



# Updates in IDH-Wildtype Glioblastoma

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Accepted: 14 May 2022

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## Abstract

Glioblastoma is the most aggressive primary brain tumor with a poor prognosis. The 2021 WHO CNS5 classification has further stressed the importance of molecular signatures in diagnosis although therapeutic breakthroughs are still lacking. In this review article, updates on the current and novel therapies in IDH-wildtype GBM will be discussed.

**Keywords** Glioblastoma · IDH-wildtype · Targeted therapy · Precision medicine · Clinical trials

## Introduction

Glioblastoma (GBM), a subtype of adult diffuse glioma, is a primary central nervous system (CNS) tumor presumed to arise from neuroglial stem cells or their progenitors in the subventricular zone [1–3]. There has been a recent paradigm shift, with increasing reliance on molecular information for diagnostic classification and prognostication within gliomas, as seen in the most recent World Health Organization (WHO) classification of CNS tumors [3]. This update further incorporated molecular markers in addition to histology for an integrated diagnosis, resulting in a clearer separation of adult and pediatric-type gliomas. As a result, GBM now refers specifically to the most aggressive form of isocitrate dehydrogenase (IDH)-wildtype diffuse adult-type astrocytoma, for which *O*<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) promoter methylation status is prognostic. Despite increased understanding of the molecular evolution of GBM, it continues to be an incurable disease with poor survival. The standard multimodal therapies of maximal safe resection and radiation therapy (RT) with concomitant and adjuvant temozolomide (TMZ) remain the backbone of treatment. Clinical trials continue to expand on novel approaches

such as targeted agents and immunotherapy. This review will provide an overview of current updates in the classification, diagnosis, and management of GBM.

## Epidemiology and Risk Factors

Glioblastoma accounts for 14.5% of all primary CNS tumors and 48.6% of all malignant primary CNS tumors, with an annual age-adjusted incidence rate of 3.23 per 100,000 population [4]. Age, sex, and race/ethnicity influence the incidence rate which exponentially increases beyond 40 years of age, with a mean age of diagnosis at 65, and peaking between 75 and 84 years at 15.30 per 100,000 population. GBM is 1.59 times more common in males and 1.99 times more common in Caucasians compared to African-American patients [3–5].

Multiple studies have tried to delineate whether environmental risk factors could be linked to GBM, but only ionizing radiation in medium-to-high doses, particularly in children, was consistently found to be a risk factor (e.g., atomic bomb survivors, therapeutic radiation cohorts) [6]. In case–control studies, the odds of identifying a family history of primary brain tumor are 2.3 times more likely in patients with adult gliomas. Although 5% of gliomas are familial, a Mendelian inherited syndrome (e.g., Lynch syndrome, neurofibromatosis, tuberous sclerosis), which commonly harbors loss-of-function mutations in tumor suppressor genes, can only be pinpointed in approximately 1–2% and 4% of adult and pediatric gliomas, respectively [6–9]. Conversely, case–control studies have also shown that a history of atopic diseases (e.g., eczema, allergies) decreases the risk of glioma by 30% [10].

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## Imaging Techniques to Aid in Diagnosis

Combined with the appropriate clinical history, imaging can help achieve an accurate pre-operative diagnosis. Structural magnetic resonance imaging (MRI) with gadolinium remains the gold standard diagnostic modality for GBM evaluation. GBM has a predilection of involving the cerebral hemispheres, with the frontal and temporal lobes most involved. Typical findings are that of an infiltrative mass with poorly delineated margins that exhibits mixed signal intensities on T1-weighted imaging (T1-WI). Heterogeneous hyperintensities are also evident on T2/fluid-attenuated inversion recovery (FLAIR)-WI, which also demonstrate indistinct margins that blend in with extensive vasogenic edema. Cysts, hemorrhage at various stages of evolution, fluid/debris levels, and “flow voids” are common findings. Radiologically, the majority of glioblastomas appear as a unifocal mass; approximately 20% are multifocal/multicentric [11–13]. In contrast to multifocal GBM which refers to enhancing foci that are embedded within the same abnormal high FLAIR signal region, the less common multicentric pattern demonstrates enhancing foci that are spatially (commonly occurring in regions separated by a tentorium) and temporally separated as most of them arise metachronously [14].

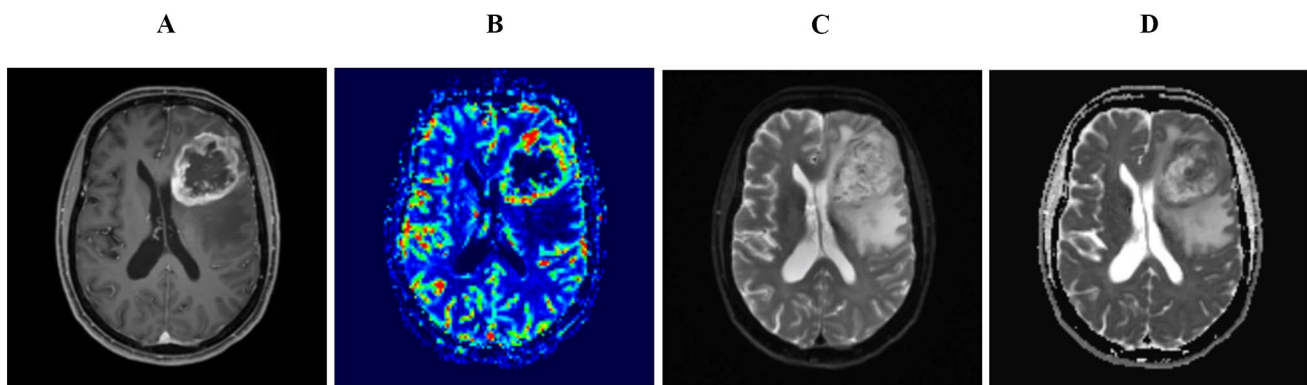
Advanced imaging techniques can help differentiate GBM from other diagnoses. These include diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI). Contrary to PWI, which reflects the state of the tumor vascular bed, DWI can be used to calculate apparent diffusion coefficient (ADC) values, which can be used to indirectly assess tumor cellularity through measuring water diffusivity. GBM has a significantly lower ADC value as compared to gliomas of lower grades [15], while primary CNS lymphoma has lower ADC values than GBM

[16]. Figure 1 shows a classic appearance of GBM on post-contrast T1-WI, MR perfusion, and DWI as well as ADC.

Dynamic susceptibility contrast MRI measures cerebral blood volume (CBV), a surrogate marker of total tumor vascularity. In contrast, dynamic contrast-enhanced MRI measures the permeability surface area which reflects the leakiness of vasculature [17]. Both parameters may differentiate GBM from lower-grade gliomas [18]. Compared to IDH-mutant tumors, IDH-wildtype tumors are associated with significantly higher relative CBV values [19, 20]. Several studies have assessed whether certain imaging signatures can be predictive of genotypic or epigenetic signatures such as IDH status and MGMT promoter methylation, respectively [21–24].

