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Further understanding of glioma mechanisms of pathogenesis: implications for therapeutic development

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Abstract

Introduction: Recent discoveries in the molecular makeup of gliomas, the relationship of certain molecular drivers, and the patient's response to therapy and overall prognosis have resulted in a paradigm shift and redefined our understanding of glioma and revealed potential vulnerabilities within this recalcitrant and lethal disease.

Areas covered: We summarize the current classification of malignant glioma in the context of the historical background, current data driven treatment strategies, and recent discoveries of the mechanisms of pathogenesis of this disease which recapitulates the developing brain. We describe the relationship to common genetic alterations found in glioma, and possible avenues to exploit these newly revealed mechanisms.

Expert opinion: Improved understanding of the molecular underpinnings of this disease has been directly translated into treatment decisions and an improved ability to counsel patients regarding their prognosis. We are beginning to see the first glimmer of a return on the investment in regard to immunotherapy in malignant glioma, with further anticipated successful exploitations of the unique pathophysiology of glioma.

Keywords: Glioma, IDH, TERT, 1p/19q, MGMT, NLGN3, tumor heterogeneity, immunotherapy , adoptive cell therapy, CART

Article highlights:

- We summarize the current classification of malignant glioma in the context of the historical background, current data driven treatment strategies, and recent discoveries of the mechanisms of pathogenesis of this disease which recapitulates the developing brain.

- A gain of function mutation in the metabolic enzyme IDH results in the formation of a novel onco-metabolite, 2-HG which interferes with transcription factor binding and disrupts the formation of chromatin loops and physical segregation of DNA, bringing together disparate genetic elements in close physical proximity to influence one another.

- Though the IDH mutation results in a gain of function, the net result is that lower substrate concentrations are available to buffer the oxidizing effect of radiotherapy: NADPH, glutathione, and deoxynucleotides are all reduced in *IDH*-mutant glioma cells

- *IDH*-mutated tumors demonstrate defective homologous recombination resulting in increased levels of DNA damage from cellular metabolism as well as alkylating chemotherapy.

- PARP inhibition may be a rational and efficacious strategy in MGMT methylated glioblastoma and IDH mutant gliomas.

- 1p-19q co-deleted gliomas have a decreased ability to produce ultra-long membrane protrusions, termed tumor-associated microtubules (TMs), which appear to be a mechanism of tumor resilience and pathogenesis. TMs enable their host cells to interconnect and form a functional resistance network, conveying resistance to surgical lesions, chemotherapy, and radiotherapy.

- Neoadjuvant pembrolizumab prior to repeat surgical resection may prolong overall survival in recurrent glioblastoma.

1. Clinical Case

A 61-year-old right-handed female experienced insidious difficulty manipulating objects with her left hand and began to drop objects. While shopping at a grocery store, she developed difficulty with ambulation followed by loss of awareness. Onlookers described a seizure. Head imaging demonstrated a large right parietal mass associated with significant vasogenic edema and midline shift. The patient underwent maximal safe debulking of the lesion, which was found to be an IDH-wild type glioblastoma without MGMT promoter methylation. Postoperatively, her left-sided weakness resolved over several weeks. She was subsequently treated at our institution with F-DOPA Positron Emission Tomography (PET), guided radiation with concomitant daily temozolomide. The patient was treated with 76 Gy of radiotherapy and 6 cycles adjuvant temozolomide with concomitant tumor-treating field device deployment. After 6 cycles of adjuvant temozolomide, the patient entered observation with continuation of the tumor-treating field (TTF) device. The patient elected to discontinue the device 10 months after completion of the adjuvant temozolomide. The patient had a prolonged period of disease stability in which she was able to work full-time and drive (ECOG score: 0).

Eleven months after discontinuation of the tumor-treating field device, the patient had asymptomatic radiographic recurrence adjacent to the prior disease site. The patient underwent repeat surgical resection and again developed a mild left hemiparesis post-operatively, which persisted. Subsequently, she was treated with abbreviated combinatorial chemo-radiotherapy with proton beam (total dose: 4,000 cGy) again under F-DOPA guidance and a repeat trial of adjuvant temozolomide with re-initiation of the TTF device. This was unfortunately followed by rapid recurrence in the contralateral hemisphere. The patient was then transitioned to bevacizumab monotherapy but continued to have multifocal disease progression. The TTF was remapped, and the patient was treated with a third course of radiotherapy again under F-DOPA PET guidance. Over the course of her salvage treatments, she developed a progressive left hemiparesis and became wheelchair bound. In the setting of relentless disease progression, the patient transitioned to hospice three and a half years after her diagnosis. She passed two months later.

