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# Diagnostic implications of IDH1-R132H and OLIG2 expression patterns in rare and challenging glioblastoma variants

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**Recent work has demonstrated that nearly all diffuse gliomas display nuclear immunoreactivity for the bHLH transcription factor OLIG2, and the R132H mutant isocitrate dehydrogenase 1 (IDH1) protein is expressed in the majority of diffuse gliomas other than primary glioblastoma. However, these antibodies have not been widely applied to rarer glioblastoma variants, which can be diagnostically challenging when the astrocytic features are subtle. We therefore surveyed the expression patterns of OLIG2 and IDH1 in 167 non-conventional glioblastomas, including 45 small cell glioblastomas, 45 gliosarcomas, 34 glioblastomas with primitive neuroectodermal tumor-like foci (PNET-like foci), 23 with an oligodendroglial component, 11 granular cell glioblastomas, and 9 giant cell glioblastomas. OLIG2 was strongly expressed in all glioblastomas with oligodendroglial component, 98% of small cell glioblastomas, and all granular cell glioblastomas, the latter being particularly helpful in ruling out macrophage-rich lesions. In 74% of glioblastomas with PNET-like foci, OLIG2 expression was retained in the PNET-like foci, providing a useful distinction from central nervous system PNETs. The glial component of gliosarcomas was OLIG2 positive in 93% of cases, but only 14% retained focal expression in the sarcomatous component; as such this marker would not reliably distinguish these from pure sarcoma in most cases. OLIG2 was expressed in 67% of giant cell glioblastomas. IDH1 was expressed in 55% of glioblastomas with oligodendroglial component, 15% of glioblastomas with PNET-like foci, 7% of gliosarcomas, and none of the small cell, granular cell, or giant cell glioblastomas. This provides further support for the notion that most glioblastomas with oligodendroglial component are secondary, while small cell glioblastomas, granular cell glioblastomas, and giant cell glioblastomas are primary variants. Therefore, in one of the most challenging differential diagnoses, IDH1 positivity could provide strong support for glioblastoma with oligodendroglial component, while essentially excluding small cell glioblastoma.**
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In 2008, whole genome analysis of human glioblastomas led to the discovery of recurrent mutations in the active site of isocitrate dehydrogenase 1 (IDH1) in secondary (those progressing from lower grade gliomas) rather than in primary glioblastomas.[1] This discovery was subsequently validated and expanded in larger series of human gliomas, establishing that roughly 80% of all WHO grade II–III infiltrating/diffuse gliomas (astrocytomas, oligodendrogliomas, and oligoastrocytomas) and secondary glioblastomas show mutations in IDH1 and to a lesser extent, IDH2;[2–4] these mutations correlate with enhanced patient survival, and by far the most common alteration is the R132H mutation in IDH1.[5,6] Immunohistochemistry (IHC) for expression of R132H IDH1 mutant protein[7] is increasingly performed in the routine pathologic evaluation of glioblastoma and lower grade diffuse gliomas because of its prognostic significance. In glioblastomas, immunoreactivity for IDH1 mutant protein suggests that the tumor is secondary, even

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without the appropriate history of a preceding lower grade glial neoplasm.[6,8] In support of this notion, such patients tend to be younger and enjoy similarly prolonged survival times as those of more classically defined secondary glioblastomas. IDH1 mutant protein expression has also proven to be diagnostically useful in the distinction of entrapped dysmorphic cortical neurons in low-grade infiltrative gliomas from gangliogliomas, the majority of which do not show IDH1 mutations.[9] Similarly, IDH1 is useful in the distinction of diffuse low-grade gliomas from reactive astrocytosis.[10,11] Lastly, immunopositivity may be useful in distinguishing diagnostically challenging diffuse gliomas from other tumor types, given that other primary central nervous system (CNS) tumors and metastatic malignancies are typically negative.[2,12–15] The only other tumor type currently known to have a significant rate of IDH1 mutations is acute myelogenous leukemia.[16,17]

Although the frequency of IDH1 mutation is well established in the most common forms of WHO grade II–IV gliomas, IDH1 mutant protein expression has not been widely examined in rare glioblastoma variants. A few limited studies have recently suggested that such mutations may be higher in some of the rare variants compared with conventional glioblastomas. For example, a recent case report of granular cell glioblastoma demonstrated R132H IDH1 mutation,[18] another study found it in 2 of 8 glioblastomas with PNET-like foci,[19] and another study found it in 31% of glioblastomas with an oligodenodroglial component.[20] R132H IDH1 mutation was recently reported in gliosarcomas at a similar rate to glioblastomas, with 2 out of 26 (7.7%) cases being positive.[21] However, larger series are still needed to confirm these results and to additionally screen other challenging subtypes, such as small cell glioblastoma and giant cell glioblastoma.

Another potentially useful immunostain in the diagnostic workup of gliomas is OLIG2. Recent work has shown that although this oligodendroglial lineage marker, a bHLH transcription factor, is restricted to oligodendroglia and their progenitors in normal human brain, it is nearly universally expressed in diffuse human gliomas.[22] Thus, OLIG2 is not useful in the distinction of astrocytomas from oligodendrogliomas as was originally hoped, but can potentially help distinguish gliomas from other high-grade CNS and non-CNS malignancies, including sarcomas and primitive neuroectodermal tumors (PNET).[22] Additionally, OLIG2 is a particularly useful diagnostic marker for infiltrative tumors, such as gliomas because the protein has nuclear expression and as such, is often considerably easier to interpret than cytoplasmic markers such as glial fibrillary acidic protein (GFAP), which tends to produce background parenchymal staining. Like IDH1, OLIG2 expression has not been widely examined in rare glioblastoma variants.

In this study, we sought to establish the expression patterns of IDH1 and OLIG2 in a series of 167 non-classic glioblastoma variants, including 45 small glioblastomas, 45 gliosarcomas, 34 glioblastomas with PNET-like foci, 23 glioblastomas with an oligodenodroglial component, 11 granular cell glioblastomas, and 9 giant cell glioblastomas.

## Materials and methods

### Case Selection and Histologic Review

This study was performed in accordance with guidelines set forth by the Institutional Review Board of the University of California, San Francisco (UCSF). For all tumor types, formalin-fixed paraffin-embedded tumor tissue was obtained from both UCSF in-house and consult cases over the last 15 years. Additional cases of small cell glioblastoma (30) and glioblastoma with PNET-like foci (19) were obtained from archived material at Washington University School of Medicine in St Louis from prior studies performed by one of the authors (AP).[23,24] Additional cases of granular cell glioblastoma were obtained from Washington University (3) and Emory University (2). Hematoxylin and eosin (H&E) stained sections were reviewed in all cases for diagnostic accuracy.