Metabolic alterations within GBM can be detected by MR spectroscopy [25]. Accelerated cellular proliferation, neuronal death, and necrosis are reflected through an elevated choline peak, suppressed N-acetyl aspartate (NAA)/creatine peaks, and increased lactate/lipid peaks, respectively. Compared to lower grades, higher grade gliomas demonstrate lower NAA and creatine levels, higher lipid/lactate levels, and higher choline/NAA and choline/creatine ratios [26, 27]. Chemical exchange saturation transfer (CEST) is an MRI technique that relies on the amide proton exchange rate between proteins and bulk water, which is inversely correlated with the tissue pH [28, 29]. A recent study including IDH-wildtype GBM found a diagnostic sensitivity and specificity of 90% when incorporating additional physiologic (ADC values) and metabolic (CEST) parameters into the model [30].

Positron emission tomography (PET) uses radiolabeled molecules to gain insight into neoplastic tissue biology. Glucose transporters are upregulated in gliomas and exhibit a high affinity to [18F]-2-fluoro-2-deoxy-d-glucose (FDG)



**Fig. 1** A 70-year-old woman who presented with headache and behavioral changes and was found to have an IDH-wildtype GBM over the left frontal region causing minimal mass effect. Contrast-enhanced T1 image shows a large necrotic mass over the left frontal

region with irregular thick rind (A). Elevated CBV on MR perfusion correlates to areas of enhancement (B). Compared to DWI map (C), cellular heterogeneity is evident within the tumor fields as more hypercellular areas demonstrates higher signal drop on ADC map (D)

tracers. Due to the similar affinity seen with inflammatory entities and the physiologic uptake by cerebral parenchyma, FDG tracers have low diagnostic specificity and low signal/noise ratio hindering their ability to delineate gliomas [31–33]. To overcome these issues, radiolabeled amino acid tracers are preferred, and the repertoire of such molecules continues to expand [34–37]. Clinical applicability includes the delineation of tumor extent, especially in normal-appearing tissue where it can aid in radiotherapy and surgical planning [38]. Assessment of treatment response by amino acid PET is gaining interest. Decreased  $^{18}\text{F}$ -fluoro-ethyl-tyrosine (FET) uptake 6–8 weeks following chemoradiation is associated with improved outcome compared to non-responders [39, 40].

## Response Assessment in Neuro-oncology

The Response Assessment in Neuro-Oncology (RANO) criteria for high-grade gliomas (WHO grade 3 and 4) is the established standard for evaluation of response or progression in GBM clinical trials [41]. To address the challenges of response interpretation in the context of changes due to disease biology and treatment effect, the RANO criteria evolved from the previously adopted Macdonald et al. criteria [42], and other response assessment frameworks [42, 43]. The modified RANO criteria for high-grade gliomas (mRANO) was later developed and uses the post-radiotherapy MRI baseline for these newly diagnosed gliomas [43]. In addition, confirmation of progression is recommended in both newly diagnosed and recurrent high-grade gliomas after the preliminary progressive disease is observed on an initial MRI scan. The RANO criteria use post-contrast two-dimensional tumor measurements based on the standardized Brain Tumor Imaging Protocol [44]. Beyond MRI-guided response assessment, other response assessment modalities are being investigated. These include the Neurologic Assessment in Neuro-Oncology, which integrates clinical response assessment [45]; immunotherapy RANO (iRANO), which addresses possible pseudo-progression related to immunotherapies [46]; and PET-RANO, which leverages metabolic imaging for response assessment in glioma [47]. Future directions in RANO include leveraging advances in imaging technology and artificial intelligence for automated volumetric measurements and response assessments [48, 49]. Additional factors that need to be weighed are the changing landscape of therapies (particularly immunotherapy) and the revised classification of gliomas. The RANO group is currently reviewing its criteria with revised criteria scheduled to be proposed later in 2022.

## Pathology, Molecular, and Genomic Signatures, and Recent Updates in Classification

Glioma classification has relied on histopathology, with the hallmarks of GBM as an astroglial tumor with features of microvascular proliferation and/or necrosis. With integrated molecular classification, updated in the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (c-IMPACT NOW) and the more recent WHO CNS5 classification, diffuse astrocytic gliomas have now been subdivided based on molecular lineage. A mutation in *isocitrate dehydrogenase 1* or *2* (*IDH1/2*) has become a defining branch point in adult-type diffuse glioma diagnosis [50]. The most common *IDH* variant in glioma is the *IDH1* R132H mutation, for which there is a rapid immunohistochemistry stain [51]. The yield of non-canonical *IDH* mutations identified with sequencing is age-dependent. Compared to the 6.3% rate in patients younger than 55 years, only 0.9% are identified in older patients with glioblastoma. This low yield has raised concerns regarding the cost-effectiveness of sequencing in that age group [52].

The distinction between *IDH*-mutant vs wildtype gliomas has also become clearer, with the omission of “secondary” or *IDH*-mutant GBM in favor of the term diffuse *IDH*-mutant astrocytoma, WHO grade 4. Diffuse gliomas that are *IDH*-wildtype and have molecular features of GBM can now be classified as the latter, even when histological high-grade features are absent [3, 53]. These molecular features include the combination of gain of chromosome 7 and loss of chromosome 10, *TERT* promoter mutations, or *EGFR* amplification.

Pediatric-type diffuse high-grade gliomas possess distinct mutations from their adult counterparts [3]. Notably, diffuse midline gliomas can carry an H3 K27 alteration and are now recognized as a separate entity from GBM. Similarly, diffuse hemispheric gliomas with an H3 G34 mutation are a separate entity found in adolescents and young adults.

