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Changing paradigms for targeted therapies against diffuse infiltrative gliomas: tackling a moving target

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Abstract

Introduction: Gliomas are highly heterogeneous primary brain tumors which result in a disproportionately high degree of morbidity and mortality despite their locoregional occurrence. Advances in the understanding of the biological makeup of these malignancies have yielded a number of potential tumor-driving pathways which have been identified as rational targets for therapy. However, early trials of agents that target these pathways have uniformly failed to yield improvement in outcomes in patients with malignant gliomas.

Areas covered: This review provides an overview of the most common biological features of gliomas and the strategies to target the same; in addition, the current status of immunotherapy and biological therapies are outlined and the future directions to tackle the challenges of therapy for gliomas are examined.

Expert opinion: The limitations of current treatments are attributed to the inability of most of these agents to cross the blood brain barrier and to the intrinsic heterogeneity of the tumors that result in treatment resistance. The recent emergence of immune-mediated and biological therapies and of agents that target metabolic pathways in gliomas have provided strategies that may overcome tumor heterogeneity and ongoing trials of such agents are anticipated to yield improved outcomes.

Keywords

gliomas; targeted therapies; tumor heterogeneity; immunotherapy; treatment resistance

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Declaration of interest

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1. Introduction

Gliomas constitute the most common primary brain tumors and are a significant cause of morbidity and mortality across the age groups. The recently revised 2016 WHO classification of brain tumors reorganized the histologic types of these tumors with a new emphasis on molecular characteristics that better define the subtypes of brain tumors including gliomas[1]. The Central Brain Tumor Registry of the United States (CBTRUS) 2009–2013 report estimates that gliomas represent approximately 24.7% of central nervous system tumors. Glioblastoma (GBM), the most aggressive of gliomas, accounts for 56.6% of gliomas and 14.7% of all brain tumors with an age-adjusted incidence rate of 3.21 per 100,000 population[2]. Despite our advanced understanding of the molecular pathogenesis of glioblastoma, mortality remains very high with 2- and 5-year survival rates of 17.4% and 5.6% respectively.[2] While lower grade gliomas comparatively have a better overall survival, they remain a cause of significant morbidity and eventual mortality in the younger adults.

The initial diagnosis of a glioma is usually triggered by the onset of neurological symptoms such as headaches, seizures or focal neurological deficits. However, these symptoms are nonspecific for malignancies and can happen in non-tumor neurological conditions. Advances in imaging techniques including MRI sequences such as diffusion and perfusion-weighted images, and MR spectroscopy have helped distinguish gliomas from other mass lesions in the brain, as well as progression of disease from complications of treatment. Advanced imaging studies also allow a better delineation of tumor heterogeneity, help outline eloquent areas of the brain using functional MRI scans and aid in potentially identifying more aggressive regions of the tumors to facilitate optimal surgical resection. Surgical intervention is uniformly recommended to distinguish tumors from non-tumor masses, to debulk the mass lesion and to obtain an accurate histological and molecular diagnosis of the tumor. The goal of surgery is maximal safe resection of the radiographically evident tumor. Imaging tools such as tractography and functional MRI scans allow for safer resections that aim to avoid the motor, speech and visual pathways [3, 4]. In selected patients, the use of awake-craniotomies allows maximal resection while avoiding eloquent areas especially in setting where neural plasticity may have resulted in migration of functional areas in the brain. Several studies have shown a survival advantage when patients undergo gross total resection of the tumor compared with biopsies.[5, 6] Hence this is the surgical approach of choice for patients with gliomas whenever there is not a medical contraindication. Debulking the tumor further helps to alleviate the clinical symptoms caused by the tumor mass effect. In lower grade gliomas, more aggressive approaches such as supramaximal resections have also been advocated although only some series have shown improvement in outcome [7].

Analysis of resected tumor tissue provides the histological diagnosis and allows further assessment of the molecular characteristics of the tumor; such markers can be both prognostic and predictive for outcomes. For example, mutation of isocitrate dehydrogenase (*IDH*) is frequently seen in low-grade and anaplastic glioma and is a marker of better prognosis [8]. Most glioblastomas on the other hand have wild type *IDH*; the presence of

IDH mutation in glioblastoma suggests an overall better prognosis or indicates progression of a lower grade astrocytoma to a secondary GBM [9, 10]. Similarly, promoter methylation of *MGMT*, a DNA repair enzyme involved in monofunctional DNA repair, which results in transcriptional silencing of the gene, is associated with an improved outcome in the setting of treatment with chemoradiation with alkylating agents such as temozolomide [11, 12].

The effect of radiation therapy on survival was first shown using whole brain radiation therapy (WBRT) in combination with nitrosoureas [13, 14]. It was later demonstrated that adequate doses of RT (50–60 Gy) confined to the location of the radiologically visible tumor can lead to improved median survival and that dose-escalation beyond that does not provide further benefit [15, 16]. Involved field RT has since become the standard radiation approach as recurrent high-grade gliomas are more likely to recur within 2 cm of the original tumor site [17].

The current standard-of-care therapy for newly diagnosed GBM is based on the landmark trial by Stupp et al [18]. Patients with newly diagnosed glioblastoma were randomized to receive radiotherapy alone (2 Gy given 5 days a week for 6 weeks, for a total of 60 Gy) or radiotherapy plus concomitant temozolomide (75 mg/m² of body-surface area daily), followed by six cycles of adjuvant temozolomide (150 to 200 mg per square meter for 5 days during each 28-day cycle). The addition of temozolomide to radiotherapy resulted in significant overall survival benefit: median survival was 14.6 months with radiotherapy plus temozolomide and 12.1 months with radiotherapy alone. The survival benefit of adding TMZ was predominantly seen in patients with methylated *MGMT* [11]. Tumor treating fields (TTF) represent another novel approach for glioma treatment. The device consists of electrodes that are applied to the patient's scalp to deliver low-intensity, intermediate-frequency (100–300 kHz) alternating electric fields. TTF showed survival benefit in patients with GBM in a multivariate analysis taking into account several known prognostic factors including age and *MGMT* promoter methylation status.[19]