### Immunohistochemistry

Sections were stained using an automated IHC staining process at the UCSF Brain Tumor Core and/or the UCSF Immunohistochemistry Laboratory. The IDH1 antibody (DiaNova, Germany) was applied at 1:750 dilution after antigen retrieval with pH 6.0 citrate buffer. OLIG2 antibody (Immuno-Biological Laboratories, Minneapolis, MN, USA) was applied at 1:250 dilution after antigen retrieval with pH 6.0 citrate buffer. IDH1 cytoplasmic staining and OLIG2 nuclear staining were scored as diffusely positive (staining in $\geq 50\%$ of tumor cells), patchy/focal positive (staining in $<50\%$ of tumor cells), or negative (staining in 0% of tumor cells).

## Results

Immunohistochemical results stratified according to the various glioblastoma subtypes are summarized in Table 1 and clarified in greater detail below.

### Small Cell Glioblastoma

As in previously published studies, our 45 small cell glioblastomas were infiltrative tumors composed predominantly of small glial cells with uniform oval mildly hyperchromatic nuclei, a brisk mitotic index despite otherwise low-grade appearing cytology, foci of microvascular proliferation and/or necrosis (often

**Table 1** Summary of immunohistochemical data in glioblastoma variants

| | *Olig2 expression* | | | *R132H IDH1 expression* | | |
|---|---|---|---|---|---|---|
| | *Total positive* | *Diffuse* | *Patchy/focal* | *Total positive* | *Diffuse* | *Patchy/focal* |
| Small cell glioblastoma | 98% (43/44) | 82% (36/44) | 16% (7/44) | 0% (0/45) | 0% | 0% |
| Gliosarcoma | | | | | | |
| Sarcomatous component | 14% (6/42) | 0% | 14% (6/42) | 7% (3/45) | 7% (3/45) | 0% |
| Glial component | 93% (39/42) | 76% (32/42) | 17% (7/42) | 7% (3/45) | 7% (3/45) | 0% |
| Glioblastoma with PNET-like foci | | | | | | |
| PNET-like foci | 74% (25/34) | 56% (19/34) | 18% (6/34) | 15% (5/34) | 12% (4/34) | 3% (1/34) |
| Glial component | 88% (29/33) | 88% (29/33) | 0% | 15% (5/34) | 12% (4/34) | 3% (1/34) |
| Glioblastoma with oligodendroglial component | 100% (21/21) | 95% (20/21) | 5% (1/21) | 57% (13/23) | 52% (12/23) | 4% (1/23) |
| Granular cell glioblastoma | 100% (11/11) | 64% (7/11) | 36% (4/11) | 0% (0/10) | 0% | 0% |
| Giant cell glioblastoma | 67% (6/9) | 11% (1/9) | 56% (5/9) | 0% (0/9) | 0% | 0% |

pseudopalisading), and lacking mucin-filled microcystic spaces and minigemistocytes (Figure 1a). Previously performed molecular testing confirmed chromosome 10q deletions in 98% and EGFR amplifications in 58% of cases. No case demonstrated 1p/19q co-deletion. Nearly all cases (98%) demonstrated OLIG2 expression, with 84% of positive cases showing diffuse (Figure 1b) and 16% showing patchy/focal expression. All 45 cases were negative for IDH1 (Figure 1c), which is consistent with the previously published hypothesis that this represents a variant of primary glioblastoma.

### Gliosarcoma

Of the 45 gliosarcomas included in our study, 22 presented *de novo* (sarcoma component already present at first surgery), while 23 first developed their sarcomatous element after recurrence of a prior glioma, most commonly glioblastoma. The glial component was often seen as nests or islands of fibrillary and/or gemistocytic astrocytoma cells (Figure 2a) admixed with sarcomatous elements composed of reticulin-rich, malignant appearing spindled cells arranged in a fascicular growth pattern (Figure 2a–f). The glial component demonstrated OLIG2 expression in 93% of cases (76% diffuse and 17% patchy/focal; Figure 2b, Table 1), while the sarcomatous component only showed patchy/focal OLIG2 expression in 14% (Figure 2e; Table 1) of cases. The remaining 86% of cases were completely negative for OLIG2 expression in the sarcomatous component (Figure 2b; Table 1).

Like primary glioblastomas, the majority of gliosarcomas (93%, 42/45 cases) were negative for the IDH1 mutant protein (Figure 2c; Table 1). However, IDH1 expression was seen in 3 (7%) gliosarcomas with strong diffuse expression in both the glial and sarcomatous components (Figure 2f; Table 1). All three of these cases were secondary gliosarcomas with prior histories of lower grade glioma. In contrast, none of the IDH1-negative gliosarcomas arose from a prior WHO grade II or III glioma.

### Glioblastoma with PNET-Like Foci

As in prior publications, the PNET-like component in our cases of glioblastoma with PNET-like component typically appeared as sharply demarcated foci within otherwise infiltrative glial neoplasms (Figure 3d); the PNET-like foci displayed markedly increased cellularity, primitive appearing cells with high nuclear-to-cytoplasmic ratios, markedly hyperchromatic nuclei, and high mitotic rates (Figure 3a and d). Neuropil formation and Homer Wright rosettes were seen in a subset (Figure 3a, inset). As a definitional requirement, the PNET-like component in all cases displayed immunoreactivity to one or more neuronal markers, including synaptophysin, Neu-N, neurofilament protein, chromogranin, and neuron-specific enolase (NSE). The extent of positivity for these markers was always greater in the PNET-like foci compared with the adjacent diffuse glioma, whereas the GFAP expression was always greater in the latter element.

Diffuse OLIG2 expression was seen in the glial component of 88% of glioblastomas with PNET-like foci. The PNET-like foci were similarly positive for Olig-2 expression in 74% of cases overall (Figure 3b and e; Table 1), including 6 focal (Figure 3e) and 19 diffuse patterns of expression (Figure 3b). Hence, the PNET-like foci showed a diffuse OLIG2 staining pattern more typical of a glioma in 56% and a focal/negative staining pattern similar to a medulloblastoma or CNS PNET in 44% of cases. No cases displayed greater OLIG2 expression in the PNET-like foci than the surrounding astrocytoma component.