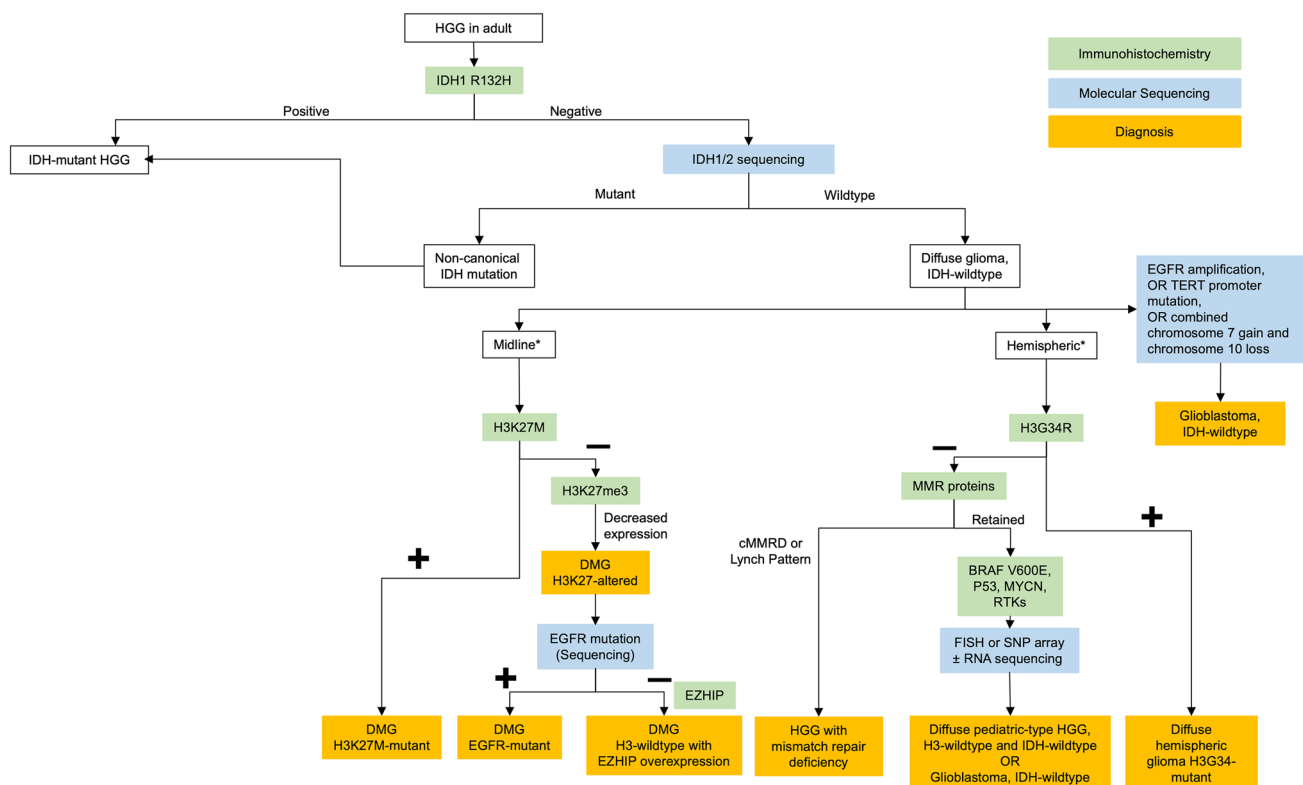
Of the *IDH*-wildtype and H3-wildtype high-grade gliomas, there are other mutations that have distinct prognostic and therapeutic implications. These include *BRAF V600E* mutations, *FGFR* alterations, or *MYB* or *MYBL1* rearrangement [54]. GBM with *BRAF V600E* mutations has been described as epithelioid GBM [55], especially with a co-occurring mutation in the *TERT* promoter and homozygous deletion of *CDKN2A/2B* [56]. While rare (<2% of GBM) [57] and difficult to distinguish from pleomorphic xanthoastrocytoma [58], recognizing the molecular, clinical, and radiological features of this subtype is important [58] due to the observed efficacy of combination BRAF/MEK inhibition in gliomas with *BRAF V600E* mutation [59]. Figure 2 provides a proposed sequential approach to

molecular testing in gliomas, although many centers now opt for targeted next-generation sequencing panels, which allows for the testing of all relevant molecular markers of GBM in one custom assay. The clinical utility of molecular testing through targeted sequencing panels remains limited beyond diagnostic confirmation and prognosis [60].

Advances in sequencing technology have led to the identification of specific somatic alterations in gliomagenesis [61, 62]. Three specific GBM subgroups with distinct somatic alteration signatures have been identified: the proneural type with cyclin-dependent kinase 4 (*CDK4*) and platelet-derived growth factor alpha (*PDGFRa*) amplification; mesenchymal type with neurofibromatosis type 1 (*NF1*) loss; and classical type with epidermal growth factor receptor (*EGFR*) amplifications and homozygous loss of *CDKN2A/B* [57, 63]. Beyond genomic alterations, profiling the epigenome and methylome has provided a more accurate classification of GBM. DNA methylation profiling has identified 7 distinct entities within GBM [64]. However, knowledge of these distinct subtypes has not yet translated into clinical utility.

## Current Approach to Newly Diagnosed GBM

The well-known standard of care for newly diagnosed GBM is maximal safe surgical resection followed by concurrent chemoradiation with TMZ, and adjuvant TMZ [65–68]. Attempts to improve the efficacy of chemoradiation for GBM have been disappointing. Alternative TMZ dosing regimens aimed to exhaust MGMT reservoirs; for example, the RTOG 0525 trial compared adjuvant dose-dense (DD) TMZ (75–100 mg/m<sup>2</sup>/day for 21 days in a 28-day cycle) to conventional TMZ. Even for patients with MGMT methylated tumors, DD TMZ did not improve outcomes and was associated with more toxicity [69]. Similarly, extending the conventional TMZ regimen beyond 6 cycles did not change survival outcomes in a randomized open-label trial (GEINO14-01) [70]. This lack of benefit even in patients with a methylated MGMT promoter was also corroborated by non-randomized studies [71, 72]. Prolonged TMZ therapy has a cumulative negative impact on bone marrow reserves [73, 74] and a cumulative increase in treatment-related fatigue [75, 76], and is not recommended in routine practice.



**Fig. 2** Proposed molecular testing algorithm for HGG. \*Consider testing in young adults. *Abbreviations:* HGG, high-grade gliomas; IDH, isocitrate dehydrogenase; FISH, fluorescence in situ hybridization; SNP, single nucleotide polymorphism; WHO, World Health Organization; DMG, diffuse midline glioma; EGFR, epidermal

growth factor receptor; RNA, ribonucleic acid; EZHIP, EZH Inhibitory Protein; MMR, mismatch repair; cMMRD, constitutional mismatch repair deficiency; BRAF, B-Raf; MYCN, N-myc proto oncogene; RTK, receptor tyrosine kinase; NTRK, neurotrophic tyrosine receptor kinase; TERT, telomerase reverse transcriptase

Tumor-treating fields (TTF) are wearable scalp transducers that deliver local low-intensity, intermediate-frequency (200 kHz) alternating electrical fields that have an anti-mitotic effect, and work synergistically with concurrent chemotherapies [77, 78]. The EF-14 trial was an open-label, non-sham-controlled randomized phase III trial investigating the addition of TTF to standard therapy and demonstrated an improvement in median OS (20.9 versus 16 months) and PFS (6.7 versus 4 months) without compromising the health-related quality of life (HRQoL) [79–81]. However, compliance with TTF might be a limiting factor for its implementation in real-world practice [82]. A sub-group analysis from the same trial [83] has shown that the minimum average compliance rate threshold is 50% for a clinical gain in PFS (HR 0.70, 95% CI 0.47–1.05) and OS (HR 0.67, 95% CI 0.45–0.99) to be attained with a stepwise increase in both outcomes with higher compliance rates. A cutoff compliance rate of at least 75% per day was found to be prognostic of improved survival that is independent of age, performance status, the extent of resection, and MGMT methylation status (HR 0.78;  $p = 0.031$ ). Other limitations have also deterred the widespread adoption of TTF despite its endorsement by the NCCN guidelines [84]. An ongoing randomized study of TTF concomitant with RT and TMZ is underway (EF-32-NCT04471844).