This case demonstrates that, despite an increased understanding of pathogenesis, glioblastoma remains a recalcitrant and lethal tumor even with gross total resection, radiation to the residual tumor and margins (standard of care being 60 Gy), concomitant and adjuvant temozolomide-alkylating chemotherapy, and the tumor-treating field device, an external transducer array that generates a rapidly oscillating electromagnetic field within the skull, and bevacizumab for symptomatic recurrence. Despite all of these therapies, the average life expectancy for patients with glioblastoma ranges between 14 and 21 months.

2. Surgery

Surgery for malignant glioma is often essential to relieve mass effect and obtain a pathologic diagnosis; however, the overall clinical significance of the extent of tumor resection in the care of patients with

glioblastoma is undetermined. Fundamental questions, such as the benefit of extent of resection and presence of functioning brain tissue within the contrast-enhancing portion of the tumor, are still debated. However, the available data for the evidence of the benefit of surgical resection, largely from the large retrospective series, demonstrate that there is, indeed, a difference in outcome when comparing biopsy versus maximal safe resection. Overall, the consensus is that maximal resection is beneficial and is the optimal treatment approach when safe (NCCN) [2, 3].

Glioblastoma is considered a whole brain disease. In 1928, Dr. Walter Dandy famously removed (several) right hemispheric gliomas with right hemispherectomies; however, the patient who survived the surgery developed tumor recurrence in the remaining (left) hemisphere and died of recurrence three and a half years later [4].

Imaging the glioma to determine the extent of surgical disease remains problematic as glioma cells diffusely infiltrate the substance of the brain. The extent of contrast-enhancing tumor burden that remains post-operatively has been associated with clinical outcome [5]. Contrast enhancement relies upon disruption of the blood brain barrier [6], whereas the extent of tumor present on the T2/FLAIR imaging is less certain due to the additional presence of edema in malignant glioma [7]. Dr. Patrick Kelly [8] performed a study in the 1980s in which 40 patients with a previously untreated malignant glioma underwent serial stereotactic CT- and MRI-assisted biopsy with >195 specimens collected. This study demonstrated isolated tumor-cell infiltration extending at least as far as the T2-FLAIR signal abnormality present on MRI. Patient-derived xenograft mouse models with human glioblastoma cell lines demonstrate that isolated glioblastoma tumor cells can be found in the contralateral hemisphere relative to the discrete tumor. Intraoperative fluorescence-guided imaging tumor visualization with 5-ALA (5-aminolevulinic acid) reveal the previous boundaries of malignant glioma to be demonstrably beyond the areas of contrast enhancement [9]. Accordingly, there is now substantial interest in supramaximal resection, particularly in temporal lobe tumors [10].
The radiographic limitations of MRI extend beyond surgical resection. Radiation oncologists need to understand the extent of tumor and which areas can be spared from high-dose RT to balance anti-tumor efficacy, cognitive function, and quality of life. 18F-FDG (18F-fluoro-dioxyglucose) PET has limited utility in the brain secondary to brain glucose metabolism, which is very high [11]. However, brain tumors have high uptake of amino-acid tracers relative to a normal surrounding brain [12]. 18-F-DOPA is a radio-labeled analog of L-DOPA and is transported across the intact blood-brain barrier, unlike gadolinium, and this technique further characterizes the extent of disease in otherwise radiographically occult disease and has been demonstrated to be superior to contrast in MRI for detection of recurrent glioma as well as more accurately identifying regions of higher/higher density disease [13]. PET radioisotopes are an area that will likely continue to be explored for glioma imaging into the foreseeable future.

3. Radiation

The benefit of radiation in the treatment of malignant glioma has been known since 1978, at which point a cooperative clinical trial published by Walker et al [14] evaluating the benefit of BCNU and/or radiotherapy in the treatment of anaplastic gliomas demonstrated a doubling of overall survival in those patients treated with 60 Gy relative to those treated without radiation, or BCNU alone. Three hundred three patients received 50 to 60 Gy to the whole brain through bilateral opposing ports. When compared to conventional care, radiation therapy demonstrated a survival benefit with evidence of a dose response [15]. Patients receiving the highest dose of 60 Gy had the highest median overall survival. Subsequent studies by Chan et al. demonstrated an apparent ceiling to the dose response; in patients with malignant glioma treated at doses from 70 to 90 Gy, those with the lower dose had better median overall survival (13.9 months) compared to those with 90 Gy, (median overall survival of 11.7 months).

Chan et al. also noted that the pattern of failure (i.e., disease recurrence) in the vast majority of patients (78 percent) was within the central field; no patients had distant failure [16].