Age and performance status are important prognostic factors for the overall survival in GBM [20]. Monotherapy with radiation or TMZ alone based on *MGMT* methylation status may be better tolerated in older individuals. In elderly patients with malignant gliomas, median survival was 8.6 months for the dose dense TMZ arm versus 9.6 months in the RT arm in the NOA-08 trial which was not statistically different.[21] Similarly, the Nordic study in the elderly with GBM reported a median survival of 8.3 months for standard dose TMZ alone and 7.5 months with hypofractionated RT which were superior to 6 months with standard RT alone.[22] Single agent TMZ treatment also showed potential benefit in elderly GBM patients with poor functional status with a median OS of 25 weeks which was better than the expected median OS of 12–16 weeks.[23] A recent randomized phase III trial demonstrated that even older patients with good functional status who can tolerate combined treatment benefit from concurrent chemoradiation therapy regardless of *MGMT* promoter methylation status [24].

First-line treatment for anaplastic gliomas has been a focus of several large randomized trials. Phase III trials of radiation therapy with neoadjuvant or post-radiation chemotherapy with the procarbazine, lomustine and vincristine (PCV) regimen demonstrated significantly

improved overall survival in patients with anaplastic oligodendrogliomas with 1p/19 codeletion in two independent studies establishing the current standard of care for these patients [25, 26]. Hence, we consider this as the appropriate evidence-based treatment for adult patients with anaplastic oligodendrogliomas who have a good functional status. However, given the concern for significant toxicity and poor tolerance of the regimen, an ongoing CODEL study seeks to compare the radiation followed by PCV regimen with radiation with concurrent and adjuvant temozolomide to determine if the latter can provide the same efficacy as PCV with reduced toxicity [27]. The optimal treatment for anaplastic astrocytomas remains to be determined. Interim analysis of the ongoing CATNON study which compares the efficacy of four treatment arms (radiation alone, radiation with concurrent temozolomide, radiation followed by temozolomide and radiation with concurrent and adjuvant temozolomide) in patients with 1p/19q non-codeleted anaplastic gliomas, showed that the arms with adjuvant temozolomide showed improved survival compared to those without leading to the termination of the arms without adjuvant temozolomide [28]. Hence, the current recommended treatment for non-codeleted anaplastic gliomas includes adjuvant temozolomide after radiation therapy although the role of temozolomide given concurrent with radiation therapy remains to be defined as the study matures.

Therapeutic options for patients with low grade gliomas have been challenging to establish due to the relative rarity of the disease and the concerns regarding toxicity related to early aggressive therapies in these predominantly younger patients. However, the growing recognition that there is inevitable tumor progression, morbidity and mortality in these patients has triggered exploration of several trials that have provided guidelines for first-line therapy of these patients. Maximal safe resection instead of biopsy alone is recommended for low grade gliomas based on cumulative evidence from several studies [29] although there are no prospective studies that definitively address this issue. Further, radiation therapy was shown to improve outcome in the management of low-grade glioma regardless of extent of resection although the optimal time of initial radiation therapy was not established after initial trials which showed that progression free survival but not overall survival was improved with radiation therapy at the time of diagnosis versus at the time of recurrence [30]. More recently, the benefit of chemotherapy was conclusively demonstrated in a recent phase III clinical trial which showed that radiation therapy followed by the PCV regimen yielded improved progression-free and overall survival compared to radiation therapy alone in patients with low grade gliomas [31]. However, whether temozolomide should replace the PCV regimen in the treatment of low grade gliomas remains to be determined; results of the CATNON and CODEL trials are expected to provide insights into these treatment options.

2. Biology of gliomas

Gliomas are heterogeneous tumors in both their phenotypic and genetic expression. Numerous genetic mutations have been identified, yet it has been nearly impossible to illuminate a singular disease-initiating event. The diversity of mutations has been an obstacle in developing glioma animal models that can closely approximate human glioma disease and consequently, has caused a universal clinical treatment plan to be elusive. The biological consequences of the genetic and epigenetic alterations gliomas that underlie their increased

survival and malignant transformation include unrestricted proliferation, invasion, neovascularization, necrosis, and resistance to apoptosis.

3. Biology of low grade and anaplastic gliomas

Low-grade diffuse infiltrative gliomas (LGG) constitute WHO grade II primary brain tumors of astrocytic and oligodendroglial lineage. Recent years have heralded a molecular revolution in the understanding of LGG tumors. LGG are generally characterized by a lower frequency of somatic mutations and structural variations per tumor, suggesting that their pathogenesis is driven by fewer genetic changes overall. Pediatric and adult LGG fundamentally differ in their underlying molecular characteristics, despite their histological similarities. Pediatric LGG show alterations in *FGFR1* and *BRAF* genes, and adult LGG are characterized by *IDH1/2* mutations, *ATRX* mutations, and 1p/19q codeletion. These molecular markers have also been incorporated into clinical guidelines to provide context for treatment decisions.[32, 33, 34] The following sections highlight some of the most common genetic alterations in infiltrative gliomas.

3.1 BRAF V600E mutation

BRAF, a Raf kinase involved in the mitogen-activated protein (MAP) kinase pathway has been identified as a driver oncogenic protein in several tumors including low grade gliomas. A point mutation in *BRAF* with valine to glutamate at position 600 (V600E), has been identified as the key oncogenic mutations in several cancers, especially in melanoma; these mutations are believed to change the protein conformation to a constitutively active state driving the downstream MAPK pathways and resulting in increased proliferation. In gliomas, *BRAF*V600E mutations have also been reported most frequently in pleomorphic xanthoastrocytoma (~70%), gangliogliomas (~20%) and dysembryoplastic neuroepithelial tumors (~30%) [35, 36]. Notably, this mutation is uncommon in other adult gliomas including pilocytic astrocytomas (~10%) except its higher occurrence in epithelioid glioblastomas (~50%) and in pediatric malignant gliomas particularly in association with *CDKN2A* [35, 37]. The development of drugs that specifically target BRAFV600E mutations such as vemurafenib and dabrafenib have made a dramatic impact in the outcome of patients with systemic malignancies harboring this mutation such as malignant melanomas; similar but more modest results have also been seen in patients with gliomas anecdotally or in small series suggesting the value of this approach in these uncommon brain tumors especially at recurrence.[38, 39]