The addition of bevacizumab (BEV), a humanized monoclonal VEGF antibody, to the standard of care was studied in two phase III double-blind randomized trials [85, 86]. Both trials showed a statistically significant advantage of 3 months in PFS with the addition of BEV but no improvement in OS. While RTOG 0825 reported worsening quality of life and neurocognitive functions, the AVAglio trial reported a longer global health status deterioration-free survival. It may be that the effects of BEV upon vascular permeability may have reduced the effective concentration or penetration of TMZ in tumor tissue. The addition of BEV to the standard of care therapy is therefore not routinely implemented.

### Approach to Elderly Patients with GBM

Patients with unfavorable prognostic markers such as advanced age or poor performance status have a worse prognosis and are vulnerable to treatment toxicities [87, 88]. Several RCTs have addressed the optimal strategy to manage these patients who cannot tolerate standard 6-week chemoradiation. It is clear that RT provides better outcomes than supportive care alone [89], while early randomized studies suggested that there was no survival disadvantage to shorter radiation schedules such as 40 Gy in 15 fractions [90] or 25 Gy in 5 fractions [91]. In larger RCTs such as the NORDIC study, there is evidence that conventional 6-week radiation may lead to worse outcomes than hypofractionated

schedules [92]. These data lead to the phase III CCTG CE.6/EORTC 26,062 study which randomized 562 patients above the age of 65 years to short-course RT (40 Gy in 15 fractions) alone or with concurrent and adjuvant TMZ. Adding TMZ resulted in an improvement in median OS with most of the benefit seen in the methylated MGMT promoter group [93]. Although there has not been a study comparing 60 Gy/30 with TMZ to 40 Gy/15 with TMZ, the latter has become a common standard of care, particularly for patients over 70 years of age.

In patients where RT may be impractical (for example distance to a treatment facility, neurological impairment), TMZ alone can be considered for those harboring a methylated MGMT promoter. In relatively fit older patients, the updated NOA-08 study results show an impressive median OS of 18.4 months for those treated with TMZ alone. These results set the stage for consideration of a study using lower RT doses, or perhaps omitting RT, in elderly patients with MGMT promoter methylated GBM [94].

### MGMT Promoter Methylation as a Predictive and Prognostic Biomarker

MGMT promoter methylation status is the most predictive and prognostic molecular biomarker and roughly 40% of IDH-wildtype glioblastomas will be methylated [95]. From a modern pooled analysis of 5 phase III trials, the median OS in MGMT methylated patients is approximately 24 months compared to 14 months in their unmethylated counterparts [96]. The optimal CpG island methylation detection assays are unclear; however, methylation-specific polymerase chain reaction (MSP) has been widely used in clinical trials [97]. A pitfall of reporting methylation status in a binary fashion using a qualitative MSP is a potential underestimation of TMZ benefit for patients categorized as unmethylated. Quantitative MSP can further refine the methylation score into high range (highly methylated), intermediate range (partially methylated or gray zone), and low range (truly unmethylated) [98]. The partially methylated group, which constitutes 10% of the “unmethylated” cohort, has a sensitivity to TMZ with a significantly improved OS (HR 0.58, 95% CI 0.43–0.78;  $p < 0.001$ ) compared to “truly unmethylated” patients [99].

MGMT methylation predicts response to standard of care therapy with TMZ with a 50% increase in median OS [69, 100]. The benefit of the addition of TMZ in unmethylated patients is less clear [98, 101–103]. In these patients, there is an urgent unmet need for effective systemic therapy. In the setting of clinical trials, it is now widely accepted that TMZ can be omitted from the standard of care; doing so optimizes the assessment of the efficacy and toxicity of new agents and eliminates the need for pharmacokinetic and dose-finding evaluations of new agents combined with TMZ.

## Current Approach to Recurrent GBM

The vast majority of all GBM recur with a median survival time of 6.2 months after the first progression [104]. There is no universally accepted salvage therapy, and treatment for recurrent GBM (rGBM) requires individualized consideration of re-operation, re-irradiation, and systemic therapy options. Clinical trial involvement is critical for drug discovery in these patients.

Surgical resection is feasible in 20–30% of rGBM patients and is indicated to reduce the mass effect and provide pathological evidence of disease recurrence versus radionecrosis. Early true progression is an indicator of aggressive tumor biology and may mitigate the advantages of a second surgery [67]. The extent of resection (EOR) in rGBM was evaluated in the DIRECTOR trial which enrolled patients with a median age of 55 years with a relatively small volume of enhancing lesion (< 10 cm<sup>3</sup>) and the majority had a KPS of 70 or more. Complete resection of the enhancing tumor was associated with an improvement in post-recurrence PFS (3.5 versus 1.9 months) and survival (12.9 versus 6.5 months) [105]. However, the generalizability of the trial results is limited as the cohort was highly selective for patients with favorable prognostic factors.

Since most GBM recur locally and in the high-dose radiation volume, the benefit of re-irradiation strategies can be limited by dose constraints and the risk of radiation necrosis. Doses in the range of 30–35 Gy delivered in 5–15 fractions are well-tolerated with an acceptable toxicity profile [106, 107]. Small tumor volumes can be managed with radiosurgery [108, 109]. Radiation for recurrence is aimed at local disease control and has a primarily symptomatic/palliative role [110]. BEV may be useful to treat radionecrosis or to mitigate the development of radionecrosis with re-irradiation. In one randomized study, re-irradiation combined with BEV compared to BEV alone was associated with an improved PFS (7.1 versus 3.8 months;  $p=0.05$ ) but no survival benefit [111]. A better understanding of patient selection factors will be key to the development of safe strategies for retreatment.

There are no attractive systemic therapies for rGBM. Nitrosoureas are commonly used, with lomustine (CCNU) preferred for its oral formulation, less frequent administration, and tolerability [112]. No novel agents tested in randomized trials have proven superior to lomustine [113]. Overall, in phase III trials, CCNU confers a benchmark of 20% 6-month PFS and 7–10 months of overall survival from the time of progression [114, 115]. A recent phase II randomized study detected improved survival with regorafenib, an oral multikinase inhibitor, compared to CCNU in rGBM; however, the findings are limited by imbalances in prognostic factors and evaluation in phase III is ongoing [116]. It is humbling to reflect upon the lack of progress beyond

CCNU in rGBM, especially when one acknowledges that the 6-month PFS associated with CCNU may be no better than supportive care alone.