4. Chemotherapy

The benefit of chemotherapy in addition to surgery and radiation was difficult to prove, and FDA-approved chemotherapeutic agents for malignant glioma are few: Lomustine (CCNU), Carmustine (BCNU), Temozolomide (TMZ), (all DNA-alkylating agents) and Bevacizumab (BEV- a monoclonal antibody-targeting Vascular Endothelial Growth Factor - VEGF). In 2002, a meta-analysis of 12 randomized controlled trials, most of which utilized CCNU or BCNU and enrolled >3,000 patients, did demonstrate a 0.85 hazard ratio for patients treated with chemotherapy with a significant *p* value in favor of radiotherapy plus chemotherapy versus radiotherapy alone [17]. However, it was not until 2005 that alkylating chemotherapy TMZ demonstrated superior efficacy over radiotherapy alone with a median survival of 14.6 versus 12.1 months. The 2-year overall survival also favored the addition of TMZ chemotherapy with 26.5 percent of patients treated with TMZ plus radiotherapy living two years compared to 10.4 percent for those treated with radiotherapy alone. The benefit was most marked in those patients with MGMT-promoter methylation (mOS 23.4 months TMZ+ RT versus 15.3 months RT alone), and in a 2009 *Lancet* 5-year trial follow-up, MGMT-promoter methylation was the strongest predictor of benefit but also most clearly demonstrated the benefits of TMZ in MGMT unmethylated patients (mOS 12.6 months versus 11.8 months RT alone) [18]. While there is a statistically significant benefit to TMZ chemotherapy across multiple studies in patients who are methylated or unmethylated [19], the clinical benefit is more marked in those who are methylated [19].

5. The Molecular Era

Historically, gliomas were classified based on their histologic appearance on the basis that cancer cells recapitulate the native glial cells in the brain: the higher the number of abnormal findings, the more aggressive the tumor and the higher the tumor grade. Glioblastoma was defined histologically as a diffusely infiltrative neoplasm with cells resembling astrocytes, but demonstrative cellular atypia, increased mitoses, abnormal microvascular proliferation and/or necrosis. Gliomas are now classifiable into five groups with prognostic implications based on the presence or absence of three genetic alterations [20] *IDH*, 1p/19q co-deletion, and *TERT* promoter mutation. A tumor with only *TERT*-promoter mutation (that is without IDH mutation or 1p/19q co-deletion) whether grade 2 or 3, will have a similar prognosis to "true" histologic glioblastomas. As such, these are commonly referred to as "molecular glioblastomas." Tumors that harbor all three genetic alterations are termed "triple-positive" tumors and have the most favorable prognosis. These "triple-positive" tumors are, by definition, oligodendrogliomas per the 2016 WHO revision [21] due to their 1p/19q mutation. *IDH*-mutant tumors (without 1p/19q co-deletion) have a relatively favorable prognosis when compared to *IDH* wild-type tumors or *TERT* mutant-only tumors but a less favorable prognosis than "triple-positive" tumors[20]. The 2016 WHO grading criteria for CNS tumors now use *IDH* to dichotomize tumors and incorporate the presence or absence of *IDH* mutation into the diagnosis [21]. This classification schema emphasizes the unique pathophysiology of tumors harboring this mutation. For example, glioblastoma is now *glioblastoma, IDH wild-type* or *glioblastoma, IDH-mutant*. *IDH*-mutant tumors (without 1p/19q co-deletion) have a relatively favorable prognosis when compared to *IDH* wild-type tumors or *TERT* mutant-only tumors but a less favorable prognosis than "triple-positive" tumors [20]. High-grade (grade 4) lesions share a fair degree of heterogeneity regarding prognosis, which again centers on the presence or absence of the *IDH* mutation, MGMT-promoter methylation, and the presence of *TERT*-promoter mutations [20]. The current hypothesis is that *IDH* is a tumor-initiating mutation in lower-grade gliomas,

which may be followed by the 1p/19q co-deletion and/or the *TERT* mutation or a *P53* and *ATRX* mutation. Triple-negative tumors are tumors without IDH mutations, 1p/19q co-deletion, or *TERT*-promoter mutation. They comprise a relatively heterogeneous group and will likely continue to be further characterized [20].

While this method of classification is a new paradigm it is not without faults. A confusing aspect of this classification is that histologic grade 2 and grade 3 tumors can be lumped together in terms of prognosis, whereas the clinical trial data to guide management of these tumors is based on the histologic grade. As such, further efforts to delineate which tumors will behave more or less aggressively has further identified CDKN2A homozygous deletion as a negative prognostic marker in IDH-mutant glioma (with or without 1p/19q co-deletion). The presence of CDKN2A homozygous deletion in oligodendroglioma has been associated with a prognosis as poor as that of histologic glioblastoma [22].