IDH1 mutant protein expression was seen in 15% of glioblastomas with PNET-like foci and in all 5 of these cases, expression was seen in both the glial (Figure 3f, inset) and PNET-like components (Figure 3f), consistent with the previously published hypothesis that the latter clone arose from the former. In one of these cases, IDH1 expression was patchy in both components, whereas the remainder showed diffuse staining in all tumor cells. Of the five IDH1-positive cases, four patients had a prior

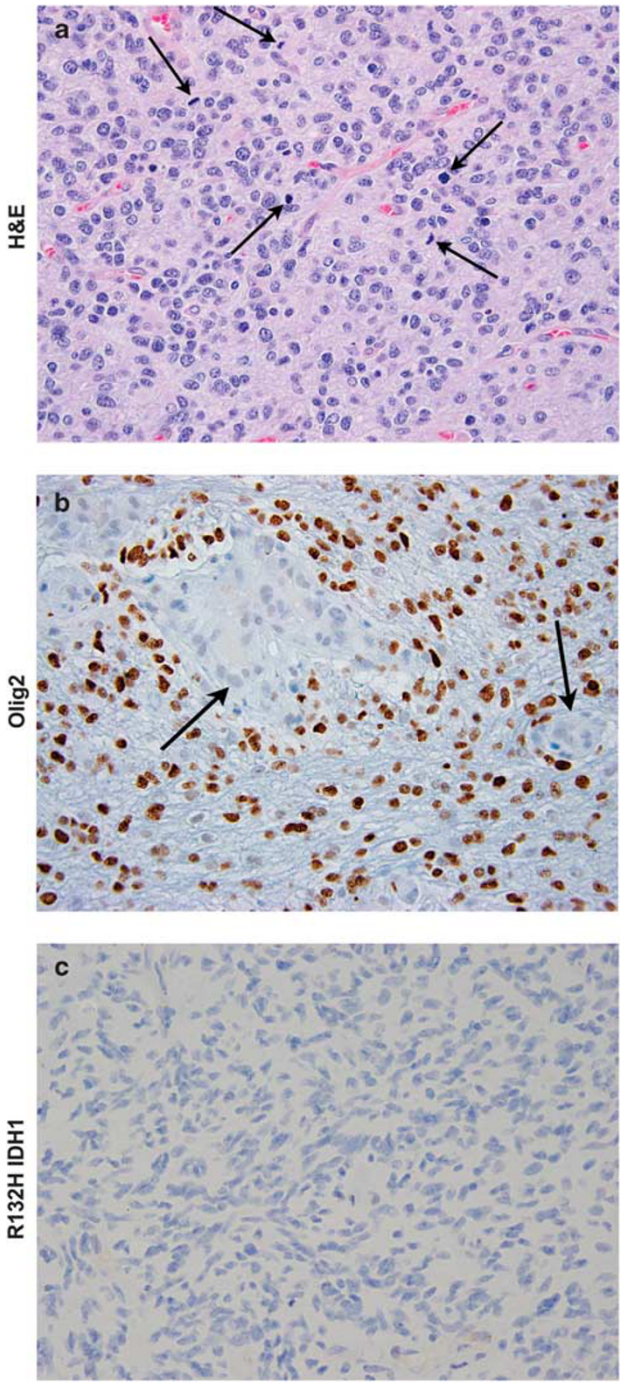


**Figure 1** Small cell variant of glioblastoma. H&E, OLIG2, and IDH1 stained sections from a case of small cell glioblastoma (**a–c**). As shown in H&E section (**a**), all cases of small cell glioblastoma showed small neoplastic astrocytic cells with uniform, bland oval nuclei and numerous mitotic figures (arrows in **a**). Chicken-wire vasculature and perinuclear halos were common histologic findings, as seen in this case (**a**), as well as microvascular proliferation (arrows in **b**). Ninety-eight percent of small cell glioblastomas demonstrated strong nuclear OLIG2 expression (**b**, Table 1), although OLIG2 expression was patchy to focal in 16% of cases (Table 1). All 45 cases were negative for R132H IDH1 mutant protein expression (**c**, Table 1).

history of low-grade glioma, consistent with the clinical definition of secondary glioblastoma. Of the remaining 85%of glioblastomas with PNET-like foci that were IDH1 negative (Figure 3c; Table 1), 2 of 29 (7%) similarly had histories of prior low-grade gliomas and fulfilled clinical criteria for a secondary glioblastoma. This suggests that these two cases may harbor other rarer forms of IDH1 or IDH2 mutations not screened for in our current study.

### Glioblastoma with an Oligodendroglioma Component

The microscopic features of 23 previously diagnosed glioblastomas with oligodendroglial component were reviewed and all demonstrated a mixture of classic astrocytic (Figure 4d) and oligodendroglial components (Figure 4a), along with increased mitotic activity, microvascular proliferation, and by definition, foci of tumor necrosis. Both astrocytic and oligodendroglial components were positive for nuclear OLIG2 expression in all 21 cases examined (Figure 4b and e; Table 1), 20 of which were diffuse (Figure 4b) and 1 of which was patchy/focal (Figure 4e).

IDH1 expression was positive in 57% (13/23) of cases, including both astrocytic and oligodendroglial components (Figure 4c; Table 1). One of the positive cases showed patchy IDH1 expression. Of the IDH1-positive cases, 46% were clinically secondary glioblastomas, as the patients had a known history of a prior lower grade glioma. Of the IDH1-negative cases, one of ten cases (10%) was a secondary glioblastoma by clinical criteria.

Flourescence *in-situ* hybridization testing for 1p/19q was performed in 22 of the 23 cases of glioblastomas with oligodendroglial component, including all 13 IDH1-positive cases and 9 of the 10 IDH1-negative cases. Interestingly, of the 13 IDH1-positive cases, only 4 demonstrated co-deletion of 1p/19q. An additional IDH1-positive case demonstrated loss of chromosome 1, but no deletion of 19q. Of the nine IDH1-negative cases, three showed 1p/19q co-deletions. An additional three IDH1-negative cases demonstrated deletion for either 1p or 19q in isolation. Therefore, considering all cases of glioblastomas with oligodendroglial component, there was detectable 1p/19q co-deletion in 7 (32%), IDH1 positivity in 13 (57%), and both in 4 (18%). Clinical and/or immunohistochemical (IDH1) evidence of secondary glioblastoma subtype was found in 14 cases (61%).