As in the upfront setting, there have been attempts to optimize TMZ dosing and schedule to leverage the depletion of MGMT by TMZ. Multiple prospective studies used the surrogate marker of MGMT activity in peripheral blood mononuclear cell (PBMC) levels and suggest depletion of MGMT with extended or dose-dense schedules of TMZ [117–119]. The DIRECTOR trial [120] demonstrated similar efficacy between a 1 week on/1 week off schedule compared to a 21-day regimen. The RESCUE study [121] was an uncontrolled single-arm study that used a continuous 28/28 regimen (50 mg/m<sup>2</sup> daily) at the time of disease progression following standard chemoradiation. Patients who were rechallenged at least 2 months after 6 cycles of adjuvant TMZ had a PFS at 6 months of 35.7% MGMT status was not predictive of outcome, perhaps suggesting that a continuous schedule of TMZ may overcome the disadvantage of unmethylated disease.

BEV dramatically alters vascular permeability and causes rapid reductions in cerebral edema and an apparent reduction in the contrast-enhancing volume of rGBM. This was the basis for its approval in some countries; however, it has not been evaluated in a controlled trial. It can be useful to improve clinical and radiological deterioration [122], and symptomatic radiation-necrosis [123], and aid with corticosteroid tapering but the survival benefits are unclear. No trials have yet compared BEV to placebo and combination treatments with CCNU [115, 124] or irinotecan [125, 126] have not shown any survival benefit. Although the usual administered dose is 10 mg/kg every 2 weeks, lower doses have been anecdotally reported to be as effective [127, 128].

## Newer Approaches to Treatment

### Surgery and Surgical Approaches

Cytoreductive surgery likely prevents neurological deterioration and improves survival but is of course noncurative as tumor cells migrate to regions distant from the contrast-enhanced (CE) lesion [129]. Earlier studies focused on concepts of gross-total resection (GTR) and subtotal resection (STR) of the CE lesion to define the extent of resection (EOR) thresholds (or its inverse correlate, residual volume) where survival benefit becomes significant. Prospective and retrospective studies have shown that there is an incremental gain in survival with an EOR of at least 78% or RV below 2–5 mm<sup>3</sup> [130–132]. In a 2016 meta-analysis [133], compared to STR, those who underwent a GTR had a superior outcome and STR, compared to biopsy, was also associated with a statistically significant, clinically meaningful improvement albeit less profound and less durable. Since

most tumors recur within 2 cm of the enhanced lesion [134], the surgical concept of maximal safe resection replaced the earlier rigid EOR thresholds [135]. A large retrospective cohort of 1229 patients (median age, 55.7 years) found that extending the resection into the FLAIR regions resulted in improved survival. Compared to less extensive resections, resection of  $\geq 53.21\%$  of the surrounding non-contrast enhancing (NCE) abnormality was associated with a significant prolongation of median OS (20.7 versus 15.5 months) [136]. Age and molecular diagnosis, independent of MGMT status, seemed to impact the benefits attained from maximal safe resection as well [65]. In patients <65 years, GTR of the CE lesion and maximal resection of NCE regions resulted in a similar median OS independent of IDH status. However, older IDH-wildtype patients did not have additional survival benefits beyond the GTR of the CE lesion. The Glioma Supra Marginal Incision Trial (G-SUMIT; NCT04737577) is a phase II pilot randomized control trial that aims to assess whether there is a survival benefit in extending the resection 1 cm beyond the CE lesion in patients with high-grade gliomas.

Laser interstitial thermal therapy (LITT) is an emerging minimally invasive cytoreductive technique that relies on heating the tumor tissue with laser light delivered through a stereotactically positioned optic fiber over a short period of time while simultaneously monitoring the tissue temperature with an intra-operative MRI. LITT techniques have gained interest for ablating small, well-circumscribed, and deep-seated lesions with an oblong morphology in both newly diagnosed and recurrent GBM. However, randomized clinical trials have not been forthcoming considering its null effect on PFS and OS from retrospective studies and the associated high rate of adverse events [137].

Different techniques have been developed to maximize the EOR while minimizing new neurologic deficits. 5-Aminolevulinic acid (5-ALA) is administered orally causing fluorescence in malignant tissue with reported specificity and sensitivity of 100% and 85%, respectively [138–140]. Compared to white light, fluorescence-guided surgery nearly doubled the rates of complete resection of CE lesions (65% versus 36%) and PFS at 6 months (41% vs. 21.1%) [141]. In experienced centers, a minimum EOR of 90% can be achieved [142]. Reliance on neuronavigation can be difficult due to the invariable intra-operative brain shift, but this can be overcome with intraoperative MRI (iMRI). In addition, iMRI provides near real-time imaging thus eliminating the risk of incomplete resections and re-operating. Complete CE lesion resection is achievable in nearly 96% of the patients with iMRI [143]. Compared to 5-ALA, iMRI is a superior surgical adjunct [144]. Multiple studies have found that combining iMRI with 5-ALA has an even higher rate of achieving GTR [145–147]. Intraoperative ultrasound is an inexpensive alternative to iMRI

with GTR rates of 77%; however, resection beyond the CE lesion is limited [148]. Although pre-operative tractography and functional MRI can aid with surgical planning, intraoperative cortical mapping (e.g., awake craniotomy) is pivotal for supramaximal resection of tumors in proximity to eloquent cortices [149–152].

## Radiotherapy

It was previously presumed that after surgery, any gross residual tumor and the surgical cavity remained relatively static throughout a 3- or 6-week course of RT. A relatively large expansion of 1.5 to 3 cm around the GTV, called the clinical target volume (CTV), is used to encompass microscopic disease not visible on MRI. However, recent data evaluated volume changes and migration distances of tumors during RT and reported shifts in tumor location of over 5 mm in half of the patients and over 10 mm in 20% of patients [153]. Others have found an increase in the size of GTV by 25% over RT, resulting in underdosing of the RT target [154–158]. Conversely, a reduction in the tumor volume or surgical cavity during RT may allow for a smaller margin but adaptive RT (whereby GTV and CTV volumes are adjusted based on updated imaging) reduces the toxicity by limiting RT dose to normal surrounding brain tissue. One form of MRI-guided Radiation Therapy uses an MR-Linac (Unity, Elekta) that combines a linear accelerator with an MRI that affords daily MRI just prior to RT delivery and allows for adaptation to interfraction changes. The UNITED study (UNItY-Based MR-Linac Guided AdapTive RadiothERapy for High GraDe Glioma; NCT04726397) is an ongoing phase II trial testing this approach in newly diagnosed GBM.

Protons have a unique depth-dose (energy-dependent) characteristic which leads to reduced dose deposition in normal tissue proximal and distal to the target volume. In addition, higher doses can theoretically be given safely, potentially improving local control and survival while at the same time reducing toxicity and improving quality of life [159]. Proton radiotherapy (PT) was compared to intensity-modulated radiotherapy (IMRT) in a small cohort phase II unblinded trial [160]. The primary endpoint, time to cognitive failure, was similar in both groups. Compared to IMRT, PT patients had less fatigue and lower rates of grade  $\geq 2$  toxicities but no difference in PFS was seen. The clinical benefit of PT, compared to standard photon RT, in GBM remains unclear.