Complementing the above classification schema, an interesting theory put forward by Venteicher et al.[23] is that the overall percentage of stem-like cells in the framework of the above mutations is what determines the grade of the tumor and that astrocytomas and oligodendrogliomas share the same cellular hierarchy. The percentages of relatively differentiated cells determine the grade of the tumor. In lower-grade glioma (e.g., *IDH* -mutant astrocytoma and co-deleted oligodendroglioma), the cell of origin is likely the same (neural progenitor cell) but with the distinguishing factor being the underlying mutations (e.g., 1p/19q versus p53 following the IDH mutation). These mutations drive the overall direction of net differentiation. The pathologic grade correlates with the percentage of stem-like cells [23]. The histologic differences observed are due to the microenvironment and tumor genetics. Therefore, the highest-grade tumors have the highest fraction of stem-like cells and are capable of rapid, remarkably creative adaptation to selective pressures (e.g., tumor associated microtubules recapitulating the neuronal growth cone, glioma cells undergoing epithelial to mesenchymal transition in the setting of hypoxia). In IDH wild-type and histologic glioblastomas are more heterogeneous and genotypically plastic with relatively favored steady-state gene expression profiles. Glioma remains a remarkably adaptive and recalcitrant disease.

6. *TERT*

Telomerase reverse transcriptase (*TERT*) is telomere-lengthening ribonucleotide reverse- transcriptase enzyme normally expressed during early development and subsequently down regulated in postnatal somatic cells. *TERT* functions to maintain telomere ends, which shorten during DNA replication and cell division, by the addition of a nucleotide repeat to telomere. Normally, telomerase expression is repressed in postnatal somatic cells, which leads to progressive shortening of telomeres with replication and cellular senescence. Cells that acquire *TERT* mutations can lengthen their telomeres and prevent cellular senescence, enabling clonal immortality. *TERT*-promoter mutations likely occur late in clonal selection of neoplastic cells. *TERT*-promoter mutation is associated with an older patient age at presentation and de novo glioblastoma formation, a more aggressive phenotype and worse prognosis [20].

7. O(6)-methyl guanidine-DNA-methyltransferase (MGMT)

The O(6)-methyl guanidine-DNA-methyltransferase (MGMT) enzyme is a suicide DNA-repair enzyme that is consumed in the process of removing alkyl adducts from the O(6) position of the nucleotide guanine This is a normal DNA-repair mechanism exploited by glioma to repair the effect of TMZ . TMZ adds a methyl group to the O(6) position of the nucleotide guanine, producing O(6) methylguanine. O(6) methylguanine pairs with thymine rather than cytosine and, in the setting of functional mismatch repair

during DNA replication, can lead to cell-cycle arrest and apoptosis [24]. Gliomas with *MGMT* gene-promoter methylation have less capacity to repair the alkylating effect of TMZ and are more treatment-sensitive to TMZ chemotherapy. *MGMT* gene-promoter methylation is present in slightly more than a third of cases of glioblastoma across multiple studies (as high as 80% in grade 2 gliomas) [24]. Mutation of the *MGMT* gene (as opposed to methylation of the gene promoter) is relatively rare, occurring in approximately 1% of gliomas. MGMT methylation is likely an early event in gliomagenesis, usually homogeneous throughout the tumor and stable through disease recurrence. Its presence or absence is not influenced by current therapies. The degree of methylation, which has traditionally been reported as a binary value (present, indeterminate, or absent), may be of prognostic significance as well; in other words, high versus low methylation versus absent (<10% methylation) may be able to further explain outliers and long-term survivors [25].As a "suicide" enzyme, MGMT is consumed when repairing O(6) methylguanine, but clinical approaches to "overwhelm" the enzyme with dose-dense TMZ have not proven superior to standard dosing in the upfront setting [26]. Alkylating chemotherapy has been the primary method to exploit MGMT-promoter methylated tumors, with intriguing results from the CeTeG study recently published in the *Lancet* detailing the benefit of the addition of CCNU to TMZ chemotherapy in patients with MGMT-promoter methylated glioblastoma. Currently the adoption of this regimen is on a case by case basis given the additional toxicity of dual alkylator therapy and complexities surrounding the reported primary outcome of overall survival and the small size of the trial [27].

PARP inhibition in MGMT promoter-methylated glioblastoma may be a viable strategy to exploit further the reduced DNA-repair capacity in the setting of coincident TMZ chemotherapy. PARP (Poly (ADP-ribose) polymerases are enzymes that catalyze the transfer of ADP-ribose to target proteins – playing a critical role in DNA repair in addition to DNA transcription, replication, recombination and chromatin modulation. In tumors with defective homologous recombination mechanisms, PARP-mediated DNA repair may be a mechanism of escape and cell survival [28]. As such, PARP inhibitors may also increase tumor sensitivity to DNA-damaging agents or push cells with defective homologous recombination (e.g., BRCA1 mutations) towards apoptosis. Recently, a clinical trial of patients with MGMT-promoter unmethylated glioblastoma treated with veliparib in addition to standard therapies in the upfront setting was underwhelming [29]. A current study (NCT02152982) utilizing veliparib as a PARP inhibitor in MGMT-promoter methylated GBM is underway. This strategy may be more effective in patients with relatively deficient DNA repair (e.g., MGMT-methylated) than in the unmethylated group. Additionally, PARP inhibitors may be a rational treatment approach in IDH-mutated tumors as well (discussed further below).

