 2.

A recent investigation of the proliferative potential of reactive human astrocytes in non-neoplastic diseases used double-label immunohistochemistry to identify Ki-67/MIB-1 among GFAP-positive astrocytes in biopsies from a variety of pathologic conditions [57]. These included infections, arteriovenous malformations, demyelinating lesions, metastatic tumors, and long-standing gliosis. Twenty-nine of the 54 cases in this study showed no evidence of Ki-67/MIB-1 labeling in reactive astrocytes. The proliferation rate was very low (0.9 %) among those cases that showed positivity. The highest proliferation was noted in progressive multifocal leukoencephalopathy, which had an average Ki-67/MIB-1 labeling index of 5.8 %. These results indicate that Ki-67/MIB-1 labeling can be seen in reactive astrocytes. Thus, the presence of Ki-67/MIB-1 labeling in atypical astrocytes cannot be used as a marker of progressive malignant gliomas. However, the proliferation index in the majority of reactive conditions that showed positivity was low. Although not directly assessed in this study, the proliferation indices of reactive astrocytes could overlap with those noted in low grade gliomas [54, 58]. Most high grade gliomas would have Ki-67/MIB-1 labeling indices well above the rates noted for reactive proliferations [24].

One caveat to consider in the interpretation of Ki-67/MIB-1 proliferation indices in the setting of prior therapy is that the treatment may lead to reduced proliferation of neoplastic cells in progressive gliomas, thereby complicating the interpretation of Ki-67/MIB-1 labeling in the differential diagnosis of reactive gliosis versus recurrence [59]. In a study of 41 patients diagnosed with GBM who were treated with radiation or chemoradiation, the Ki-67/MIB-1 was studied in both the primary and progressive neoplasm. Rather than quantifying the % positive cells, this investigation categorized tumor cells as 0 (no staining), 1 (single positive cells representing less than 2 %), 2 (positively labeled cells up to 10 %), 3 (up to 50 %) and 4 (more than 50 %). In the group of GBMs treated with radiation, but not those treated with chemoradiation or those that were untreated, there was a significant reduction in the proliferation score in the recurrence, going from 2.44 before radiation to 1.50 after radiation ($P = 0.0015$). In this study, progressive GBMs had a wide range of Ki-67/MIB-1 indices, from 0 to 4 (absent to over 50 % positive). Thus, while the Ki-67/MIB-1 labeling indices in reactive astrocytes are generally low, there can be overlap with those seen in progressive gliomas. Therefore, proliferation indices should be used cautiously in the attempt to distinguish progressive gliomas from reactive astrocytosis associated with radiation necrosis. The evidence supporting the use of proliferation markers the diagnosis of progressive malignant gliomas is provided in Table 2.

Immunohistochemical and molecular testing

The diagnosis of progressive GBM can, in some instances, be aided by the use of immunohistochemical stains and/or molecular genetic testing. The specific genetic alterations

that have been defined for each histologic category of glioma have been previously described and are beyond the scope of the current review [60]. Specific genetic alterations that have been most thoroughly documented include *IDH1, IDH2, PTEN* and *TP53* mutations, *MDM2* and *EGFR* amplification, $p14^{ARF}$ and *p16(CDKN2A)* deletion, 1p/19q deletions and promoter methylation status of *MGMT* [21, 22, 61, 62]. Some genetic alterations have been used in the diagnostic setting, either to provide assistance with pathologic classification or to provide independent prognostic information [63]. *IDH1* and *IDH2* mutations have been recently described as frequent alterations in grade II and III astrocytomas, oligodendrogliomas and oligoastrocytomas [64]. They are also present in GBMs that progress from lower grade gliomas (secondary GBMs). Allelic losses on chromosomes 1p and 19q are strongly associated with oligodendrogliomas and the morphologically pure, or classic, forms of oligodendrogliomas have the highest frequency of combined 1p/19q loss [65–69]. Gliomas with genetic alterations that include 10q (PTEN) loss, *TP53* mutation, gains on chromosome 7, including EGFR amplifications, are more closely associated with astrocytic differentiation. *PTEN* loss, *EGFR* amplification and *p16(CDKN2A)* deletion are most often seen in high grade astrocytomas, including GBM [70, 71]. Genetic testing of malignant gliomas is becoming a routine component of pathologic diagnosis and there are some specific examples when such tests can provide diagnostic utility in the diagnosis of progressive malignant glioma. The evidence supporting the use of ancillary test in the diagnosis of progressive malignant gliomas is provided in Tables 3, 4, 5, 6 and 7.

