




REVIEW

Current status of precision oncology in adult glioblastoma

Johannes Weller¹, Anna-Laura Potthoff² , Thomas Zeyen¹, Christina Schaub¹, Cathrina Duffy¹, Matthias Schneider²  and Ulrich Herrlinger¹ 

¹ Department of Neurooncology, Center for Neurology, University Hospital Bonn, Germany

² Department of Neurosurgery, University Hospital Bonn, Germany

Keywords

biomarker; glioblastoma; molecular profiling; personalized medicine; precision oncology; targeted treatment

Correspondence

U. Herrlinger, Department of Neurooncology, Center for Neurology, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany
Tel: +49 228 287 31241
E-mail: ulrich.herrlinger@ukbonn.de

(Received 16 November 2023, revised 5 April 2024, accepted 28 May 2024, available online 20 June 2024)

doi:10.1002/1878-0261.13678

The concept of precision oncology, the application of targeted drugs based on comprehensive molecular profiling, has revolutionized treatment strategies in oncology. This review summarizes the current status of precision oncology in glioblastoma (GBM), the most common and aggressive primary brain tumor in adults with a median survival below 2 years. Targeted treatments without prior target verification have consistently failed. Patients with BRAF V600E-mutated GBM benefit from BRAF/MEK-inhibition, whereas targeting EGFR alterations was unsuccessful due to poor tumor penetration, tumor cell heterogeneity, and pathway redundancies. Systematic screening for actionable molecular alterations resulted in low rates (< 10%) of targeted treatments. Efficacy was observed in one-third and currently appears to be limited to BRAF-, VEGFR-, and mTOR-directed treatments. Advancing precision oncology for GBM requires consideration of pathways instead of single alterations, new trial concepts enabling rapid and adaptive drug evaluation, a focus on drugs with sufficient bioavailability in the CNS, and the extension of target discovery and validation to the tumor microenvironment, tumor cell networks, and their interaction with immune cells and neurons.

1. Introduction

In recent years, there has been a rapidly expanding amount of information on the molecular vulnerabilities of cancer cells, informing the development and application of targeted drugs. Actionable targets may comprise the

genomic, genetic, epigenetic, transcriptional, proteomic, and metabolomic properties of tumor cells and may vary from one individual tumor to the next. Early studies of targeted treatment determined target engagement and

Abbreviations

ALK, anaplastic lymphoma kinase; AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CAR, chimeric antigen receptor; CCNU, lomustine; CD95L, CD95 ligand; CDK4/6, cyclin-dependent kinase 4/6; CDKN2A, cyclin-dependent kinase inhibitor 2A; CNS, central nervous system; CSF-R1, colony-stimulating factor 1 receptor; CXCL12/SDF1, CXC chemokine ligand 12/stromal cell derived factor 1; CXCR4, CXC chemokine receptor 4; EGFR, epidermal growth factor receptor; EGFRvIII, EGFR variant III; EV, extracellular vesicle; FGFR, fibroblast growth factor receptor; FLT, FMS-like tyrosine kinase, member of VEGFR family; GBM, glioblastoma; HER2, human epidermal growth factor receptor 2; HGF/SF, hepatocyte growth factor/scatter factor; IDH, isocitrate dehydrogenase; IL13R, interleukin 13 receptor; IL4R, interleukin 4 receptor; MDM2, mouse double minute 2 homolog; MDM4, mouse double minute 4 homolog; MEK, mitogen-activated protein kinase kinase; MGMT, O⁶-methylguanine-DNA-methyltransferase; mTOR, mammalian target of rapamycin; NF1, neurofibromin 1; NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer; NTRK, neurotrophic tropomyosin receptor kinase; ORR, objective response rate; OS, overall survival; PARP, polyADP-ribose-polymerase enzymes; PD-1, programmed cell death protein 1; PDGFR, platelet-derived growth factor receptor; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PFS2/PFS1, ratio of 2nd PFS/1st PFS; PI3K, phosphatidylinositol 3-kinase; pSTAT3, phosphorylated signal transducer and activator of transcription 3; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; TGF β , transforming growth factor β ; TKI, tyrosine kinase inhibitor; TME, tumor-associated microenvironment; TMZ, temozolomide; VEGFR, vascular endothelial growth factor receptor.

therapeutic efficacy in *post hoc* subgroup analyses of larger non-selective and target-agnostic trials. Precision oncology goes in the opposite way, analyzing an individual tumor to inform treatment tailored to its biological characteristics [1,2]. Target detection is a prerequisite, but the decision to apply a drug to an individual patient also requires antitumor efficacy based on – at minimum – biological plausibility, key insights from preclinical research, or clinical data from other tumor types or optimally the same tumor entity harboring the targeted vulnerability. Several classifications formalize the grading of clinical significance and actionability of molecular targets [3–5].