8. Isocitrate dehydrogenase (*IDH*)

Isocitrate dehydrogenase-1 (*IDH-1*) is a cytosolic NADP+-dependent enzyme involved in cellular metabolism, familiar to many from rote memorization of the (Kreb's) citric-acid cycle. Somatic gain of function mutations in the *IDH-1* (cytosolic) and *IDH-2* (mitochondrial) genes initiate events in the majority of low-grade gliomas [30], whereas somatic IDH mutant mosaicism is seen in Ollier disease/Maffucci syndrome [31] and is associated with the formation of enchondromas, chondrosarcomas, glioma and spindle-cell hemangiomas. In its wild-type state, *IDH* normally functions to catalyze isocitric acid to alpha ketoglutaric acid, while also producing NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate. The canonical mutations are *IDH-1* mutations, and the majority of these are R132H mutations in which the highly conserved amino acid arginine (R7) in position 132 of the amino-acid sequence is substituted with histidine (H) in the binding site for isocitrate [30].This substitution increases the function of the IDH mutation, which further catalyzes α-

ketoglutarate to the pathogenic “onco-metabolite” D-2-hydroxyglutarate (2-HG) while oxidizing NADPH to NADP+. The spectrum and significance of non-canonical IDH mutations comprise an ongoing field of study.

In cells harboring IDH mutation there are blanket genome-wide effects, the best described and most relevant of which is an inhibitory effect on multiple enzymes within the cell including the Ten-eleven translocation (TET) class of DNA demethylases [32]. Simplistically, global inhibition of DNA demethylases results in a net *increase* in DNA methylation including methylation of CpG islands. The methylation of CpG islands (referred to as the G-CIMP phenotype) interferes with the tertiary structure of DNA by interrupting the binding site for CTCF. CTCF is an insulator protein and transcription factor that binds to thousands of sites across the genome, where it interacts with transcription factors involved in the maintenance of chromatin loops. The insulator function of CTCF holds the DNA in a specific spatial orientation, forming chromatin loops, and spatially separating genes and transcriptional regulatory elements. Interference with CTCF binding disrupts the formation of chromatin loops and therefore disrupts the physical segregation of DNA, bringing together disparate genetic elements in close physical proximity to influence one another. This is a newly discovered mechanism of oncogenesis, elucidated in glioma and, presumably identical in other *IDH* mutation-associated malignancies (chondrosarcoma, cholangiocarcinoma, Acute Myeloid Leukemia, and Ollier disease/Maffucci syndrome). In cases other than glioma, it is associated with a worse prognosis [32].

Given that the *IDH* mutation is key to gliomagenesis in low-grade glioma and that *IDH* mutations (such as the R132H) are found only in tumor cells, where they represent a driver mutation, IDH small-molecule inhibitors might be beneficial [33] as well as *IDH*-targeted vaccines [34] and adoptive cell therapy. The efficacy of this approach in gliomas remains to be seen in multiple highly anticipated studies in recurrent low grade IDH mutant gliomas and is the focus of several early phase clinical trials specific to IDH mutant glioma [35, 36], e.g. NCT02481154, NCT03343197, NCT03030066. Other trials include patients harboring solid tumors with IDH mutations [35]. Of note, some data indicate subsequent genetic drift from this mutation, meaning that although this mutation is instrumental in glioma-genesis, it is not a mutation on which the glioma is dependent. As a result, targeting the IDH mutation after surgical resection or biopsy with an IDH inhibitor or vaccine may be most effective as exposure to alkylating chemotherapy and radiotherapy may introduce further mutations and inadvertently contribute to malignant progression of lower histologic grade tumors [37].

IDH-mutated tumors demonstrate defective homologous recombination resulting in increased levels of DNA damage from cellular metabolism as well as alkylating chemotherapy [38]. Again, PARP inhibition is potentially a rational strategy in *IDH*-mutated tumors [39]. Though the IDH mutation results in a gain of function, the net result is that lower substrate concentrations are available to buffer the oxidizing effect of radiotherapy: NADPH, glutathione, and deoxynucleotides are all reduced in *IDH*-mutant glioma cells [38]. Intriguingly, reducing NADPH in the *IDH* wild-type glioblastoma by targeting the non-mutated *IDH*1 enzyme proved an effective adjunct to radiotherapy *in vitro,* where it was found that *IDH* silencing in GBM cells decreased the concentrations of NADPH, deoxy-nucleotides, and glutathione and increased radiotherapy-induced cellular senescence [40]. Therefore, *IDH* inhibition may prove an effective strategy in both *IDH*-mutant and *IDH* wild-type gliomas.