### Granular Cell Glioblastoma

Our 11 cases of granular cell glioblastoma were all either completely or predominantly composed of large foamy clear to granular cells with variable mitotic activity and nuclear enlargement, irregularity, and mild-to-moderate hyperchromasia

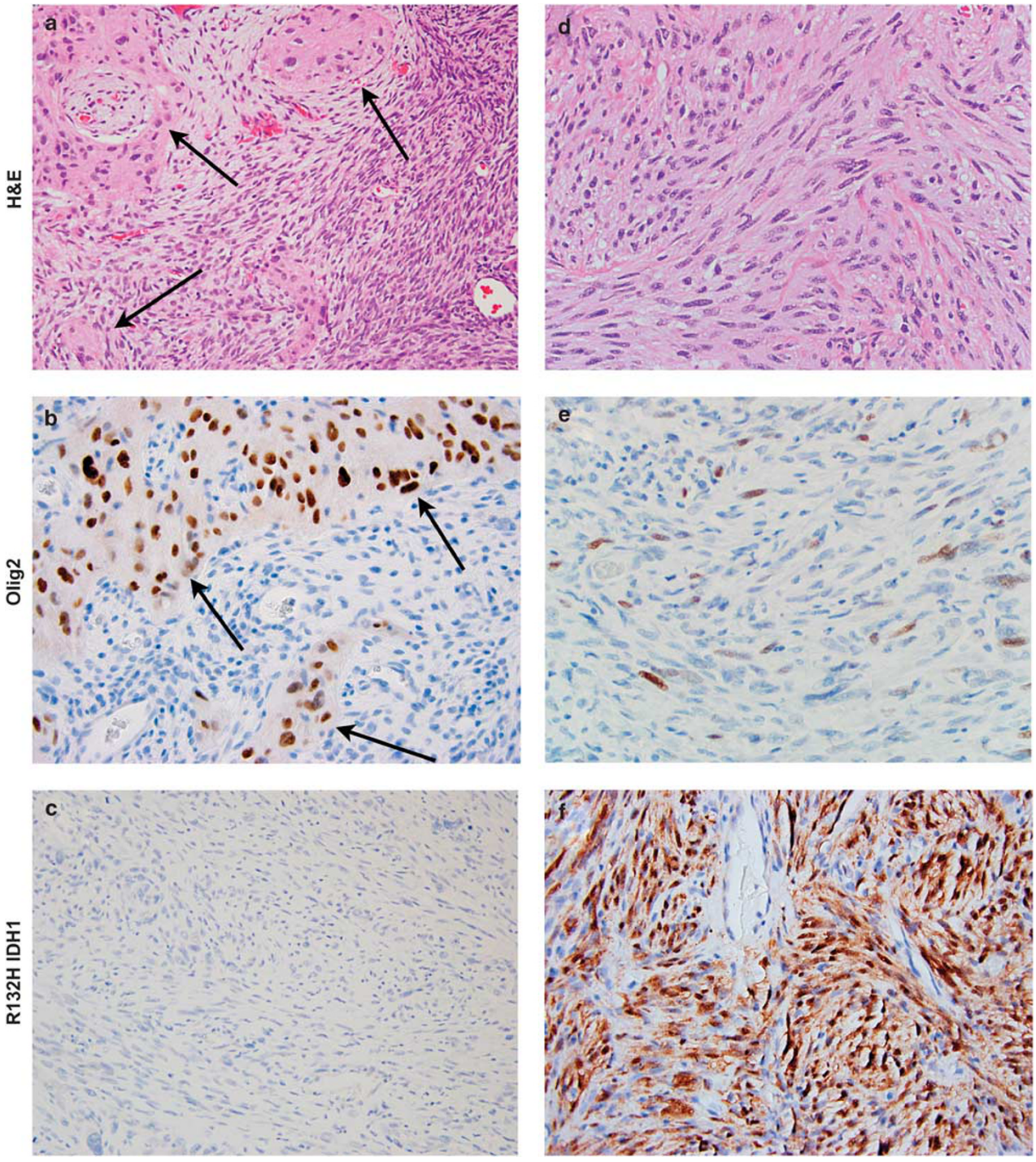


**Figure 2** Gliosarcoma. H&E, OLIG2, and IDH1 stained sections from two different examples of gliosarcomas; one example that was negative for R132H IDH1 mutant protein (**a–c**) and one example that was positive for R132H IDH1 (**d–f**). Both cases demonstrated a prominent spindle cell component of neoplastic cells arranged in fascicles (**a**, **d**). The arrows in (**a**) and (**b**) highlight the glial islands that are often seen admixed with the sarcomatous components. These glial regions showed nuclear OLIG2 expression (**b**) in 93% of cases (Table 1). The sarcomatous component showed patchy/focal OLIG2 positivity in only 14% (**e**, Table 1) and the remaining 86% of cases were negative for OLIG2 within the sarcomatous areas (**b**, Table 1). Diffuse staining for R132H IDH1 mutant protein was seen in 7% of gliosarcomas (**f**, Table 1) and was negative in the remaining 93% of cases (**c**, Table 1).

(Figure 5a and d). Tumor cells were variably positive for both CD68 and GFAP. All 11 cases showed nuclear OLIG2 expression (Figure 5b and e; Table 1), 7 being diffuse and 4 showing patchy/focal staining. Additionally, all cases were negative for R132H-IDH1, consistent with the observation that these typically arise *de novo*, rather than from progression of histologically classic lower grade astrocytomas.