P53

TP53 mutations are frequent in grade II and III astrocytomas and the GBMs that progress from them (secondary GBMs) and also occur at lower frequency in primary GBMs [22, 72]. The p53 protein accumulates in the nuclei of *TP53* mutant glioma cells, since the mutant form is not degraded as rapidly as the wild type. Thus, immunohistochemistry can be used to detect nuclear p53 in most *TP53* mutant astrocytomas. These characteristics provide a rationale for the potential use of p53 immunohistochemistry in discriminating neoplastic astrocytes from reactive astrocytes, and in particular, neoplastic astrocytes of progressive GBMs from the reactive gliosis associated with radionecrosis. To explore the question of whether p53 immunoreactivity could be used as a discriminating marker for neoplastic astrocytes, Yaniji et al. performed an IHC study of p53 in 110 CNS lesions including 22 grade II astrocytomas, 12 grade III astrocytomas, 42 GBMs, and 56 reactive astrocytic lesions of various etiologies [73]. They found that 54 % of grade II astrocytomas, 75 % of grade III and 90 % of GBMs were immunoreactive for p53. Among the reactive astrocytic lesions, only five (9 %) cases of were immunoreactive for p53 and these were all examples of progressive multifocal leukoencephalopathy. It was concluded that p53 immunostaining was uncommon in reactive gliosis and could potentially be used to distinguish it from astrocytomas (see Table 3).

A second study performed by Kurtkaya-Yapicier et al. [74] reached a different conclusion. They studied p53 immunoreactivity in 60 nonneoplastic CNS lesions, including gliosis (n = 12), infarction (n = 9), demyelinating disease (n = 23), progressive multifocal leukoencephalopathy (n = 11), and herpes simplex virus encephalitis (n = 5). Grade II-IV astrocytomas (n = 50) were also included. p53 immunoreactivity was noted in all reactive lesions, including 100 % of the cases of gliosis. The immunoreactivity was generally of low level, most being 1+ within a range of 0 to 3+ in the cases of gliosis. All astrocytomas exhibited p53 immunoreactivity. Thus, the authors concluded that p53 immunoreactivity could be present in reactive lesions and well as astrocytomas and that it should be used cautiously and as a part of a battery of stains (including Ki67/MIB-1 and the histiocytic marker KP-1) in making the distinction between reactive gliosis and astrocytoma (see Table 3).

The difference in results and conclusions of these two investigations were substantial. They may be explained, at least in part, by technical differences in immunohistochemistry protocols used, such as antibody dilution, epitope retrieval and incubation times. Also, there may have been differences in the threshold level for detecting immunoreactivity. Nonetheless, there was wide variation in levels of p53 immunoreactivity noted between the two laboratories, raising serious questions regarding the appropriateness of p53 as a marker that can distinguish gliosis from gliomas. It should also be considered that radiation causes up regulation of p53 in non-neoplastic cells, most likely due to its DNA-damaging capacity [75, 76]. Thus, irradiated cells of gliosis associated with radiation necrosis could potentially show p53 expression due the effects of radiation, which would make them difficult to distinguish from progressive malignant glioma on this basis. While the time course of effects of some forms of radiation on p53 upregulation in non-neoplastic cells appears to be short and transient, the impact of ionizing radiation on p53 in CNS cells including reactive gliosis has not been well documented (see Table 3) [77–79].

WT1

The unexpected expression of the Wilms' tumor gene product (WT1) by astrocytic tumor cells raised the