IDH-mutated gliomas have demonstrated reliance on glutaminase for glutamate biosynthesis. Glutamate critically provides carbon atoms for the generation of TCA intermediates, nucleotides, and glutathione. Branched chain amino acid transaminase (BCAT) catalyzes the conversion of branched chain amino acids to glutamate, and BCAT expression and activity is diminished in IDH mutant gliomas [41].

This has been attributed to BCAT1 promoter hypermethylation and silencing secondary to excess tumor 2-HG [42]. CB-839 is a novel, potent, selective and reversible inhibitor of glutaminase activity [43]. CB-839 depletes intracellular glutamate and glutathione in IDH mutant glioma models in vitro, and this is associated with an enhancement in cell lethal effects of radiation therapy in these tumors [41]. Therefore, glutaminase inhibition with drugs like CB-839 may be a promising strategy for the treatment of IDH mutant gliomas. Currently a NCI CTEP supported phase I clinical trial (NCT03528642) is investigating the safety and tolerability of CB-839 when combined with radiation/TMZ in patients with previously untreated IDH mutant grade II/III astrocytomas[44, 45].

9. 1p/19q Co-Deletion

Whole-arm 1p-19q co-deletion and oligodendroglioma histology have long been associated with a better prognosis relative to other gliomas without this co-deletion [46]. As defined by the 2016 WHO classification of gliomas, 1p-19q whole-arm co-deletion (i.e., deletion of the short arm of chromosome 1 and the long arm of chromosome 19), most often actually occurs as a trans-location, and is required for the diagnosis of oligodendroglioma. The addition of procarbazine, CCNU (and vincristine) chemotherapy to radiotherapy in anaplastic and low-grade gliomas has resulted in markedly improved overall survival in the RTOG 9402 [47] and the EORTC 26951 [48] trials (both anaplastic oligodendroglioma) as well as the RTOG 9802 trial (high-risk, low-grade glioma) [49].

Recently, it has been demonstrated that 1p-19q co-deleted gliomas have a decreased ability to produce ultra-long membrane protrusions, termed tumor-associated microtubules (TMs), which appear to be a mechanism of tumor resilience and pathogenesis [50]. TMs enable their host cells to interconnect and form a functional resistance network with tumor microtubules, conveying resistance to surgical lesions, chemotherapy, and radiotherapy [1]. Cells that harbor TMs demonstrate the ability to engage a pathologic healing response with cell densities that significantly exceed those of non-lesioned brain regions over time when compared to adjacent non lesioned areas [1]. TMs serve as routes for brain invasion, proliferation, and a means for inter-connection over long distances within the host. TMs are highly dynamic structures, similar to the neuronal growth cone present in radial glial-cell processes seen during brain development. Of absolute relevance, those cells within astrocytoma models connected by these TMs appear to be protected within the network from chemotherapy and radiotherapy compared to astrocytoma cells not sufficiently connected by TMs. Unconnected tumor cells are much more susceptible to therapy in mouse models. The mechanisms by which these inter-connected TMs resist therapy include the ability to buffer calcium in the setting of radiotherapy and the ability to re-supply damaged cells and organelles such as mitochondria and even nuclei via TMs [1]. These tumor microtubules are able to act as a functional syncytium. This interconnectedness appears to be inversely related with 1p/19q co-deletion. 1p/19q co-deleted cells have reduced expression of GAP43, which is a driver or neuronal growth-cone formation [1].

TMs are connected via connexins, specifically connexin 43. Of note, the expression of connexin 43 is inversely related to the stemness of the tumor cell. Glioma initiating stem cells express very low levels of connexin 43, and when this protein is restored the stem cell phenotype of glioma stem cells is reversed and the self-renewal capacity, resistance to standard therapies and invasive capacity is reduced [51]. The discovery of this pathogenic mechanism has already prompted tremendous excitement and a drive to develop rapid through-put techniques to exploit potential vulnerabilities in astrocytoma and glioblastoma cells. A recent publication of interest detailed targeting connexin 43 via chimeric antigen-receptor T-cell therapy [52]. The teleological explanation for why 1p-19q co-deletion is selected for and seems to remain present upon recurrence is unclear.