3.2 BRAF-KIAA1549 fusions

While BRAF mutations are frequently seen in the subtypes of gliomas outlined above, other alterations in BRAF driven signaling pathway such as the fusion of the can also provide oncogenic pressure in the setting of certain gliomas [36]. Although they occur in about 2% of gliomas overall, their incidence in certain pediatric and young adult low grade gliomas especially in pilocytic astrocytoma (~30%) has been noted to be higher [40]. In addition, rates of *BRAF* fusion appear to vary depending on tumor location in the central nervous system (CNS); ~75% of cerebellar pilocytic astrocytomas exhibit *BRAF-KIAA1549* fusions in contrast with only 33% of supratentorial astrocytomas harboring this alteration [41]

Several other novel BRAF fusions have been described and although their incidence is small, their biological behavior is believed to be similar to *BRAF-KIAA1549* fusions [42].

3.3 Isocitrate Dehydrogenase 1/2

Genome-wide profiling studies unexpectedly identified heterozygous mutations in the metabolic enzymes isocitrate dehydrogenase 1 and 2 (*IDH1/2*) in >70% of grade II and grade III gliomas and more than 90% of secondary GBM bringing to light the involvement of metabolic genes in the pathogenesis of lower grade infiltrative gliomas [8]. Such mutations were notably absent in the majority of grade 1 gliomas, ependymomas and primary glioblastomas. *IDH1/2* are homodimeric enzymes that catalyze the conversion of isocitrate to α -ketoglutarate by oxidative decarboxylation in a NADP⁺ dependent manner in the mitochondria and cytoplasm. The mutations occur in the isocitrate binding pocket of the IDH protein resulting in neomorphic enzyme activity changing its specificity to α -ketoglutarate and generating high levels of 2-hydroxyglutarate (2-HG), an oncometabolite that drives pathological alterations in a variety of cellular processes especially - ketoglutarate-dependent dioxygenases, including the TET group of DNA demethylases [43]. Inhibition of TET function, for instance, results in widespread epigenetic alterations including hypermethylation of a subset of genes and changes in chromatin structure resulting in gliomagenesis. Recent studies have shown that *IDH1/2* mutations cause genome-wide changes in multiple histone marks causing altered chromatin structure which after sustained exposure leads to irreversible genomic and epigenetic alterations in gliomas. Further, it was seen that mutant *IDH1* can result in the generation of a CD24⁺ stem like cell population that can drive tumor growth [44]. Additional gene expression analysis studies have provided These findings have resulted in a focused effort to develop inhibitors of *IDH1/2* [43] although it remains to be seen if such targeting may be aiming at an early oncogenic event whose relevance may be overshadowed by subsequent changes that maintain tumor growth or drive tumor progression to more malignant states through downstream genetic and epigenetic alterations.

3.4 1p19q co-deletion

Oligodendrogliomas were recognized initially as a chemo-sensitive subtype of gliomas [45]; subsequent studies showed that approximately 70% of gliomas that are morphologically consistent with oligodendrogliomas harbored co-deletions of chromosomes 1p and 19q which correlated with their sensitivity to treatment [46]. It was subsequently discovered that 1p/19q codeletion results from an unbalanced translocation involving the centromeric regions of chromosomes 1p and 19q [47]. Given the strong association of 1p/19q codeletion with a classical oligodendroglial morphology as well as to treatment sensitivity [25], molecular reclassification of gliomas currently defines oligodendrogliomas by the combined presence of *IDH* mutation and 1p/19q codeletion [48]. The association of 1p/19q codeletion with other mutations such as in *CIC*, *FUBP1* and *TERT* promoter has further refined the molecular assessment of oligodendroglial tumors [49].

3.5 ATRX

ATRX (α thalassemia/mental retardation syndrome X-linked) is a SWI/SNF family DNA helicase involved in DNA repair and chromatin remodeling protein which works in

conjunction with the death-associated protein 6 (DAXX), a histone chaperone, to deposit histone H3.3 monomers on to the chromatin [50]. Large scale genome sequencing of glioblastoma in pediatric and young adult population identified mutations in ATRX in astrocytic gliomas; mutations in components of this complex including ATRX, H3.3 and DAXX were seen to facilitate alternative lengthening of telomeres (ALT) in malignant cells which can result in preservation of telomere length and enhanced tumor cell survival [51]. ATRX mutations were also noted in adult diffuse astrocytomas with *IDH1* and *TP53* mutations being seen in 33% of low grade astrocytomas, 46% of anaplastic astrocytomas and 80% of secondary glioblastoma [52]. ATRX loss by immunohistochemistry which is associated with ATRX mutations has hence served as a marker of a astrocytic gliomas and is mutually exclusive from 1p/19q codeletions.

3.6 TERT mutations:

The protection of chromosomal ends from DNA damage depends on the integrity of telomere sequences which are repetitive DNA stretches at the end of the chromosome. Telomere length can undergo progressive shortening with successive mitoses that eventually leads to senescence, a process that is countered by the activity of telomerase, a complex of telomere reverse transcriptase (TERT) with an RNA component. Telomerase activity can restore telomere length in stem cells but *TERT* is epigenetically silenced in differentiated cells which hence have limited ability to replicate [53]. Cancer cells are characterized by reactivation of telomeres resulting in replicative immortality mostly driven by point mutations in the promoter region of *TERT* gene which increases telomerase expression, thereby maintaining telomere length and enabling repeated cell division.[53] These mutations have been identified in glioblastomas and LGG; in LGG, *TERT* promoter mutations are predominantly observed in oligodendrogliomas (63–78%) and less frequently (0–32%) in diffuse astrocytomas [54, 55]. The clinical impact of TERT promoter mutations in lower grade and anaplastic gliomas appear to depend on the IDH status; in IDH mutated astrocytomas, TERT promoter mutations appear to confer a longer progression free and overall survival. Conversely, patients with IDH wild type astrocytomas which also exhibited TERT promoter mutations have a shorter progression free and overall survival.[55, 56]