Table 3 Immunohistochemical and molecular testing—p53

Author (year)	Description of study	Data class	Conclusions
Yaziji (1996) [73]	Retrospective review of p53 immunoreactivity in110 CNS lesions from 108 patients; Grade II astrocytomas (n = 22), Grade III astrocytomas (n = 12), GBMs (n = 42), Reactive astrocytic lesions of various etiologies (n = 56)	III	Immunoreactivity for p53 was noted in 54 % (12/22) of Grade II astrocytomas, 75 % (9/12) of Grade III astrocytomas, 90 % (38/42) of GBMs. Of the 56 reactive astogliosis lesions, 9 % (5/56) were immunoreactive. p53 is uncommon in reactive gliosis and can be of potential utility in distinguishing it from astrocytomas
Kurtkaya-Yapicier (2002) [74]	Retrospective comparative immunohistochemical study of p53 in 60 CNS lesions; Gliosis (n = 12), infarction (n = 9), demyelinating disease (n = 23), PML (n = 11), HSV encephalitis (n = 5) compared to Grade II – IV astrocytomas (n = 50)	II	Immunoreactivity for p53 was reported in 100 % (12/12) of Gliosis, 100 % (9/9) of Infarction, 100 % (23/23) of demyelinating disease, 100 % (11/11), 83 % (5/6) of HSV. While p53 staining was low level in reactive conditions, it was present in the large majority. Therefore, p53 should be used with caution for discriminating neoplastic from reactive astrocytes

possibility that it might be a marker that could distinguish neoplastic astrocytes from those of reactive astrocytic proliferations, such those seen in response to radiation-associated necrosis [80]. Schittenhelm et al. investigated the utility of IHC for WT1 in distinguishing gliosis from gliomas using paraffin-embedded sections from normal brains (28 cases), reactive gliosis that arose in response to a variety of etiologies (48 cases) and astrocytomas, grades I–IV (219 total cases). In normal brains and reactive gliosis, the authors reported that only the endothelial compartment showed appreciable expression of WT1, while normal and reactive astrocytes did not. In contrast, WT1-positive tumor cells were demonstrated in pilocytic astrocytomas (66.7 %), grade II astrocytomas (52.7 %), grade III astrocytomas (83.4 %) and GBMs (98.1 %). Establishing a cut-off value of 0 % immunoreactive tumor cells served to recognize neoplastic astrocytes with 100 % specificity and 68 % sensitivity and was associated with positive and negative predictive values of 1 and 0.68, respectively. This investigation concluded that WT1 expression in astrocytes strongly favored their neoplastic nature rather than reactive or normal and suggested this marker could be used to differentiate reactive astrocytes from astrocytomas (see Table 4).

In a follow-up investigation by the same investigative group, a larger and more diverse set of non-neoplastic diseases with reactive astrocytes was studied for WT1 expression [81]. In this set of 120 cases, WT1 expression was noted in 17 % of the non-neoplastic cases and was especially noted in the gliosis surrounding inflammatory lesions. Thus, the authors concluded that the specificity of WT1 for neoplastic astrocytes was not as high as previously reported and that WT1 should not be considered a tumor specific marker. This investigation did not specifically address the role of WT1 expression in post-therapy gliomas and therefore its ability to assist in the distinction between radiation necrosis and progressive malignant gliomas remains unsettled (see Table 4).

IDH1

Mutations in IDH1 are frequent in grade II and III astrocytomas, oligodendrogliomas, and oligoastrocytomas, as well as the GBMs that progress from these lower grade lesions [64, 82]. Importantly, over 90 % of IDH1 mutations in the diffuse gliomas occur at a specific site and are characterized by a base exchange of guanine to adenine within codon 132, resulting in an amino acid change from arginine to histidine (R132H). Because of this consistent protein alteration, a monoclonal antibody has been developed to the mutant protein, allowing its use in paraffin-embedded specimens (mIDH1R132H) [83]. The initial characterization of this antibody was performed on 186 gliomas of various histologies and grades that had been previously sequenced for IDH1 mutations. Of the 95 cases that had IDH1 R132H mutations, 94 showed immunoreactivity using the antibody. Seventy-seven wild type cases and six cases with other forms of IDH1mutations did not demonstrate immunoreactivity for mutant IDH1. Among cases that were initially identified as wild type by sequencing, there were eight that showed immunoreactivity for the mutant protein. Upon resequencing these wild type tumors using the tissue that showed the highest level of immunoreactivity, all eight were found to have R132H mutations. The sensitivity for identifying IDH1 mutant gliomas of any kind using this monoclonal antibody was