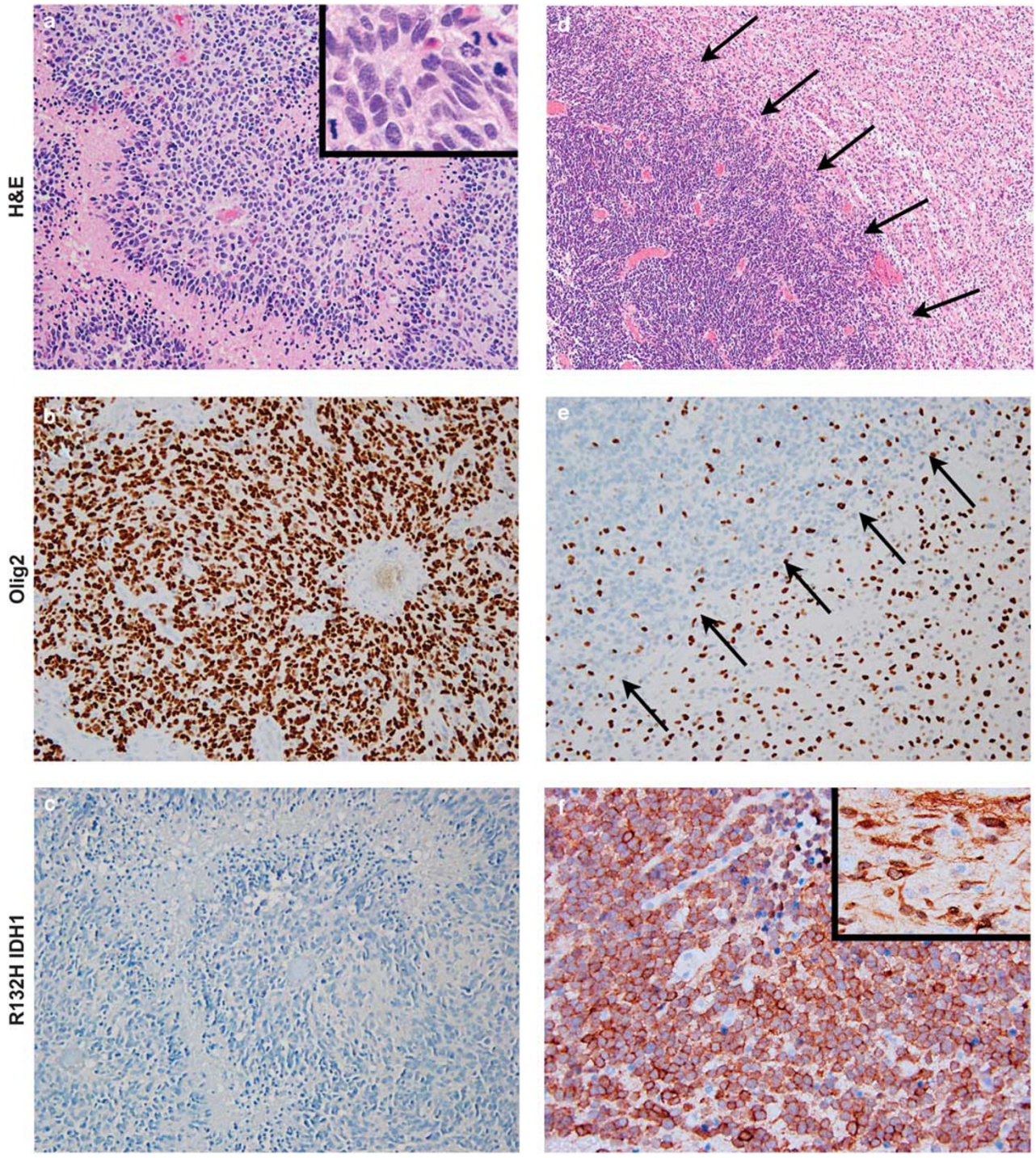


**Figure 3** Glioblastoma with PNET-like foci. H&E, OLIG2, and IDH1 stained sections from two different examples of glioblastoma with PNET-like foci; one example that was negative for R132H IDH1 mutant protein (**a–c**) and one example that was positive for R132H IDH1 (**d–f**). All cases demonstrated foci of primitive neuroectodermal cells exhibiting markedly elevated cellularity with areas of geographic necrosis (**a**, **d**). The primitive tumor cells have high nuclearto-cytoplasmic ratios, enlarged, hyperchromatic oval nuclei and frequent mitotic figures, whereas the presence of Homer Wright rosettes was variable (high magnification inset in **a**). The arrows in (**d–f**) highlight the sharp demarcation between the glial component on the right and the PNET-like component on the left. The majority of tumors demonstrated strong nuclear OLIG2 expression in both glial and PNET-like components (**b**) although OLIG2 expression was patchy to focal in 19% of cases, generally with less expression in the PNET-like foci (**e**). The majority of cases (84%) were negative for R132H-IDH1 (**c**, Table 1), but 15% of cases showed R132H IDH1 expression in both PNET (**f**) and glial (**f,** inset) components.