10. Intra-tumoral heterogeneity

Glioblastoma cells from the same tumor may harbor different genetic mutations or exhibit distinct phenotypic or epigenetic states. A striking example of this is that tumor cells isolated from different locations within the same tumor may have more phenotypically and genotypically in common with tumors from different subjects than other tumor cells within the original tumor. This intra-tumoral genetic and epigenetic heterogeneity, often constituted by a multitude of redundant signaling pathways, is presumably the reason that multiple trials of targeted agents in small-molecule inhibitors have not shown benefit in glioblastoma. It stands to reason that, with the intra-tumoral heterogeneity of glioblastoma, a single selective pressure will produce an adaptive resistance. This is frustrating but not unexpected as the selective pressures present within glioblastoma in its native state are fairly dramatic. For example, the histologically diagnostic feature of pseudo-palisading necrosis, which experimentally is essentially a necrotic core of dead tumor with glioma cells escaping toward an oxygen gradient (i.e., oxygen-rich adjacent tissue, lining up to form a pseudo-palisade [53]. In order for a glioma cell to be able to migrate, as in a pseudo-palisade, the hypoxia must activate an epithelial-to mesenchymal transition, which has been shown to be hypoxia-inducible factor-dependent. The epithelial-to-mesenchymal transition describes cell adoption, fibroblast-like morphology, and a migratory phenotype, which are commonly present during embryonic development. The utilization of the epithelial-to-mesenchymal toolkit, as it is utilized in glioblastoma, is a critical process to cancer progression. The universal presence of pseudo-palisading necrosis in glioblastomas suggests that this pattern of hypoxia induced necrosis is instrumental in the spread and invasion of glioblastoma. Pseudo-palisades are not highly proliferative areas. They are actually significantly lower in proliferation than the surrounding areas. Experimentally, using the epithelial-to mesenchymal transition, glioblastoma cells have been shown to migrate away from hypoxic regions toward oxygen-rich regions and actually proliferate more robustly upon arrival to an oxygen-rich environment. In this manner, glioblastoma cells are exposed to cyclic hypoxia that switches their phenotype between migratory and proliferative: “go or grow” phenotypes [53]. Both hypoxia and bevacizumab can select for an invasive mesenchymal phenotype by up-regulating hypoxia-inducible factor 1-alpha [54]. The selective pressure that bevacizumab induces upon glioblastoma is already present in the tumor in the form of areas of pseudo-palisading necrosis. The failure of bevacizumab to provide a meaningful improvement in overall survival and subsequent demonstration that bevacizumab may actually drive tumor cells more towards an invasive phenotype as opposed to a proliferative phenotype is not unexpected given that these selective pressures are present in the glioblastoma in its native state. Bevacizumab may contribute to hypoxia within the tumor microenvironment by reducing microvascular proliferation [54]. Increasingly, the tumor microenvironment is being invoked as a principal driver of glioma resistance [55]. The highest-grade tumors have the highest fraction of stem-like cells and are capable of rapid, remarkably creative adaptation to selective pressures.

11. Neuronal activity and glioma growth

A recently described mechanism of a direct neuronal activity to a glioma mitogen link implicates neuronal activity-dependent cleavage on a synaptic-adhesion molecule, neuroligin-3 (NLGN3), which binds to glioma cells similarly as it does for the native oligodendrocyte or oligodendrocyte progenitor cell and stimulates the PIK3-mTOR pathway [56]. NLGN3 sends the glioma cell a mitogenic signal that predominantly facilitates an oligodendrocyte progenitor-cell gene- expression profile pattern. Additionally, NLGN3 offers positive feedback, which results in glioma- cell surface expression of NLGN3, which can then be cleaved. NLGN3 is cleaved from neurons and oligodendrocyte-progenitor cells via ADAM10 sheddase, which can be inhibited by ADAM inhibitors such as INCB3619. ADAM10 sheddase

inhibitors prevent the release of neuroligin-3 into the tumor microenvironment and block glioma growth in xenograft models [56]. The association of ADAM10 with glioblastoma-cell migration and invasion has previously been demonstrated. Notably, the up regulation of ADAM10 sheddase has been demonstrated in more invasive glioblastoma cell lines and in human specimens [57]. The ADAM10-expression levels have correlated with tumor progression as well as the grade of malignancy. Inhibitors of the ADAM family of enzymes have been used in lymphoma and breast cancer [58]. There is very importantly a direct neuronal activity to glioma link. Clinical trials are to be underway in the very near future with agents targeting ADAM10 in human glioma. Currently, the ADAM10 inhibitor INCB7839 is currently being explored for use in glioma [56].