Table 4 Immunohistochemical and molecular testing—WT1

Author (year)	Description of study	DATA class	Conclusions
Schittenhelm (2008)[a] [80]	Retrospective diagnostic comparative study of paraffin-embedded sections of n = 295 cases (Normal brains (n = 28 cases), reactive CNS (n = 48), astrocytic neoplasms (n = 219 cases))	II	WT1 positive tumor cells were noted in pilocytic astrocytomas (66.7 % of cases), WHO grade II astrocytomas (52.8 %), WHO grade III astrocytomas (83.4 %), WHO grade IV glioblastomas (98.1 %). Statistically significant difference in staining scores of astrocytic tumors combined versus reactive CNS ($P < 0.0001$).Statistically significant differences of WT1 expression in astrogliosis versus astrocytomas ($P < 0.05$). Statistically significant difference of WT1 expression between normal brains compared to astrocytomas ($P < 0.0001$). Statistically significant difference of WT1 expression between gliosis and astrocytomas subtypes ($P = 0.0001$). Using score of 0, neoplastic astrocytes: 100 % specificity, 68 % sensitivity; PPV and NPV of 1 and 0.68. Using score 1 (<1 % immunoreactive cells), neoplastic astrocytes: 100 % specificity, 52 % sensitivity; PPV and NPV of 1 and 0.52
Capper (2010) [81, 83]	Retrospective study of n = 139 cases (reactive gliosis (n = 120), WHO II post-therapy gliomas (n = 6), WHO II post-therapy gliomas (n = 13))	III	WTI positive cells were noted in 17 % (20/120) of the cases of reactive gliosis. WT1 is not a tumor-specific marker and cannot dependably differentiate reactive gliosis from low-grade glioma

PPV positive predictive value, *NPV* negative predictive value

[a] Staining score: 0 (no staining), 1 (singular positive cells <1 %), 2 (2–19 %), 3 (20–50 %) and 4 (>50 %)

94 % and the specificity was 100 %. The ability of the antibody to detect only a minor component of the tissue as mutant may give this method greater sensitivity than sequencing for identifying R132H mutant gliomas (see Table 5)

The high degree of specificity of mutant IDH1 to diffuse gliomas makes it a potential biomarker for distinguishing glial neoplasms from reactive gliosis and other non-neoplastic lesions. Horbinski et al. [84] used a PCR-based assay on formalin-fixed paraffin-embedded material from 75 glial neoplasms and 57 nonneoplastic conditions that can mimic gliomas including radiation changes, viral infections, and infarcts. In their analysis of the gliomas, they found that 37 (49 %) were positive for *IDH1* or *IDH2* mutations and that the most common mutation was *IDH1* (97 %). Importantly, none of the nonneoplastic cases, including the examples of reactive gliosis associated with prior radiation, were positive for *IDH* mutations. They concluded that PCR-based methods to detect *IDH1/2* mutations could be performed in a clinical setting to enhance the accuracy of diagnosis of gliomas (see Table 5).

The ability of the monoclonal antibody to sensitively and specifically detect mutant IDH1 protein provides the opportunity to use immunohistochemistry rather than PCR-based sequencing in distinguishing gliomas from reactive conditions. One of the first studies to explore this possibility performed immunohistochemistry for mutant IDH1 on a set of 21 WHO grade II astrocytomas and 20 examples of reactive conditions representing a diverse set of etiologies [85]. They detected immunoreactivity for IDH1 in 10 of the 21 astrocytomas, but in none of the reactive conditions, suggesting that the specificity of IDH1 immunohistochemistry for neoplastic disease was high. This study did not include PCR-based sequencing of the IDH1 gene for comparison of sensitivities for detecting mutation. However, the percentage of IDH1 mutant cases of grade II astrocytoma in this study was lower than most PCR-based investigations [64, 83, 86].

A second study of the utility of immunohistochemistry for mutant IDH1 also demonstrated excellent sensitivity for this method of detecting mutant IDH1 and for distinguishing diffuse gliomas from non-neoplastic lesions of the brain [29]. This investigation also specifically addressed the question of whether this antibody could distinguish between glioma and therapy-induced changes. Using the mIDH1R132H antibody, 120 reactive gliosis specimens of various etiologies were examined for immunoreactivity for