4. Biology of glioblastoma

Glioblastomas are characterized by intra- and inter-tumoral genetic and epigenetic heterogeneity that drive their aggressive behavior and treatment resistance [57, 58]. Several genetic subtypes of glioblastoma have been described although their relevance to clinical outcome remains to be delineated [59, 60]. The neural subtype is the least clearly defined of these tumors and show predominantly neuronal and synaptic markers and resembles normal brain. The proneural subtype is characterized by *PDGFRA* alterations, *TP53* mutations, *IDH1* mutations and *PIK3CA/PIK3R1* mutations and is associated with longer median survival. The classical subtype demonstrates EGFR overexpression, *EGFRvIII* mutation and *CDKN2A* loss. Similar in survival to the classical subtype is the mesenchymal subtype which shows *NF1* loss or mutations, expression of genes associated with epithelial-to-mesenchymal transformation and the presence of inflammatory infiltrates and angiogenesis.

Several markers of biological and prognostic relevance have been identified in these tumors as follows.

4.1 MGMT promoter methylation:

Hypermethylation of the *MGMT* gene promoter results in epigenetic silencing of the gene and is predicted to decrease the levels of the protein thus reducing the ability of the cell to repair DNA lesions from monofunctional alkylating agents such as temozolomide. *MGMT* promoter methylation is seen in approximately 45% of patients with newly diagnosed GBM and appears to be a prognostic rather than a predictive factor in these patients who receive chemoradiation therapy with temozolomide.[11] The relation between *MGMT* promoter hypermethylation and protein expression is not clearly established; in some studies, protein expression did not appear to be a surrogate to methylated promoter status in terms of its impact on clinical outcome whereas others showed that immunohistochemical assessment of *MGMT* protein strongly correlated with overall survival [61, 62]. The prognostic relevance of a methylated *MGMT* promoter has also been shown in elderly GBM patients as well as those with unresectable GBM [24, 63]. Of note, the *MGMT* promoter contains a CpG island with 97 CpG sites stretching across the minimal promoter, first noncoding exon, and minimal enhancer but it has been reported that the methylated sites relevant to prognosis includes nine CpG sites that partially cover the first noncoding exon and the minimal enhancer but do not necessarily correlate with protein expression [61].

4.2 Isocitrate dehydrogenase 1/2

As noted in the previous sections, point mutations in the *IDH1* and *IDH2* genes are uncommon in primary glioblastomas but are frequently seen in secondary glioblastoma [8]. However, *IDH1/2* mutations are associated with the gCIMP subtype of glioblastoma and by their effect on α -ketoglutarate dependent dioxygenases such as TET demethylases which may be causative of the increased methylation seen in this subtype of glioblastoma [64]. Gliomas with a mutated *IDH1* or *IDH2* are associated with better prognosis compared to their wild-type counterparts. However, a recent report analyzing IDH mutant GBM (comprising of ~10% of the tumors) reported a poorer prognosis for patients of male gender and those with IDH mutant tumors which had wild type TERT promoter, suggesting further nuances in the implications of IDH mutation in patients with GBM.[65]

4.3 EGFR overexpression and EGFR variant III (EGFRvIII)

Alterations of EGFR, a membrane bound tyrosine kinase, are among the most common tumor-specific genetic changes seen in GBM. Approximately 50% of primary GBMs demonstrate overexpression of wild type EGFR which can drive tumor proliferation and survival. Additionally about 30% of primary GBM also express the *EGFR* variant III (*EGFRvIII*) which results from deletion of exon 2–7 of *EGFR* and is a constitutively activated mutant receptor which cannot bind any known ligand [66]. More than half the GBMs with EGFR overexpression also co-express EGFRvIII; additionally *EGFR* amplification is seen in all GBM expressing EGFRvIII [67]. This co-expression appears highly relevant to the activation of this pathway given that the wild type and mutant proteins appear to heterodimerize and constitutively activate receptor tyrosine kinase signaling. Trials of the EGFR-VIII targeted trial using rindopepimut, a peptide vaccine, reported that a subset

of tumors recurrent after treatment with the vaccine showed loss of EGFRVIII expression which was interpreted as a sign of efficacy of the vaccine in eliminating EGFRVIII expressing cells.[68] A similar result has been noted in patients treated with a EGFRVIII targeted CAR-T cell therapy.[69] However, it should be noted that while EGFR amplification and overexpression is retained at tumor recurrence, a subset of recurrent tumors loses EGFRVIII expression after first line standard treatment which does not target this alteration.[70]

5. Targeted therapies and its challenges in gliomas:

5.1 Angiogenesis

Vascular proliferation and endothelial hyperplasia are histological hallmarks of glioblastoma which are drivers of the pathophysiology and clinical course of these tumors [1, 71, 72]. Therefore, inhibiting neoangiogenesis was an early focus of targeted therapy. Antiangiogenesis strategies that have been translated into clinical practice include targeting vascular endothelial growth factor VEGF and/or its receptors VEGFR with antibodies, or its subsequent downstream signaling pathway with tyrosine kinase inhibitors (TKIs).