## Updates in Systemic Therapies

There are over 100 systemic therapies currently under investigation for GBM [161]. Broad strategies include drug

repurposing, use of targeted therapies (to intrinsic GBM targets or microenvironmental targets), metabolic therapies, immunotherapies, and viral therapies. Several investigational agents utilize more than one of these strategies, and our review will focus on a subset of these therapies.

## Chemotherapy

While chronotherapy has been most extensively studied in other solid cancers, its role in GBM is emerging. Gene transcription [162], cell cycle regulation [163], metabolism [164], and DNA repair [165] are dynamic processes that fluctuate throughout the day and appear to be paced by the hypothalamic suprachiasmatic nucleus, known as the “circadian rhythm pacemaker.” Based on data from non-CNS tumors [166–168] and the particularly relevant findings of diurnal fluctuations in MGMT activity [169, 170], Dalmato et al. retrospectively analyzed OS in 166 patients according to the timing of TMZ (AM versus PM) [171]. AM dosing was associated with longer survival than PM dosing with a more pronounced effect in MGMT methylated patients. A phase II trial randomizing patients to either AM (before 10) or PM (after 8) (NCT02781792) is ongoing.

Microtubule-targeting agents (MTAs) disturb microtubule function resulting in mitotic arrest and cell death through the spindle assembly-checkpoint pathway. In addition, cells in mitotic arrest have a heightened sensitivity to radiotherapy [172, 173]. Avانبulin (BAL27862) is a synthetic microtubule-destabilizing drug with a similar binding site to colchicine [174] and is active in GBM stem-like cells resistant to other MTAs [175]. Lisavanbulin (BAL101553) is a water-soluble lysine prodrug of avانبulin. In GBM patient-derived xenograft models, it improved survival as a monotherapy and showed a synergistic effect with RT/TMZ independent of MGMT status [176]. Lisavanbulin has already entered phase I/IIa clinical trials for the treatment of non-CNS solid tumors [177]. A phase I trial to establish the maximally tolerated dose in unmethylated GBM in combination with RT is in progress (NCT03250299).

The formation of O<sup>6</sup>-methylguanine, if not removed by MGMT, has a cytotoxic effect facilitated by the DNA mismatch repair (MMR) pathway. However, 90% of TMZ methylation occurs at N<sup>7</sup>-guanine and N<sup>3</sup>-adenine sites which are efficiently repaired by the base excision (BER) pathway [178, 179]. VAL 083 (dianhydrogalactitol) is an orphan molecule that readily crosses the blood–brain barrier (BBB) and has a long half-life [180]. It is a bifunctional alkylating agent that introduces interstrand-crosslinks at the N<sup>7</sup>-guanine sites that are resistant to MGMT and induces cytotoxicity independent of the MMR pathway [181–184]. Dose-escalation data from a phase I/II clinical trial [185, 186] have shown that doses in the range of 30–40 mg/m<sup>2</sup>/day (IV for 3 days in a 21-day cycle) are well tolerated and active

in both methylated and unmethylated disease. Currently, it is being evaluated in the GBM AGILE trial (see later) in newly diagnosed methylated and unmethylated patients as well as in recurrent disease.

Focused ultrasound (FUS) causes intravenously administered microbubbles to oscillate in the applied low-energy ultrasound field, thereby increasing the BBB permeability by the shear stress mechanism. This modality is gaining interest because it can allow chemotherapeutic agents, especially those with a large molecular weight, to achieve higher concentrations in the tumor tissue [187]. The safety and feasibility of FUS were first established in a study of 5 patients with high-grade gliomas. The study revealed a 15 to 50% increase in enhancement on T1-WI that was durable up to 20 h and an increase of TMZ tissue concentration with sonification [188]. Multiple studies followed and confirmed the safety and feasibility of delivering different therapeutic agents in recurrent and newly diagnosed GBM with promising results regarding survival [189–193]. Multiple open-label phase I/II studies are currently ongoing to better characterize its safety and clinical efficacy.

## Targeted Therapies and Precision Oncology

With increase in access to sequencing panels at some institutions, and its value in diagnostic confirmation [194], targeted gene testing has become prevalent in clinical practice with some optimized workflows reporting final gene panels in less than 1 week from tissue biopsy [195]. In addition, some centers have the capacity of integrating whole-exome sequencing (WES) or whole-genome sequencing for deeper molecular profiling. While knowledge of the evolving molecular landscape in glioma has grown exponentially [196–198], targeted therapy options remain limited and have not significantly changed the overall outcome in GBM. A selected review of published trials investigating some intrinsic GBM targets is summarized below.

There have been a number of clinical trials with selection based on molecular biomarkers, the most notable of which is *EGFR* amplification or *EGFRvIII* mutation, which is commonly found in GBM. Strategies targeting *EGFR* have unfortunately fallen short, including the *EGFR* inhibitor erlotinib [199], the *EGFRvIII* targeted neoantigen peptide vaccine rindopepimut [200], and the antibody–drug conjugate (depatuximab mafotidin) [201–203]. Clinical trials investigating *EGFRvIII*-directed chimeric antigen receptor (CAR) T cells in recurrent GBM are currently ongoing [204, 205].

*TERT* promoter mutations are one of the most common alterations (~80%) [206], and targeting telomerase is an attractive strategy in GBM [207]. Inhibition of telomerase in vivo has been shown to reduce cell proliferation in GBM



xenografts [208]; however, effective translation to patients with GBM has so far been unsuccessful. Novel strategies could include the use of small molecules such as the purine nucleoside analogue 6-thio-2'-deoxyguanosine (6-thio-dG) to induce alteration of the structure of telomeres leading to cell death [209]. Eribulin, a mitotic inhibitor with activity against *TERT* promoter-mutant lines [210], may also represent another opportunity to target telomerase and is currently under investigation.

Targeting alterations in *FGFR* have also been of interest in GBM, where about 3% of tumors can harbor an oncogenic fusion with the coding domains of *TACC 1* or *TACC 3* [211]. The use of the *FGFR* inhibitor erdafitinib has been reported to inhibit glioma cells harboring *FGFR3-TACC3* fusion in vitro and in vivo and has shown some activity in two reported patients [212]. The pan-*FGFR* kinase inhibitor infigratinib is currently being investigated in an early-phase clinical trial in recurrent GBM (NCT04424966).

About 2% of GBM harbor a mutation in *BRAF V600E*. While previous strategies have included monotherapy with RAF inhibitors such as vemurafenib [213], with a modest response seen in high-grade gliomas, the ongoing ROAR trial (NCT02034110), a phase II open-label multicenter study now closed to accrual, investigated the role of dual BRAF/MEK with dabrafenib and trametinib in gliomas (WHO grade 1–4) harboring a *BRAF V600E* mutation. There was clinically meaningful activity seen in both low- and high-grade glioma patients (31 of which were GBM patients) with the latter showing a 33% objective response rate [59].