Table 5 Immunohistochemical and molecular testing—IDH1

Author (year)	Description of study	Data class	Conclusions
Capper (2010) [81, 83]	Retrospective diagnostic comparative study of n = 139 cases (reactive gliosis (n = 120), WHO II post-therapy gliomas (n = 6), WHO II post-therapy gliomas (n = 13))	III	mIDH1R132H showed 100 % specificity in detecting cells from diffuse astrocytic and oligodendroglial tumors. mIDH1R132H immunohistochemistry reliably differentiates between reactive and neoplastic glial cells originating from astrocytic or oligodendroglial grade II and III gliomas with IDH1R132H mutation
Horbinski (2009) [84]	Retrospective comparative study of 132 neoplasms (n = 75) and nonneoplastic lesions (n = 57) to detect IDH1/2 in FFPE sections	II	IDH1 or IDH2 mutation was noted in 49 % of neoplastic cases (37/75); 97 % (36/37) of positive cases were mutant for *IDH1*. None of the non-neoplastic cases were mutant
Camelo-Piragua (2010) [85]	Retrospective study of biopsy results to detect mutant IDH1 in 41 cases, including diffuse astrocytoma WHO grade II (n = 21) and reactive conditions (epilepsy (n = 10), infarcts (n = 7), evacuated hematoma (n = 2), traumatic brain injury (n = 1))	III	IDH1 mutant protein was seen in WHO grade II astrocytomas 42.9 % (9/21) and in 0 % of reactive samples

FFPE formalin-fixed paraffin-embedded

Table 6 Immunohistochemical and molecular testing—EGFR/Chromosome 7

Author (year)	Description of study	Data class	Conclusions
Okada (2007) [92]	Retrospective review of pre and postradiation gliomas (n = 15) and non-neoplastic brain tissue (n = 4) using FISH to detect EGFR gene on chromosome 7	III	*EGFR* copy number alterations were noted in progressive gliomas (100 %), but not in non-neoplastic irradiated brain tissue, (0 %)
Burel-Vandenbos (2011) [93]	Retrospective review of gliosis (n = 28) and diffuse low-grade gliomas (n = 39; 23 astrocytomas and 16 oligodendrogliomas) to detect EGFR using IHC on paraffin-embedded sections	III	Weak EGFR expression in 82 % (23/28) of gliosis and strong expression in 0 %. Strong EGFR expression was noted in 100 % (39/39) of low-grade gliomas

FISH fluorescence in situ hybridization

Table 7 Diagnostic panel approach

Author (year)	Description of study	Data class	Conclusions
Camelo-Piragua (2011) [94]	Retrospective study of WHO Grade II diffuse astrocytomas (n = 21) and reactive conditions with astrocytosis (n = 20) to determine patterns of p53 expression, mutant IDH1 expression, IDH1/2 mutations, TP53 mutations, and chromosome 7 numeric alterations	II	FISH for chromosome 7 was the single most sensitive test for identifying astrocytomas as compared to reactive gliosis. Optimal overall sensitivity was noted with a panel that included IHC for p53 and R132HIDH1, as well as FISH for chromosome 7 (See Table 6)

FISH fluorescence in situ hybridization

mutant IDH1 and then compared the results to immunohistochemistry for WT1 and p53 expression, which have both been used as markers to distinguish reactive gliosis from glioma. All non-neoplastic cases investigated were negative for mIDH1R132H, while 17 % of cases showed immunoreactivity for WT1 and 63 % showed immunoreactivity for p53. The investigators also specifically chose 19 gliomas (6 WHO II, 13 WHO III) that had been previously treated with radiation therapy and contained extensive reactive changes. They were able to detect immunoreactive cells in 13 of these lesions, indicating progressive neoplasm. Of note, there was 100 % concordance between the immunoreactive status for mIDH1R132H in the pre-treatment and post-treatment biopsies among those cases where both were available for study. In five of the post-treatment cases, progressive neoplasm was not detected by H&E staining, yet was demonstrated by immunohistochemistry for mutant IDH1. This study demonstrated that IHC for mutant IDH1 was tumor-specific and less frequently positive in non-neoplastic diseases than WT1 or p53. However, since IDH mutations are not frequent in primary GBMs, IHC for the mutant protein would not be of assistance in distinguishing gliosis from IDH wild type primary or progressive GBMs (see Table 5).