Vascular endothelial growth factor (VEGF A-D) and its receptors (VEGFR 1, 2 and 3) are key mediators of angiogenesis associated with tumors [73, 74]. In fact, VEGF expression was found to correlate with grade of glioma [75]. Bevacizumab, a humanized monoclonal antibody against VEGF-A, received accelerated approval by the FDA in 2009 as monotherapy for recurrent GBM based on results of two phase-II clinical trials [76, 77]. Based on these results, bevacizumab was assessed in the setting of newly diagnosed GBM in subsequent phase III clinical trials but these failed to show improvement in overall survival when bevacizumab was added to standard chemoradiation [78, 79]. However, recognizing the improvement in quality of life related to the rapid and substantial reduction in cerebral vasogenic edema and mass effect as well as increased PFS, the FDA granted full approval to this agent in adults with recurrent GBM. Several completed and ongoing clinical trials are aimed at combining bevacizumab with radiotherapy as well as various chemotherapeutic and immunomodulatory agents to assess the potential for synergistic effects [71, 72]. Of note, bevacizumab therapy can result in pseudoresponses with significant reduction in the enhancing component of gliomas due to the restoration of the blood-brain barrier while the nonenhancing component of the tumor remains unchanged. Recognizing this issue, the RANO criteria has provided revised guidelines requiring a combination of enhancing, non-enhancing and clinical metrics to define responses and progression.[80]

In addition to targeting the ligand, several trials have focused on VEGFR TKIs including cediranib, vatalanib, pazopanib, cabozantinib, sunitinib, sorafenib and vandetanib [72]. Cediranib is a relatively selective pan-VEGF receptor (VEGFR) TKI that showed promising results in a phase II clinical trial in recurrent glioblastoma [81]. This prompted a randomized phase III trial that compared cediranib to lomustine monotherapy or to the combination of both agents in patients with recurrent GBM; however, the study failed to show significant survival benefit to the addition of cediranib to lomustine or of cediranib alone [82].

Several molecules other than VEGF have emerged as relevant mediators of angiogenesis; of these, angiopoietins are associated with robust angiogenesis in tumors [83]. Angiopoietin-2 (Ang-2) is a member of the angiopoietin family that is upregulated in GBM [84, 85]. Ang-2 signals primarily through TEK receptor tyrosine kinase (Tie-2) [86]. Studies have shown that increased Ang-2 expression can contribute to resistance to anti-VEGF therapies in GBM through its proangiogenic properties [87]. Dual inhibition of VEGFR and Ang-2 has been shown to inhibit tumor growth and prolong survival compared with VEGFR inhibition alone in murine glioma models [86].

Various other mechanisms of resistance to anti-angiogenic therapy have been suggested. Antiangiogenic agents induce tumor hypoxia causing transcriptional upregulation of HIF1- α which in turn can activate several alternative proangiogenic pathways. Hypoxia has also been implicated in proliferation of cancer stem cells and infiltration of the tumor by bone-marrow derived myeloid cells [88, 89]. Metabolic reprogramming through glucose transporter-3 upregulation was also suggested as a way for cancer cells to adapt to decreased glucose in the de-vascularized environment [90]. Efforts are ongoing to combine antiangiogenic and other approaches to overcome resistance and also develop biomarkers to stratify the subset of patients who are most likely to respond to antiangiogenic therapy.

5.2 Growth factor receptor signaling

Activation of growth factor receptor pathways is thought to play a role in carcinogenesis via inducing cell proliferation, angiogenesis and survival of cancer cells. Receptor tyrosine kinases (RTKs) regulate growth factor signaling. Gene expression profiling of GBM through the Cancer Genome Atlas project demonstrated a high degree of heterogeneity as a result of several genetic and epigenetic alterations including amplification, gain-of-function mutations, copy number loss or gain and methylation that promote growth factor receptor signaling, and loss-of-function mutations in tumor suppressor genes (e.g. PTEN and P53) [57, 59, 60]. As discussed above, EGFR is among the most commonly mutated genes; approximately 50% of GBMs have amplification of EGFR [66, 91]. *EGFRvIII* is the most common EGFR gain-of-function mutation and hence the most common mutation in GBM [92]. Other mutated RTKs include platelet derived growth factor receptor alpha polypeptide (PDGFRA), c-MET and fibroblast growth factor receptor (FGFR) [91, 92].

The impact of EGFRvIII on survival in patients with glioblastoma (GBM) has been debated [93, 94]. Nevertheless, different targeted therapies against EGFR have been studied including antibodies, tyrosine kinase inhibitors and vaccines. Antibodies that have been tested in clinical trials include cetuximab, nimotuzumab and the radiolabeled antibody 125 I-Mab 425 [95]. Cetuximab was the first chimeric monoclonal antibody developed against EGFR and has shown clinical activity in several malignancies. However, the agent had limited activity in patients with recurrent high-grade glioma even after stratification based on EGFR amplification status in a single-agent phase II trial [96]. Similarly, a randomized Phase III study of nimotuzumab, a humanized monoclonal antibody, found no significant difference of adding this agent to standard GBM therapy in newly diagnosed GBM patients [97]. Subgroup analysis, however, suggested possible benefit in patients with residual tumor with unmethylated MGMT promoter.

Small molecule EGFR inhibitors have thus far proven ineffective in GBM in contrast to success in EGFR-mutant lung cancer. A trial of gefitinib was not associated with significant improvement in OS or PFS as adjuvant therapy in patients with newly diagnosed GBM [98] and showed limited activity as a single agent in patients with recurrent high-grade glioma [99]. Similarly, trials of erlotinib and lapatinib showed no significant activity in patients with GBM [100, 101].

Limitations using antibodies and TKIs may be because of inability to cross the blood brain barrier (BBB) due to large molecular weight and presence of efflux transporters on endothelial cells respectively [95]. Lapatinib was shown not to reach therapeutic intratumoral drug levels in the CNS [102]. Moreover, intratumoral heterogeneity of EGFR presents a challenge to targeted therapy as different subsets of cells within the tumor may develop different therapeutic sensitivity [92].

Interestingly, mutation and loss of PTEN as well as PTEN phosphorylation mediated by both FGFR and Src-family kinases (SFKs) were linked to EGFR TKI resistance in GBM patients [103]. SFKs have a close relationship with RTKs becoming activated following stimulation of RTKs and integrins and in turn augmenting important downstream signaling pathways that include PI3K/Akt and Ras/MAPK pathways [104]. Dasatinib is a potent SFK inhibitor that also has activity against c-kit and PDGFR which has been tried in clinical trials against GBM; however, it failed to show efficacy in recurrent glioblastoma when used alone or in combination with CCNU [105, 106]. Integrins, which are transmembrane adhesion receptors important for cell-to-cell signaling act synergistically with RTKs to maintain cell survival, proliferation and cell adhesions. Cilengitide is a specific αV integrin inhibitor that was studied in newly diagnosed MGMT methylated GBM patients and was the target of clinical studies based on promising preclinical and early phase clinical trial data. In a phase III multicenter randomized trial, cilengitide did not improve outcomes when added to standard chemoradiotherapy [107].