Another rare but druggable target in GBM is the alterations in the neurotrophic tyrosine receptor kinase (*NTRK*) genes. Mutations in *NTRK1*, *NTRK2*, and *NTRK3* have been described in solid tumors and genomic rearrangement and fusions trigger oncogenic signaling [214]. Larotrectinib has received tissue-agnostic FDA approval in 2018 for adult and pediatric solid tumors with *NTRK* gene fusions. However, *NTRK* fusions are most commonly found in pediatric gliomas and are present in molecularly diverse gliomas [215], and their incidence remains relatively low in adult GBM (~1%).

*MET* fusions occur in about 3% of gliomas [215]. These mutations have been targeted with the drug crizotinib in pediatric GBM [216]; however, significant response has not been observed in adults. Another target of interest in GBM is the PI3K/AKT/mTOR pathway; however, studies with the mTOR inhibitor CCL-779 [217] and the pan-*PI3K* inhibitor buparlisib [218] showed a lack of activity of both agents. Targets to the retinoblastoma (pRB) pathway have also been investigated, for example, a phase II trial of palbociclib in recurrent RB1-positive GBM [219] which did not show a survival benefit. A phase II trial of abemaciclib in patients with recurrent RB-wildtype GBM with *CDKN2A/2B*

activation or *CDK4* or *CDK6* amplification is currently accruing (NCT02981940).

Clinical trial designs exploring molecularly matched therapies include the open-label multicenter phase I/IIa trial N2M2 [220], which stratifies patients with MGMT unmethylated GBM to their respective targeted agents based on matching alterations. In addition, this trial randomizes patients without matching alterations among some of the treatment arms to aid in the development of predictive biomarkers. While targeting specific mutations is one proposed strategy, assessing other molecular biomarkers as potential predictors of response may be helpful. This is being explored in two different large-scale adaptive Bayesian multi-arm platform trials, the Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGHt) trial (NCT02977780) [221] and the global multi-arm phase II/III GBM Adaptive Global Innovative Learning Environment (GBM AGILE) trial (NCT03970447) [222]. In these designs, patients are initially randomized to multiple experimental arms with a targeted agent vs a control arm, and patient subtypes including biomarkers are identified with adaptive randomization based on cumulative real-time efficacy data. Table 1 summarizes the experimental drugs used in the beforementioned trials.

There are many barriers to the development of effective molecular therapies in GBM including intra- and inter-tumor molecular heterogeneity [57], redundant signaling pathways, and a lack of active and brain-penetrant targeted therapies [223]. Combinatorial approaches based on molecular biomarkers studies and/or the use of modalities aimed at increasing BBB penetration may represent future directions to fulfill the potential of targeted therapies.

## Immunotherapies

Tumor eradication through cancer immunotherapy has garnered great interest in GBM, and the immunosuppressive paradigm in GBM has been well described [224]. There are several challenges to the development of effective immunotherapies in GBM including a paucity of tumor infiltrating effector lymphocytes and their exhaustion, predominantly related to the immunosuppressive microenvironment induced by the tumoral cells' surface proteins and secreted cytokines. In addition, the immunosuppressive nature of standard therapies and the frequent use of corticosteroids further complicate the effectiveness of immunotherapies.

Immunotherapy in GBM has thus far been disappointing [225] but could be a result of a lack of proper patient selection, microenvironment inhibition or specific immunogenicity assessments [161]. Immune checkpoint inhibitors (ICIs) such as anti-programmed cell death protein-1 (PD-1)/programmed death ligand-1 (PD-L1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have been

**Table 1** Summary of the drugs used in the three ongoing trials for GBM as of 2022

Trial Overview	Adjuvant Experimental drugs	Mechanism of action
<b>N<sup>2</sup>M<sup>2</sup> (NOA-20; NCT03158389) [220]</b> Germany; multi-center, open-label, parallel group, nonrandomized phase I/IIa trial for newly diagnosed GBM <b>MGMT promoter:</b> Unmethylated <b>Drug assignment:</b> Molecular matching	Alectinib Idasanutlin Palbociclib (concurrent and adjuvant) Vismodegib Temsirolimus	Oral ALK inhibitor Oral MDM2 inhibitor Oral CDK4/6 inhibitor Oral SHH inhibitor IV mTOR inhibitor
<b>INSIGHt (NCT02977780) [221]</b> USA; multi-center, open-label, multi-arm, randomized phase II trial for newly diagnosed GBM <b>MGMT promoter:</b> unmethylated <b>Drug assignment:</b> adaptive randomization	Neratinib CC-115 (concurrent and adjuvant) Abemaciclib	Oral pan-ErbB tyrosine kinase inhibitor Oral mTOR and DNA-PK inhibitor; radiosensitizer Oral CDK4/6 inhibitor
<b>GBM AGILE (NCT03970447) [222]</b> Global; open-label, multi-arm, randomized phase II/III trial for newly diagnosed and recurrent GBM <b>MGMT promoter:</b> unmethylated and methylated <b>Drug assignment:</b> adaptive randomization	Regorafenib Paxalisib VAL-083 Troriluzole VT1021	Oral multikinase inhibitor Oral PI3K inhibitor IV Bi-functional alkylating agent Oral modulator of glutamate metabolism First-in-class compound that blocks the CD46 immune checkpoint and influences CD36 activity

*SHH* sonic hedgehog, *ALK* anaplastic lymphoma kinase, *MDM2* mouse double minute 2 homolog, *CDK* cyclin-dependent kinases, *mTOR* mechanistic target of rapamycin, *DNA-PK* DNA-dependent protein kinase, *ErbB* Erythroblastic leukemia viral oncogene homolog, *CD* cluster of differentiation, *PI3K* phosphoinositide 3-kinase

investigated in GBM. Unfortunately, these did not show any survival benefit in newly diagnosed [226] or recurrent GBM [226, 227]. Despite these discouraging results, there may be a role for ICI in the neoadjuvant setting or in combination with other therapies. For example, a study in which 35 patients with recurrent surgically resectable GBM were given pembrolizumab prior to and/or following surgical resection showed that neoadjuvant PD-1 blockade was associated with significantly longer overall survival [228]. Another subset of GBM patients who may benefit from ICI are patients with hypermutated tumors resulting from a germline DNA MMR deficiency [229, 230] as they may exhibit tumor-associated neoantigens that trigger an immune response. The genomic signatures associated with PD-1 response in pediatric patients with germline DNA replication repair deficiency including CNS tumors were recently described [231].

Neoantigen vaccines as a means to induce immune surveillance against glioma cells through vaccination are also being explored. Previous trials utilizing this strategy included the ACT IV study, where the addition of rindopepimut, a peptide vaccine targeting *EGFRvIII* to the standard of care chemoradiation, did not improve survival in newly diagnosed GBM [200]. There are many possible reasons for the failure of this approach, and other strategies have included multi-epitope personalized neoantigen GBM vaccines that were found to generate a neo-antigen specific T cell response

[232]. Personalized neoantigens derived from next-generation sequencing analyses of individual GBM transcriptomes and immunopeptidomes have therefore been studied in small patient cohorts with good feasibility data [233]. Larger trials are required using this approach and in combination with other immunotherapy strategies and a few are under way (NCT04015700, NCT02287428).