EGFR/chromosome 7

Amplifications of *EGFR* occur in approximately 40 % of GBMs and 10 % of anaplastic astrocytomas and can be detected by FISH, CGH, or PCR-based tests [21, 87, 88]. Either wild type or mutated forms of *EGFR* can be amplified, and in either case, both mRNA and cell surface protein levels are markedly increased. The most common *EGFR* amplification is a mutated form lacking exons 2–7, which results in a truncated cell surface protein with constitutive tyrosine kinase activity (EGFRvIII) [88–90]. Although *EGFR* amplifications are much less frequent in low grade astrocytomas and are considered a late genetic event in the progression of tumors to GBM, numeric gains of chromosome 7, where *EGFR* resides, are reported in up to two-thirds of lower grade astrocytomas [91]. Given the assumption that copy number alterations or *EGFR* gene amplifications would not be expected in reactive gliosis, there have been a small number of investigations that have pursued these markers to distinguish reactive gliosis from astrocytic neoplasia.

Okada et al. [92] used FISH to investigate *EGFR* copy number in 15 samples of post radiation progressive gliomas, which included one grade II astrocytoma, four grade III astrocytomas, seven GBMs and 3 with tumor necrosis only, as well as 4 post radiation samples that were non-neoplastic. They found that samples that had progressive neoplastic tissue harbored numerical aberrations of *EGFR*, ranging from 3 copies to those that were greatly amplified. In the four cases of post-radiation gliosis, which were from reactive brain tissue surrounding metastatic neoplasms that were radiated, no *EGFR* copy number alterations were noted. The authors concluded that *EGFR* copy number as determined by FISH could assist with the distinction between progressive malignant gliomas following radiation therapy, when the alternative diagnostic consideration was reactive gliosis (see Table 6).

Given that *EGFR* amplification and increased copy number correlate with increased EGFR protein expression detected by immunohistochemistry and that increased EGFR protein expression has been previously demonstrated in astrocytomas of various grades, Burel-Vandenbos et al. [93] investigated whether EGFR immunoreactivity could be used to distinguish reactive gliosis from astrocytic neoplasms. EGFR expression was investigated in 28 cases of gliosis and 39 diffuse low-grade gliomas (23 astrocytomas and 16 oligodendrogliomas) using IHC on paraffin-embedded sections. EGFR immunostaining was then performed on 22 biopsies of proliferative glial lesions of indeterminate diagnosis to determine if it could be used to distinguish reactive gliosis from a glial neoplasm. Weak EGFR expression was detected in 23 of 28 cases of gliosis, but strong staining was not seen. EGFR expression in gliomas showed a consistent pattern of strong staining in neoplastic cells in all 39 cases. In the follow-up study of indeterminant cases, 14 of the 22 were classified as gliomas and eight as gliosis by two pathologists who used EGFR IHC as a diagnostic aid. Concordance with the initial diagnosis established by the reference center and concordance between the pathologists were 100 %. The results indicated that strong EGFR expression was specific to neoplastic glial cells and that EGFR immunohistochemistry could be useful in distinguishing between reactive gliosis and neoplastic tissue.

Diagnostic panel approach

Because no single biomarker is completely sensitive or specific for neoplastic astrocytes, some authors have advocated using a panel of markers when the differential diagnosis consists of reactive gliosis and glial neoplasms [94]. In a recent study, the status of p53, IDH1 and IDH2, and chromosome 7 was investigated in biopsy specimens from 21 grade II diffuse astrocytomas and 20 reactive conditions. The authors found that the single most sensitive test for identifying astrocytoma was FISH for chromosome 7 gain, which was positive in 76 % of cases, followed by IHC for mutant IDH1 (48 %) and immunoreactivity for p53 (48 %). Each of these tests were found to be 100 % specific for astrocytoma, since there were no cases of gliosis found to be positive for polysomy chromosome 7, immunoreactive for mutant IDH1 or for p53. The combination of IHC for p53 and mutant IDH1 provided a higher sensitivity (71.4 %) than either test alone. The best overall sensitivity (95 %) for astrocytoma was achieved when a panel was used that consisted of FISH for chromosome 7 gain and IHC for p53 and mutant IDH1. It should be noted that this study was conducted on grade II astrocytomas as compared to reactive gliosis, and higher grade astrocytomas were not included (see Tables 7, Table 8).

Therefore, in the diagnostic consideration of progressive malignant gliomas versus reactive gliosis associated with radiation necrosis, a panel approach may be helpful, yet the sensitivity and specificity of these markers may be different.