PDGFR is another RTK which function similar to EGFR in driving the growth of gliomas activating similar downstream pathways [108]. The Cancer Genome Atlas Research Network further reported PDGFR amplification in 13.1% in GBM samples [57]. Imatinib, a multikinase inhibitor which targets PDGFR, c-KIT and BCR-ABL was tested in recurrent GBM patients in several phase II and subsequently a phase III clinical trial but there was no significant improvement in outcome when combined with hydroxyurea [109, 110, 111]. The PI3K is an important mediator of one of the main signaling pathways utilized upon RTK activation by multiple cell surface receptors including EGFR, PDGFR and VEGFR and triggers activation of Akt and mTOR [112]. Efforts to directly inhibit Akt were limited by toxicity to normal tissue; hence attempts to identify a targetable downstream member of the RTK pathways led to assessment of the roles of mTOR and protein kinase C (PKC), key downstream hubs of RTK signaling. However, trials of enzastaurin, a PKC inhibitor, and everolimus, an mTOR inhibitor, did not show clinical benefit in patients with malignant glioma [113, 114, 115].

A key reason for failure of single target inhibition in gliomas appears to be the coactivation of other RTKs which sends redundant inputs that maintain the downstream signaling

pathways allowing the cancer cells to survive even if one upstream signaling receptor is blocked. This suggests the need for inhibition of multiple RTKs to block cell signaling and reduce cell survival [116]. In this context, a few agents that have multitargeted kinase inhibition activity have also been assessed against gliomas e.g. vandetanib, a dual EGFR and VEGFR inhibitor, sunitinib, a VEGFR, PDGFR, c-KIT and FLT-3 inhibitor and sorafenib, a RAF, VEGFR, PDGFR, c-KIT and FLT3 inhibitor. However, tumor heterogeneity inherent to gliomas has rendered these combinations of targeted agents ineffective in providing a significant survival advantage in patients with gliomas [91, 117].

Given the challenges with targeting RTKs and their downstream pathways, efforts have also been focused on genetic and epigenetic targets against gliomas. Such targets have included p53, a key regulator of DNA repair and cellular decisions to activate apoptosis, which is commonly dysregulated in GBM either through mutations or loss. Relevant to p53 function, its cellular inhibitory partners, MDM1, 2 and 4 are noted to be overexpressed through amplification and cause inactivation of p53 function [57, 118]. Preclinical studies of MDM2 inhibitors have shown promising activity in glioma models [119, 120]. Similarly, preclinical efforts to target mutant IDH1 and MYC amplification have been translated to early trials which are ongoing [121]. Mutations of H3.3 histones, not observed in the Cancer Genome Atlas Project, were nevertheless identified independently in midline and brain stem gliomas and constitute new targets in gliomas for development of therapies. Studies of histone deacetylase (HDAC), an epigenetic modifier of gene expression are also ongoing; despite encouraging preclinical data [122, 123], vorinostat, an HDAC inhibitor, has not shown significant effect in recurrent glioblastoma when used alone or in combination with bevacizumab [124, 125].

6. Biological therapies and immunotherapies

Recent advances in biologic therapies and immunotherapy, which have been effective treatments for several types of cancer, have raised hope that similar advances could also be realized against gliomas. Preliminary data from various immunotherapy and biological therapy trials have shown anecdotal responses and stability in patients with glioblastoma suggesting that with appropriate patient selection, such treatments may provide effective options for patients with gliomas.

6.1 Virus-based therapy

Virus-based therapies against gliomas have included several approaches: replication-deficient viral vectors, which deliver genes with therapeutic activity to the tumor environment, and replication competent and oncolytic viruses, which can infect and reproduce themselves within a tumor cell, eventually causing tumor cell death and infecting other tumor cells. Several such constructs have been tried in clinical trials against gliomas. The herpes simplex type I virus-thymidine kinase construct (HSV-TK) which allows a “suicide gene transfer” leading to tumor specific expression of thymidine kinase which converts systemically administered ganciclovir into an active, phosphorylated analog which is incorporated into the DNA of replicating malignant cells inducing cell death [126]. Clinical trials of this combination yielded modest benefit in early studies but cumulative

evidence did not support a definite improvement in outcome from this agent [127]. Toca-511, currently in clinical trials against gliomas, is a genetically engineered non-oncolytic replication competent retrovirus, which carries the gene for yeast cytosine deaminase capable of converting 5-flucytosine (administered to patients orally) to 5-fluorouracil within the infected tumor cells where it exerts a cytotoxic effect and also diffuses to exert a bystander effect on surrounding tumor cells [128]. Preliminary results of the clinical trial against recurrent GBM showed promising durable responses and final results of the study are awaited.[129]

In addition, both replication-deficient and replication-competent viral therapies have the added benefit of immune activation, resulting in glioma cell destruction through secondary immune effects. DNX-2401, a replication competent oncolytic adenovirus engineered to exploit the interaction between the virus and the retinoblastoma (Rb) protein pathway, replicates in cells with an impaired Rb pathway (seen in >80% of GBM cells) but not in those with an intact Rb pathway (normal brain cells) [130]. Results of a phase I trial of this agent against recurrent GBM have shown early promising with some long term survivors; ongoing studies will provide more insights into the magnitude and extent of benefit from this agent in the general population of patients with recurrent GBM [131]. Another strategy involves intratumoral injection of an inducible adenoviral vector, Ad-RTS-hIL-12, which expresses interleukin-12, a pro-inflammatory cytokine, under the regulatory control of an engineered transcription factor complex consisting of two fusion proteins, Gal4-EcR and VP16-RXR. Binding of an orally administered activator ligand, vemidex, to the EcR portion of the fusion protein stabilizes the heterodimerization of the two proteins forming an active transcription factor that transcribes IL-12 causing expression of the cytokine and consequent activation of NK cells and induction of cytotoxic T-cell targeting of glioma cells. Early results of a trial of this strategy have demonstrated safety of the agent and preliminary evidence of efficacy that is being explored in ongoing trials.[132] Several other engineered viruses including the measles virus, New Castle disease virus, parvovirus, cytomegalovirus, reovirus and herpes simplex virus are being investigated at various stages of clinical development and with expectation of efficacy that can overcome tumor heterogeneity.[133]