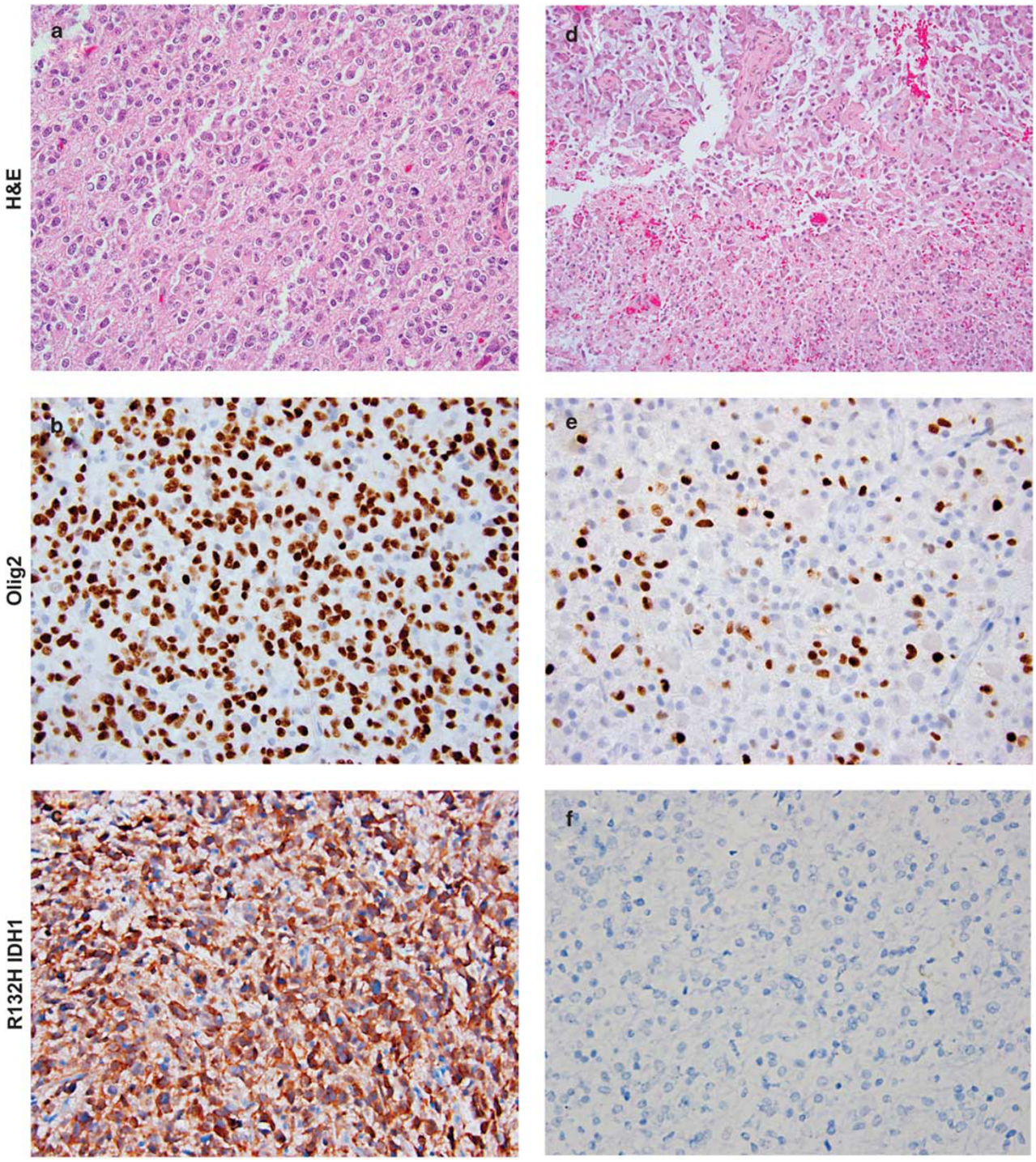


**Figure 4** Glioblastoma with oligodendroglial component. H&E, OLIG2, and IDH1 stained sections from examples of glioblastoma with oligodendroglial component. Images from (**a**) (oligodendroglial component) and (**d**) (astrocytic component) were derived from the same case. All tumors demonstrated strong diffuse nuclear OLIG2 expression (**b**, Table 1), though one case showed patchy OLIG2 expression (**e**, Table 1). Fifty-five percent of cases showed diffuse R132H IDH1 expression in both components (**c**, Table 1) and forty-five percent of cases were negative for R132H IDH1 (**f**, Table 1).

## Giant Cell Glioblastoma

Our nine cases of giant cell glioblastoma all showed prominent bizarre multinucleated giant cells with abundant eosinophilic cytoplasm (Figure 6a and d). Additionally, these tumors demonstrated increased mitotic activity, microvascular proliferation, and foci of tumor necrosis. Six of the nine cases showed nuclear OLIG2 expression (Figure 6b and e), one with diffuse expression and the other five with patchy or focal

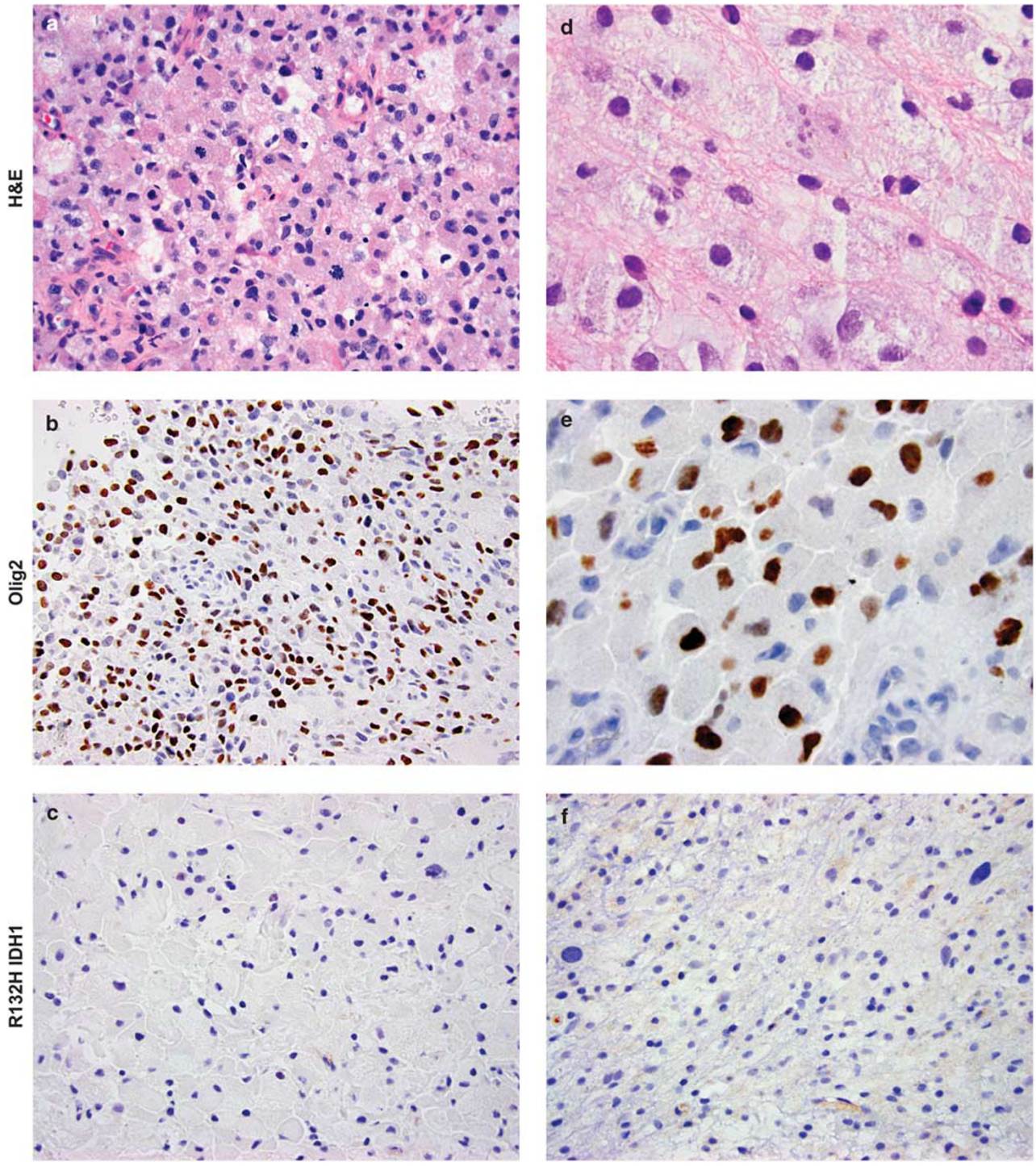


**Figure 5** Granular cell glioblastoma. H&E, OLIG2, and IDH1 stained sections from two examples of granular cell glioblastomas (**a–c** and **d–f**). As shown in H&E sections (**a**, **d**), all granular cell glioblastomas showed large neoplastic cells with abundant eosinophilic granular to clear foamy cytoplasm and frequent mitotic figures. All granular cell glioblastomas demonstrated nuclear OLIG2 expression, including six diffuse (**b**, **e**, Table 1) and three patchy/focal OLIG2 expression patterns. All cases were negative for R132H IDH1 mutant protein expression (**c**, **f**, Table 1).

expression. OLIG2 expression was more often seen in small tumor cells, though giant cells were occasionally positive. All nine cases were negative for R132H-IDH1, consistent with the observation that these typically arise *de novo*, rather than from progression of histologically classic lower grade astrocytomas.