Neuropathology summary

The current pathologic diagnosis of progressive GBM relies on the histopathologic examination of H&E stained slides prepared from biopsied or surgically resected tissue. A diagnosis should be established in a multidisciplinary setting with knowledge of the previous pathologic diagnosis, clinical information including the type of therapy, neurosurgical impression, and neuroimaging findings. Morphologic criteria for classifying and grading gliomas

Table 8 Diagnostic panel approach—sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) reported by Camelo-Piragua et al.

p53	Mutant-specific (R132H) IDH1	IDH1	FISH, chromosome 7	Sensitivity (%)	Specificity (%)	PPV	NPV
•	–	–	–	47.6	100	100	64.5
–	•	–	–	47.6	100	100	64.5
–	–	•	–	66.7	100	100	74
–	–	–	•	76.2	100	100	80
•	•	–	–	71.4	100	100	76.9
•	–	•	–	76.2	100	100	80
•	–	–	•	80.9	100	100	83.3
–	•	•	–	66.7	100	100	74
–	•		•	95	100	100	95
–	–	•	•	95	100	100	95
•	•	•	–	76.2	100	100	80
•	–	•	•	95	100	100	95
•	•	–	•	95	100	100	95
–	•	•	•	95	100	100	95
•	•	•	•	95	100	100	95

• test applied, *FISH* fluorescence in situ hybridization, *PPV* positive predictive value, *NPV* negative predictive value

can be found in the WHO Classification of nervous system tumors. In the setting of prior radiation and chemotherapy, the effects of therapy on the neoplasm need to be strongly considered since therapies can lead to vascular pathology and necrosis. Thus, strict application of histologic criteria for microvascular proliferation and necrosis are required in order to establish a diagnosis of a GBM. Both the degree of necrosis and the amount of viable progressive GBM should be indicated in the surgical pathology report, since the degree of necrosis is associated with the prognosis for patients with progressive GBM. Proliferation studies using Ki-67/MIB-1 staining can provide correlative data that may be beneficial in clinical management and may in some instances be helpful in distinguishing between progressive GBM and atypical reactive gliosis associated with radiation necrosis. This distinction may also be aided by immunohistochemistry for p53, mutant IDH1, WT1, or by FISH studies for *EGFR* or chromosome 7 numerical alterations. The results from such ancillary tests need to be carefully evaluated in the context of the morphologic features of the progressive GBM.

Key issues for future investigation

Large scale molecular studies, such as those performed by the Cancer Genome Atlas Project, and other genome-wide sequencing studies have rapidly expanded our understanding of GBM and will likely alter the way we practice [95–97]. Already, we have begun to consider GBMs in terms of their gene expression classes as established by the TCGA. The proneural, neural, classic and mesenchymal classes appear to be robust and each may provide diagnostic markers and targets for therapy in the future. While these expression classes are partially defined by genetic and epigenetic alterations, it is not clear if they are fixed or could evolve or shift over time with progression or following treatment [96]. For example, lower grade gliomas, such as those that are grades II and III, are enriched for the proneural expression class and relatively depleted for the mesenchymal class [97]. It remains to be seen if tumor progression from a lower to a higher grade is associated with a shift to another gene expression class. Moreover, it will need to be determined if progressive GBMs maintain their initial gene expression class, shift within the four expression classes, or form new, distinct types of expression classes following therapy.

The discovery of *IDH1* and *IDH2* mutations in lower grade gliomas and the GBMs that progress from them has had a substantial impact on neuro-oncology research and neuropathology practice [64, 98]. Since *IDH* mutations are such a strong prognostic factor, many centers are determining the *IDH* status of diffuse gliomas routinely. It now is apparent that these *IDH* mutant tumors are strongly associated with the hypermethylated phenotype (G-CIMP) and are likely best considered as a molecularly and biologically distinct family of gliomas [99, 100]. The rapid conversion of the discovery of *IDH* mutations to the development of antibodies specifically directed toward the mutated protein has greatly aided diagnosis. The ability to detect IDH1 mutant protein in cells of diagnostically challenging cases, such as those that could represent

progressive gliomas or radiation necrosis has had a marked impact neuropathologic practice [81, 83, 101].

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