6.2 Vaccine therapy against gliomas

Using both a cell-based and non-cell-based approach, vaccine therapy has been another way to stimulate the immune system as an additional defender against gliomas. In cell-based vaccine approaches (e.g. DCVax-L®), dendritic cells, which are immune cells with very efficient antigen-presenting properties, are obtained from glioma patient and exposed to the tumor tissue obtained at the time of glioma surgery. The DC cells are then engineered to produce a tumor-specific immune response when they're administered back to the patient. A Phase I trial showed this treatment to be safe and showed a promising overall survival [134]. A polio virus based immune strategy recently showed promising improvement in survival in a subset of patients who received, by intratumoral convection-enhanced delivery, a recombinant polio-rhinovirus chimera (PVSRIPO) which engages the poliovirus receptor CD155 expressed in GBM cells [135]. A phase II trial to further assess the efficacy is beginning shortly.

Another immune-stimulating strategy involves autologous vaccination using modification of glioma cells or patient immune cells (typically T-lymphocytes) and introduction of the altered cells into the patient to induce immune responses. An example of this strategy is the use of autologous formalin-fixed tumor vaccines in which T-cells are sensitized to the tumor. A recent study employed this vaccine in newly diagnosed glioblastoma patients during radiation therapy with median OS of 19.8 months and median PFS of 7.6 months [136].

6.3 Peptide vaccines

An example of a non-cell-based vaccine includes peptide vaccines. For instance, EGFRvIII is a constitutively active mutant form of the epidermal growth factor receptor which is present in one-third of glioblastoma specimens [137]. Rindopepimut, an anti-EGFRvIII peptide vaccine was studied in sequential phase I and phase II clinical trials and showed promising improvement in PFS and OS in these early trials [138, 139, 140]. Building on these results, an international double-blind phase III study was conducted but failed to demonstrate a survival benefit for patients treated with rindopepimut and standard chemoradiation therapy in newly diagnosed GBM compared with control although robust anti-EGFRvIII immune response was noted in the study [141].

6.4 Heat-shock protein vaccines

Heat-shock proteins are considered to be crucial to the survival of cancers such as glioblastoma due to their key roles in (a) stabilizing proteins, (b) facilitating protein conformational change, (c) protein trafficking and (d) breakdown as well as control of apoptosis [142]. They are activated by the “stress” environment found in tumor beds and consisting mainly of hypoxia and inflammation [143]. A Phase I study in patients with recurrent GBM using an autologous heat-shock protein peptide complex-96 (HSPPC-96) vaccine, which utilized peptides bound to a chaperone protein isolated from glioma tissue, to immunize patients with recurrent disease, showed the potential for improved outcome in patients who had an immune response [144]. A subsequent phase II study further demonstrated the safety and potential efficacy of this approach in adults with recurrent GBM with the additional findings that patients who were lymphopenic at baseline had worse survival [145].

7. Immune checkpoint inhibitors

The immune system uses cell surface proteins that serve as checkpoints by regulating the immune response directed towards foreign antigens and to prevent damage to normal cells. Several of these checkpoint molecules also regulate cytokine production and cellular responses in the relevant context, for instance, by reducing IL-2 production, IL-2 receptor expression, as well as lymphocyte cell division [146] and enhancing T suppressor cell function [147, 148]. The earliest of the targeting strategies aimed at checkpoint proteins was against PD-1, a protein expressed by cytotoxic and regulatory T-cells (Tregs) [149], B-cells and NK cells [150] whose expression serves as a “brake” on the immune response [151, 152]. Inhibitors of the interaction of PD-1 on T cell surface with its ligand, PDL-1 on the tumor cell, such as nivolumab and pembrolizumab, allows removal of this brake and consequent T cell activation and cell kill. Another early target was CTLA-4 which is also

expressed on T cells and can be targeted by ipilimumab, a monoclonal antibody which blocks its interaction with CD80 or CD86 on the antigen presenting cells including tumor cells allowing cytotoxic T-cell activity. The combination of ipilimumab and nivolumab has shown potent activity in melanoma. There are several ongoing trials of agents targeting PD-1 including nivolumab, pembrolizumab, atezolizumab and durvalumab [153]. Single agent or early combination trials have shown lack of efficacy in an unselected patient population with recurrent or newly diagnosed GBM highlighting the limitations in our understanding of the immune microenvironment in gliomas [154]. Inhibitors of targets other than PD-1/PDL-1 axis including TIM3, OX40 and LAG-3 are being explored for their potential for activity against gliomas.[155] The readers are further referred to comprehensive reviews of immune strategies against gliomas.[156, 157]

8. Genetically engineered T-cells

The possibilities of immune therapies for glioma have been further broadened by the development of chimeric antigen receptor (CAR) technology. In this system, T-cells are engineered to recognize antigens on tumors by fusing an extracellular binding domain to the intra-cellular signaling domain of the T cell receptor [158]. The extracellular domain is derived from an antibody to a tumor-associated antigen. CAR technology offers important advantages when compared to other immune therapies, including better penetration into the tumor than other engineered components of the immune system [159]. A recent report of a sustained and dramatic response of a patient with recurrent GBM and leptomeningeal spread to intratumoral and intrathecal CAR-T cells targeting the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13R α 2) has raised hopes for efficacy of this approach in selected patients [160]. Several additional passive and active cell based immunotherapies are currently being tested and the readers are referred to comprehensive reviews of this topic. [161, 162]