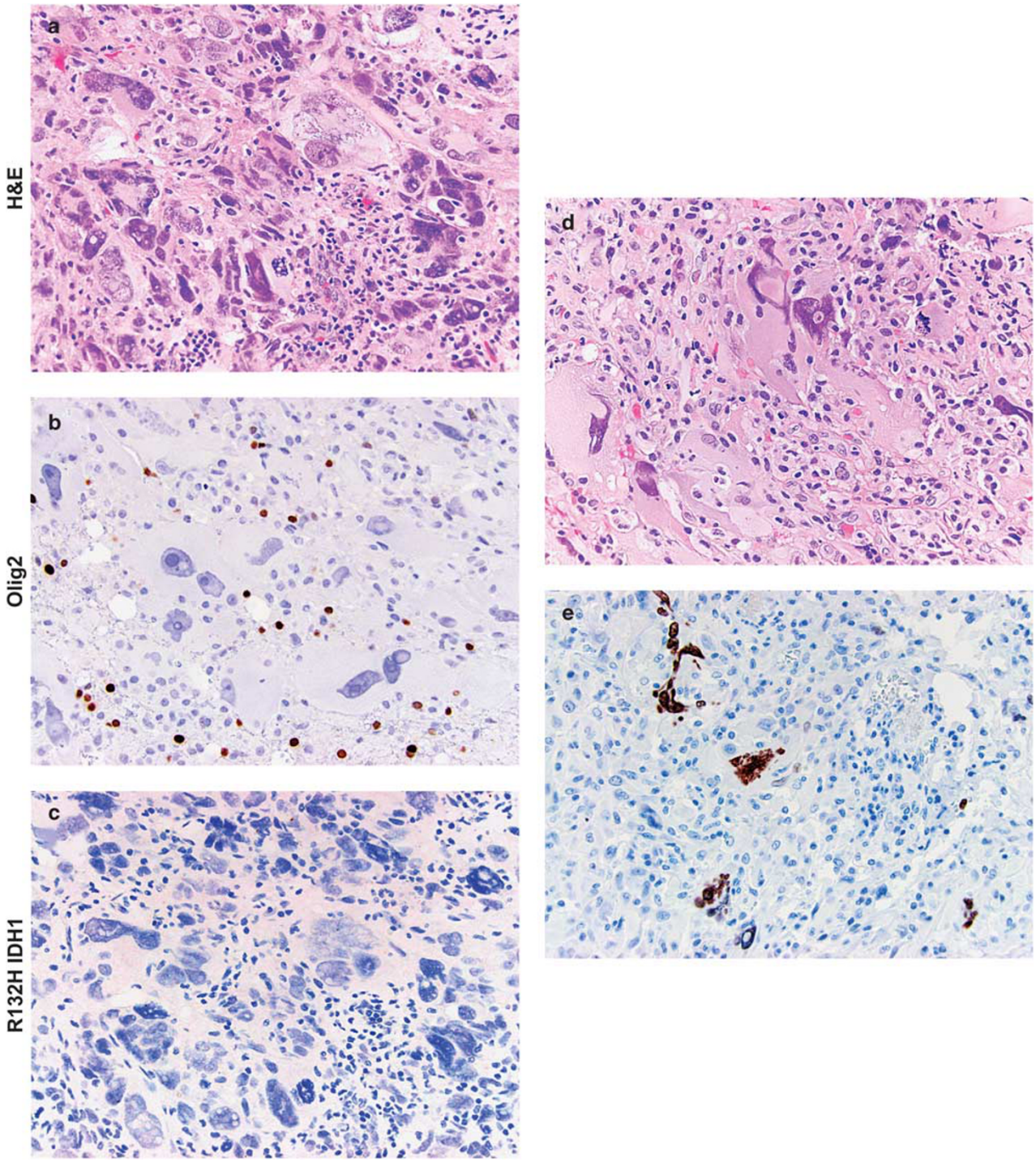


**Figure 6** Giant cell glioblastomas. H&E, OLIG2, and IDH1 stained sections from examples of giant cell glioblastomas. As shown in H&E sections (**a**, **d**), all giant cell glioblastomas contained bizarre multinucleated astrocytoma cells with large, variably admixed with smaller but more classic astrocytoma cells. OLIG2 expression was seen in 6/9 cases (**b**, **e**, Table 1), usually only in small tumor cells or entrapped oligodendrocytes (**b**) but occasionally within scattered giant cells as well (**e**). All cases were negative for R132H IDH1 mutant protein expression (**c**, Table 1).

## Discussion

We have examined the staining patterns of two relatively new diagnostic and prognostic immunohistochemical markers, OLIG2 and IDH1, in 167 rare variant glioblastomas. These data provide new insights and diagnostic utility for challenging cases of glioblastoma as discussed below.

### Small Cell Glioblastoma

The small cell variant of glioblastoma is a rare aggressive variant comprising ~5–10% of glioblastomas. Small cell glioblastoma shows considerable histologic overlap with anaplastic oligodendroglioma (WHO grade III), which poses a critically important diagnostic challenge as the latter has a dramatically superior survival advantage and enhanced therapeutic sensitivity.[23,25] Both gliomas have relatively uniform and bland round to oval nuclei with smooth nuclear contours and mild hyperchromasia. Both can also show delicate chicken-wire vasculature, perinuclear halos, perineuronal satellitosis, and calcifications. In general, the finding of brisk mitotic activity in the presence of small cell size and bland cytology favors small cell glioblastoma, while increased cell size, nucleolar prominence, presence of minigemistocytes, and mucin-filled microcystic spaces favor anaplastic oligodendroglioma. Nonetheless, sufficient morphologic overlap makes this distinction extremely difficult in a subset of cases. Thus, molecular testing is often needed to distinguish these two entities. 1p/19q co-deletions are common in anaplastic oligodendrogliomas and absent in small cell glioblastoma, whereas EGFR amplification and chromosome 10q deletions are common in small cell glioblastoma and rare in anaplastic oligodendrogliomas.[23]

Based on our data, small cell glioblastomas express OLIG2 nearly uniformly, but are always negative for R132H IDH1 mutation, consistent with the primary form of glioblastoma. As such, the latter finding is particularly useful, as up to 80% or more of anaplastic oligodendrogliomas express the R132H IDH1 mutant protein.[2] In this regard, during our search for cases to include in this study, we discovered a UCSF in-house case with an indeterminate final diagnosis of anaplastic oligodendroglioma *vs* small cell glioblastoma. The neuropathologist ultimately favored anaplastic oligodendroglioma because subsequent molecular studies demonstrated deletion of 1p19q. This particular patient is still alive 13 years after his first glioma diagnosis in 1999. We stained this case for R132H IDH1 alongside our study cases and found diffuse IDH1 positivity, providing further support for anaplastic oligodendroglioma. Thus, IDH1 IHC represents an additional assay of particular diagnostic value in such cases and it is simpler, cheaper, and more readily available than the genetic assays. These same immunohistochemical and genetic distinctions are also true in the differential diagnosis of small cell glioblastoma with anaplastic oligoastrocytomas and glioblastoma with an oligodendroglial component (see Discussion below), although the 1p19q co-deletions are much less common, while IDH1 expression is slightly less common in these mixed glioma subtypes than in the pure oligodendrogliomas.