12. Immunotherapy and the tumor microenvironment (TME)

Our current, improved molecular understanding of glioma, along with the intra-tumoral heterogeneity of glioblastoma, will hopefully lead to better, more rational treatment strategies in the future that do not expose the tumor to a single, selective pressure. Immunotherapy as a treatment strategy is rational in central nervous system tumors. Multiple sclerosis indicates CNS immunosurveillance by T cells, whereas paraneoplastic neurologic syndromes are indicative of naturally occurring anti-tumor immunity and immune surveillance of the central nervous system. Cases of severe immunosuppression may result in CNS infections such as progressive multifocal leukoencephalopathy. Previously considered devoid of lymphatics, the CNS actually has a lymphatic system that drains to the deep cervical lymph nodes [59]. As such, immunotherapy has become an exciting area of active research in neuro-oncology. There have been some very exciting case reports with the use of check-point inhibitors in patients with microsatellite instability [60] as well as CAR-T therapy in a patient primarily with leptomeningeal glioblastoma [61]. Frustratingly, these cases have been followed by clinical trial failures of immune checkpoint therapy in glioblastoma. In the Checkmate 143 study (NCT 02017717) utilizing nivolumab versus bevacizumab in patients with recurrent glioblastoma, there was no difference in overall survival, and progression-free survival was greater in patients treated with bevacizumab as presented at the WFNOS meeting in 2017 [62, 63]. One possible explanation is the relatively low mutational burden and relatively low number of neo-epitopes in gliomas as compared to melanoma or NSCLC [64]. Additionally, the central nervous system immune microenvironment differs dramatically from that of the peripheral immune system [65].

Two interesting studies utilizing PD1 inhibitors in patients with recurrent glioblastoma were recently published. One was presented at the ASCO meeting in 2018 from the MD Anderson group [66, 67] as a window-of-opportunity study that collected peripheral blood and tumor biomarkers. Macrophages—as opposed to T cells—diffusely infiltrated the tumor, and the blood-brain barrier itself was lined with macrophages with immunosuppressive markers including CD163 and CD68. The T-cell influx into the resected glioblastoma tissue was low and not associated with expression of activation or effector markers. This study concluded that T cell-effector responses in glioblastoma were not modulated by anti-PD1 therapy and that a significant population of CD68-positive macrophages highly expressed immune-inhibitory molecules. Of note, the median overall survival in this cohort (without a control) was 20 months. Interestingly, the group from UCLA demonstrated survival benefit of patients treated with a single dose of neoadjuvant pembrolizumab followed by adjuvant pembrolizumab was very similar to those receiving adjuvant pembrolizumab alone following resection of recurrent glioblastoma [68]. This was nearly identical to the MD Anderson study; however, the UCLA study had two arms, which clearly demonstrated a survival advantage rather than merely comparing to historical controls for PFS. The patients who received one dose of neoadjuvant pembrolizumab prior to repeat resection for recurrent GBM followed by adjuvant pembrolizumab had a median overall survival of 417 days, compared to

228.5 days in those patients who underwent repeat resection and received adjuvant pembrolizumab (but not a dose of neoadjuvant pembrolizumab) [68]. Immunotherapy targeting CD68-immunopositive cells and strategies (such as surgery, biopsy, or, potentially, radiotherapy) that induce T-cell influx into the tumor via should be prioritized. They may be more efficacious.

The use of immunotherapy in central nervous system tumors is uniquely affected by the use of steroids such as dexamethasone, which are frequently used in patients with central nervous system malignancies due to their effect on vasogenic tumor-associated edema and resultant mass effect. Steroid use can produce neurologic symptomatology in patients with brain tumors. In several failed studies utilizing checkpoint inhibitors, there has been an association between poorer survival and patients receiving corticosteroid [63]. A similar deleterious effect from systemic dexamethasone administration has also been seen in patients treated with checkpoint inhibitors and stereotactic radiosurgery for central nervous system metastases of systemic tumors [69]. Therefore, these high mutational load-associated tumors (e.g., melanoma) are highly "visible" to the immune system, even in the CNS, and the effects of immunotherapy may be abrogated by corticosteroids, which are potentially over-used in the management of patients with CNS malignancy. This is increasingly relevant given the first signals of efficacy of immunotherapy in this disease process. There is some rationale that VEGF blockade with bevacizumab may enhance immunotherapy as has been demonstrated in patients with melanoma [70].

13. Expert opinion

Recent discoveries in the molecular makeup of gliomas, the relationship of certain molecular drivers, and the patient's response to therapy and overall prognosis have redefined our understanding of glioma. Improved understanding of the molecular underpinnings of this disease has been directly translated into treatment decisions and an improved ability to counsel patients regarding their prognosis. Additionally, with an improved understanding of the pathogenesis of this recalcitrant and remarkably stubborn disease, we are beginning to see the first glimmer of a return on the investment in regard to immunotherapy, along with anticipated successful exploitations of the unique glioma pathophysiology.

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