9. Tumor adaptations to treatment

A particularly challenging aspect of gliomas is their heterogeneity which has confounded attempts to generate effective therapies. This heterogeneity manifests itself not in terms of high mutational burden; in fact, gliomas are noted to have a relatively modest number of mutations compared to several other malignancies. Instead, it appears to be the diversity of the biological pathways including activation of parallel processes[163] that negate the effect of single pathway or dual target inhibition as has been demonstrated by the failure of several clinical trials of such agents against gliomas. In addition, both spatial and temporal genetic heterogeneity seems to be critical to such adaptations with accumulation of specific genetic alterations that provide new pathways for tumor survival both during spontaneous evolution as well as in response to therapy. For instance, the same tumor has been seen to harbor populations that meet the criteria for more than one genetic subtype of gliomas.[164, 165, 166] Additionally, distant and local recurrences appear to evolve differently in terms of their genetic alterations which may have implications in terms of development of resistance.[167] Tumor mutation burden was postulated to increase immunogenicity of tumors and make them more sensitive to immunotherapy. Although this has been noted in highly immunogenic tumors such as melanoma, the role of such mutation burden in gliomas has

been uncertain. A recent report that analyzed the correlation between tumor mutation burden and response to immunotherapy noted that, unlike many other malignancies, higher tumor mutation burden in gliomas was associated with a trend towards poorer survival. This was attributed to the generation of poorly immunogenic subclonal mutation by first line treatment using temozolomide although the immunoprivileged nature of the brain was also considered to be a possible factor.[168] Overcoming both intrinsic and treatment generated tumor heterogeneity is hence emerging as a critical target for advancing glioma treatment strategies.

10. Conclusions

Little advancements have been made in glioblastoma management over the past few decades despite enormous research efforts. Various efforts and multiple approaches- including targeted agents, immunotherapies and viral therapies- have not achieved success in glioblastoma treatment. This emphasizes the complexity of the disease that is in part due to tumor heterogeneity. Novel and new treatment approaches in immunotherapies and treatments tackling the metabolic machinery of the tumor are on the horizon and represent the next aspiration in neuro-oncology.

11. Expert opinion

Despite our greater understanding of glioma pathogenesis and driving pathways, targeted therapy trials utilizing small molecule inhibitors of receptor tyrosine kinases, angiogenesis components or signaling pathway molecules, biological therapies such as viruses and immunotherapeutic agents including vaccines, antibodies and checkpoint inhibitors have not shown survival benefit in glioma patients, unlike in other solid tumors. A key reason for failure of single target inhibition in gliomas appears to be the existence of complex network of downstream signaling pathways as opposed to single driver mutations tumors. Moreover, tumor heterogeneity inherent to gliomas has rendered combinations of targeted agents ineffective in providing a significant survival advantage in patients with gliomas. Previous classification of gliomas into classical, neural, pro-neural and mesenchymal subtypes was recently challenged by single cell RNA sequencing study of glioblastoma samples that showed variable expression across individual cells within one tumor suggesting that such genetic classification of gliomas is insufficient in informing treatment strategies.[166] Similar to targeted therapy, immunotherapy utilizing vaccines and checkpoint blockade has not yielded the anticipated positive results. It has become evident that the effects of such therapies cannot be generalized across tumor types and that context-specific strategies which recognize the unique nature of the brain environment and the intrinsic tumors that develop within this context are more relevant to achieve improvement in outcome. This is highlighted by recent promising results seen with pre-treatment of surgical patients with recurrent GBM with pembrolizumab followed by resection and continued checkpoint blockade who had near doubling of survival compared to those who received pembrolizumab after surgery alone.[169]

Various approaches for rational targeting of the numerous well-recognized pathways relevant to both the tumor as well as the microenvironment have highlighted the enormous

heterogeneity in GBM and its tumor microenvironment and emphasized the need to further understand the intricacies of signaling, survival and resistance pathways in GBM. A special Think Tank meeting held at the 2018 Society of Neuro-Oncology meeting in New Orleans to tackle the complexity of GBM heterogeneity recognized the multifaceted nature of intrinsic and treatment specific resistance mechanisms in gliomas. It was noted that with the tremendous expansion of data related to genomic, epigenomic and more recently microenvironmental composition of GBM, new strategies designed to identify and target underlying mechanisms that govern multiple interrelated pathways related to adaptive resistance specific to tumors and not activated in normal cells were crucial to effectively target gliomas. Such strategies include developing treatments that are selective for specific genetic or epigenetic vulnerabilities of GBM in smaller subsets of selected patients and targeting metabolic or immunological aspects of tumors that bypass the heterogeneity of these tumors. Combinations of targeted therapies and immunotherapies as well as more novel approaches to immunotherapy such as using CAR-T cells are on the horizon aiming for the long-awaited paradigm shift. Biological therapies including early phase trials of TOCA 511, DNX-2401 and more recently the poliovirus have shown promise and the larger trials are ongoing or expected to launch soon. The results of IDH1/2 inhibitor trials are also eagerly awaited given that IDH mutations are believed to be the initial event in tumorigenesis of IDH mutant gliomas and invariably exist across the lifetime of IDH mutant gliomas although it remains to be seen if the benefits of such targeting may be negated by subsequent changes that maintain tumor growth through downstream genetic and epigenetic alterations. These newer treatment approaches being developed against this malignancy bear promise to overcome the failures of current therapies against GBM.

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Article highlights:

- Despite our greater understanding of glioma pathogenesis and driving pathways, targeted therapy trials have not shown survival benefit in glioma patients.
- Different targeted therapies against VEGF and EGFR have been studied including antibodies, tyrosine kinase inhibitors and vaccines. However, none have shown significant overall survival in glioblastoma. Tumor heterogeneity plays a role in glioma resistant to targeted therapy.
- Viral based therapies have shown encouraging results in early phase trials in glioblastoma and phase II and III trials of these agents are underway.
- Data from single agent or combination checkpoint blockade trial failed to show overall survival benefit in recurrent glioblastoma.
- The neuro-oncology field eagerly anticipates the results of IDH1/2 inhibitor trials and looks forward to novel treatments targeting the metabolic pathways of gliomas.