### Gliosarcoma

Gliosarcoma accounts for ~2% of glioblastomas. This variant shows a biphasic growth pattern with intertwined but distinct glial and sarcomatous components. The glial component is typically GFAP positive and reticulin negative while the sarcomatous component is typically GFAP negative and reticulin positive.[25] Molecular analyses of the two components have demonstrated identical genetic alterations in both elements suggesting a monoclonal origin of this tumor.[25–27] Our data suggest that in most cases, gliosarcomas demonstrate OLIG2 expression only in the glial component and the majority are R132H IDH1 negative. As such, OLIG2 would only occasionally be helpful in distinguishing a sarcoma-predominant gliosarcoma from a pure sarcoma. Interestingly, we did encounter R132H IDH1 mutation in three cases; all of these patients had a prior history of low-grade glioma years earlier. However, because the majority of gliosarcomas are IDH1 negative, this mutation is of limited value in the differential with a pure sarcoma. The rarity of this mutation in gliosarcomas also provides further evidence that most cases are related to the primary form of glioblastoma despite the rarity of EGFR amplification in this subtype.

### Glioblastoma with PNET-Like Foci

Glioblastomas with primitive neuroectodermal-like (PNET-like) foci represent far less than 1% of glioblastomas and are thought to often arise as secondary glioblastomas, given that at least a fourth of cases have prior histories of lower grade gliomas.[24] This glioblastoma variant shows both glial and primitive neuronal (PNET-like) components. The glial component is usually astrocytic in nature but can occasionally show oligodendroglial features; foci of sarcoma are also seen in about 10% of cases.

In our current series, most cases of glioblastoma with PNET-like foci expressed OLIG2 in both glial and PNET-like components, a feature that contrasts with CNS PNET, the main differential diagnostic consideration. Although these tumors often have clinicopathologic features of secondary glioblastoma, R132H IDH1 expression was only found in 15% of our cases. It remains possible, however, that other mutations in IDH1 or IDH2 may be present in subsets of glioblastomas with PNET-like foci and additional molecular studies would be needed to further address this possibility.

### Glioblastoma with Oligodendroglial Component

Glioblastomas with an oligodendroglioma component comprise anywhere from $<1$ to $>10\%$ of glioblastomas depending on definitions and philosophical preferences. Analogous to conventional glioblastomas, they can either arise from a lower

grade oligoastrocytoma ('secondary glioblastoma with oligodendroglial component') or *de novo* ('primary glioblastoma with oligodendroglial component'). Potentially, this distinction is prognostically important, as the overall survival of primary glioblastomas is known to be worse than secondary glioblastomas; currently, it is unclear whether the better prognosis of glioblastoma with oligodendroglial component is simply because oligoastrocytomas generally do better than pure astrocytomas of the same grade or because a higher fraction of glioblastomas with oligodendroglial component are genetically secondary rather than primary subtypes. Since earlier studies have found R132H IDH1 mutations in 80% of secondary glioblastomas and only in <10% of primary glioblastomas (most of which are now thought to be secondary glioblastomas without documentation of a prior low-grade precursor), IHC for IDH1 may help answer this question.[1] In our 23 glioblastomas with oligodendroglial component, OLIG2 was strongly positive in both oligodendroglial and astrocytic components, and 57% of our cases expressed R132H IDH1 mutant protein (roughly half of which had a clinical history suggesting secondary glioblastoma). One case fulfilled criteria for secondary glioblastoma clinically, but was IDH1 R132H negative. Therefore, when combining the clinical and immunohistochemical data, 14 of the 23 cases (61%) were secondary rather than primary, a dramatically higher fraction than that of classic or purely astrocytic glioblastomas. Interestingly, of the 13 IDH1-positive cases, only 4 demonstrated 1p/19q co-deletion, a prior marker of favorable outcome. Thus, IDH1 may prove to be a more sensitive marker of the glioblastoma with oligodendroglial component subtype than 1p/19q co-deletions. Of the eight IDH1-negative cases that were tested for 1p/19q co-deletion, three demonstrated co-deletion for 1p/19q. Therefore, our data do not demonstrate a significant correlation between IDH1 and 1p/19q status.

### Granular Cell Glioblastoma

Granular cell glioblastomas constitute far less than 1% of all glioblastomas and can be extremely diagnostically challenging when associated with only minimal nuclear atypia.[28,29] Generally, the use of GFAP and macrophage-specific markers (eg, CD163) substantiates the glial lineage. However, CD68 immunoreactivity can be misleading since the lysosome-rich glioma cells are often positive and GFAP may be difficult to interpret in cases with high background staining. Only rare genetic studies of this tumor type have been reported, often showing similar alterations to glioblastoma in general, with the exception that EGFR gene amplifications have not been found.[30] Our data suggest that this rare variant is typically OLIG2 positive and R132H IDH1 negative. As such, the former can be diagnostically useful in ruling out macrophage-rich processes, while the latter is most consistent with the primary form of glioblastoma. This fits with previously reported data of patients virtually always presenting with a newly detected mass lesion, rather than progression from a more classic lower grade glioma. Additionally, clinical follow-up demonstrates that even when the histology looks lower grade (WHO grade II or III), the average survival time is less than a year, in keeping with a primary glioblastoma variant. One recent case report demonstrated a granular cell glioblastoma case that showed R132H IDH1 mutation.[18] However, the authors reported a highly unusual molecular pattern with the coexistence of EGFR gene amplification with IDH1 mutation in the same case, alterations which are normally mutually exclusive. A second recently published case was reportedly IDH1 R132H negative,[31] which is similar to our data, suggesting that this is the much more common pattern.

### Giant Cell Glioblastoma

Giant cell glioblastomas account for <5% of glioblastomas. Usually, these tumors do not represent a diagnostic challenge, although some may be confused with non-glial tumors, such as metastatic carcinoma or a pleomorphic sarcoma. All nine of our giant cell glioblastomas were negative for R132H IDH1 mutation, consistent with the primary form of glioblastoma. Interesting, only one case demonstrated diffuse OLIG2 expression, despite the glial nature of these tumors. Although an additional five cases showed patchy/focal OLIG2 expression, the giant cells were typically OLIG2 negative with smaller tumor cells, as well as entrapped oligodendrocytes being OLIG2 positive. Therefore, this marker appears to be of less assistance with this glioblastoma variant than it is with most other diagnostically challenging subtypes.

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## Disclosure/conflict of interest

These authors declare no conflict of interest.

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