Viral therapies have been investigated in GBM in the form of oncolytic viruses (invading and/or replicating selectively in tumor cells) or gene therapy (delivery of anticancer cDNA into tumor cells) [234, 235]. Both strategies thereafter activate an immune response through cytotoxicity and the release of tumor antigens and are therefore theoretically further enhanced with the addition of ICIs. Examples of such approaches include the convection-enhanced delivery of an engineered poliovirus (PVSRIPO; NCT02986178) [236], the combination of gene-mediated cytotoxic immunotherapy (GMCI-AdV-Tk) with valacyclovir and chemoradiation in newly diagnosed GBM (NCT03576612) [237], and Toca 511, an intracavitary injection of a retrovirus that delivers a cytosine deaminase cDNA that provides chemosensitivity to 5-fluorocytosine (NCT02414165). The primary endpoint of the phase III Toca 5 trial was however not met as Toca 511 and Toca FC did not improve OS compared to standard therapy in recurrent high-grade gliomas [238].

CAR T cells are engineered T cells that express antigen-binding receptors that lead to target cell killing of tumor

cells. There are a number of current efforts in GBM to exploit this immunotherapy strategy [205, 239], including targeting multiple neoantigens, in combination with ICI or through direct intratumoral delivery.

Future directions in immunotherapy could include more personalized and precise approaches based on genomic or immunogenic biomarkers, neoadjuvant approaches, and a combination of immunotherapy with local therapies such as radiosurgery or local chemotherapy as well as multi-modality approaches (ICI in combination with vaccine therapy/viral therapy/cellular therapy) [240].

### Drug Repurposing

Repurposing approved drugs as cancer-directed therapies has been of great interest to the oncology community. Selected drugs that have been investigated in this context include antiepileptic medications, as up to 60% of patients with high-grade gliomas have seizures. Valproic acid (VPA) has histone deacetylase inhibitor activity at high concentrations in vitro and has been studied for its impact in GBM. While post hoc analysis of the Stupp trial data indicated longer survival for patients on VPA [241], this was not confirmed in a larger analysis [242] although smaller prospective studies indicate a potential benefit in younger patients [243]. Recently, two separate studies demonstrated that neuron-glioma synapses could be activated through the AMPA receptor contributing to glioma proliferation [244, 245]. In a study looking at four different anticonvulsants (levetiracetam, VPA, carbamazepine, and perampanel), only perampanel was found to have antitumoral effects [246]. Two clinical trials testing the AMPA receptor blocker talampanel showed contrasting results [247, 248] and larger randomized controlled trials targeting AMPA receptors are needed to explore the possible benefits of this strategy in GBM.

Another example of drug repurposing was the recent discovery in vitro that the highly CNS penetrant selective serotonin reuptake inhibitor antidepressant fluoxetine could inhibit sphingomyelin phosphodiesterase 1 resulting in dose-dependent GBM cell death through inhibition of *EGFR* signaling [249]. Clinical studies are however required to validate these findings in patients with GBM.

Anti-glycemic agents targeting the Warburg effect have been of interest in GBM [250]. The effect of metformin, in particular, has been investigated in pooled retrospective analyses of clinical trial data [251], with negative results.

Combinations of multiple approved drugs in specific “treatment protocols” such as CUSP9 (a combination of aprepitant, artesunate, auranofin, captopril, copper gluconate, disulfiram, ketoconazole, nelfinavir, sertraline) have also been used anecdotally [252] but beyond in vitro studies [253] remain of questionable efficacy and are being further validated in an ongoing clinical trial [254].

In the future, drug repurposing could be made more efficient by machine learning and artificial intelligence strategies [255] to identify matches based on large data repositories and therefore accelerate validation of preliminary data through GBM-specific clinical trials.

### Future Directions

The history of neuro-oncology has not been kind to recurrent glioblastoma. Our field has failed to develop a meaningful improvement in therapy beyond older alkylating agents, which in themselves may have little to no activity. Some two to three decades ago, the failure of phase III clinical trials was felt to be the result of selection bias in earlier studies. Indeed, even well-known prognostic factors were poorly accounted for in phase I and II trials, leading to over-estimation of clinical efficacy, and failure in later randomized trials. As a next step, learning from the significant influence of selection bias, attempts were made to use well-matched historical controls in phase II work. Even relatively recent negative phase III studies such as the CENTRIC study [256] were based upon promising non-randomized data. These well-intentioned efforts consumed large financial and patient resources with a drug development cycle approximately a decade long from concept to drug failure. Advances in imaging, surgery, and radiation delivery likely account for the modest shift in the overall survival seen in completed phase III studies; for example, the historical median survival of 14.3 months in the pivotal Stupp study has shifted now to 18 months in more recent trials [257]. In many instances, the use of historical controls has not adapted well and often the older survival data are still inappropriately used as comparators.

Such shortcomings in clinical trial design and therapeutic evaluation should now be behind us, and the stage is ready for more rapid drug discovery in “window of opportunity” studies and adaptive platform clinical trial designs. It remains implausible to think that one single targeted therapy will move the needle in the overall survival from recurrent GBM [258]; but our field has made little effort to understand whether specifically defined subgroups of patients may demonstrate benefit despite negative phase III results. The proverbial “throwing out the baby with the bathwater” approach to failed clinical trials should not be an acceptable outcome of these efforts. Clinicians, together with our patients, would highly value an effective treatment, even if it only worked in a fraction of all cases (assuming that fraction can be identified a priori). We need to encourage the drug development industry to pay attention to these small victories. While a fractional share of a rare disease provides little commercial incentive, we feel that the most likely way forward is a series of cumulative successes in clinical trials enriched for patients most likely to respond to novel therapies.

Having said that, it is noteworthy that not only the quantity but also the “quality” of the diseased patients influences the research question of clinical trials. Inequalities and inequities spanning the spectrum of social determinants of health such as race, ethnicity, and socioeconomic status [259, 260] are front and center targets that stakeholders should address to ensure equitable access [261]. Telehealth modalities are quickly taking over the post-pandemic healthcare scene and these may provide the ultimate solution for the inclusion of the abovementioned population. Thus, contextualizing patient care into clinical trials can be easily and cost-effectively secured.

## Conclusions

The most significant recent advances in GBM have been in our understanding of the molecular pathways, oncogenic drivers, and tumor microenvironment distinguishing these tumors from other CNS neoplasms. Future clinical trials leveraging this newly gained knowledge and correlative scientific efforts to understand patterns of response and resistance will hopefully translate into improved outcomes for this population.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s13311-022-01251-6>.

**Acknowledgements** We would like to thank Aimee Chan for her editorial assistance.

**Required Author Forms** [Disclosure forms](#) provided by the authors are available with the online version of this article.

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