Recent clinical trials, including molecularly targeted agents and immunotherapies, have achieved unprecedented survival in solid cancers enriched with specific molecular alterations. The cancer types that have benefited the most are molecularly defined subgroups of melanoma, non-small cell lung cancer (NSCLC), and breast cancer. In melanoma, high response rates (> 60%) and prolonged progression-free survival (PFS) and overall survival (OS) were achieved [6–10]. In NSCLC, targeted treatment based on defined molecular alterations became the standard of care for first-line therapy [11]. In breast cancer, targeted treatment is also well established, and several compounds are available and sequentially applied [12–17]. Targeted treatment is increasingly established for other cancer entities [18–22]. Of note, genetic tumor cell vulnerabilities are not restricted to a single cancer entity but may occur in many different entities, albeit at a low percentage. This opens the door for the application of drugs registered for one entity to be used for tumors from other cancer entities that harbor the actionable alteration, e.g. in NSCLC and gliomas [23,24]. Accordingly, entity-agnostic clinical trials and drug registrations focusing on the presence of a particular alteration have been initiated [25]. However, cross-entity comparisons illustrate that the response to a certain compound may vary significantly, owing to the differential activation of concurrent pathways, the occurrence of different mutations, and the differing tissue permeability for the respective drug [26].

This review explores the current status and perspective of precision oncology in isocitrate dehydrogenase (IDH) wildtype glioblastoma (GBM), the most common and aggressive malignant CNS tumor in adults.

2. GBM therapy: current status and potential therapeutic targets

2.1. Current standard of care in the newly diagnosed and progressive setting

Maximum-safe tumor resection [27], radiotherapy of the tumor region [28,29], and concomitant and

adjuvant temozolomide (TMZ) [29], an alkylating agent with high CNS penetrance and bioavailability, represent the current standard of care for first-line GBM treatment [30]. Tumor treating fields applying alternating electrical fields through the scalp are an additional treatment option [31]. Furthermore, nitrosoureas such as lomustine (CCNU), also an alkylating agent with similarly favorable CNS penetrance, are available for first- or further-line treatment [32,33]. In some countries, the anti-vascular endothelial growth factor (VEGF) A antibody bevacizumab is used as a non-selective second-line therapy, although it prolongs only PFS but not OS [34]. The portfolio of further line therapies is enriched only by re-resection [35], re-irradiation [36], and possibly the multi-kinase inhibitor regorafenib (see Section 3.1 [37]), while the aggressive course of disease limits the time for further treatment lines. Thus, GBM patients need new and effective treatment options that should ideally be guided by predictive molecular characteristics to ensure that medications with a high likelihood of effectiveness are applied.

2.2. Molecular predictors of therapeutic benefit from alkylating chemotherapy

O⁶-Methylguanine-DNA-methyltransferase (MGMT) is a DNA repair enzyme that confers resistance to alkylating chemotherapy. Its expression is mainly regulated by epigenetic modification, and the *MGMT* gene promoter methylation status was introduced more than 15 years ago as a prognostic and predictive factor strongly associated with benefit from TMZ and CCNU [38,39]. Patients with an *MGMT* promoter-methylated (MGMT-methylated) GBM receiving TMZ had a significantly longer median OS of up to 31.4 months [33,39–41] compared to approximately 17 months in patients without *MGMT* promoter methylation (MGMT-unmethylated) [42]. As some benefit from TMZ cannot be excluded in patients with an unmethylated *MGMT* promoter [39], it is accepted that the *MGMT* promoter methylation status is not a prerequisite for applying TMZ in GBM first-line treatment. This notion has some notable exceptions, indicating the first step toward a molecularly informed treatment of GBM. First, in patients > 65 years with an *MGMT*-unmethylated GBM, TMZ showed just under no significant benefit ($P = 0.055$), and therefore, radiotherapy alone is a valid therapeutic option in this subgroup [43]. The low activity of TMZ in patients with *MGMT*-unmethylated GBM prompted selective clinical trials for this subgroup, allowing the comparison of experimental treatment arms to placebo without

TMZ in the standard arm [42,44]. Second, the CeTeG/NOA-09 phase III trial demonstrated an increased median OS of 48.1 months in patients with *MGMT*-methylated GBM receiving CCNU/TMZ, rendering this combined chemotherapy a treatment option selectively for patients belonging to this molecularly defined subgroup [33].

Beyond *MGMT* gene promoter methylation analysis, the subclassification of brain tumors in general and GBM in particular into DNA methylation-based subgroups has spawned hopes to identify further treatment-guiding predictive patterns [45]. The RTK II subgroup is enriched for epidermal growth factor receptor (EGFR) amplification and chromosome 10 loss, which corresponds to the ‘classic’ gene expression subtype described by Verhaak et al. [46]. The RTK I subgroup is characterized by platelet-derived growth factor (PDGFR) A amplification, corresponding to the ‘proneural’ expression subtype. The MES subgroup (‘mesenchymal’ expression subtype) frequently bears NF1 and PTEN alterations [46–48]. Comparing radiotherapy versus TMZ in elderly GBM patients, the NOA-08 trial found the prognostic impact of *MGMT* promoter methylation status was limited to GBM of the RTK II subgroup and was absent in the RTK I and MES subgroups.

In addition, a biosimulation study predicted individual differential responses to CCNU/TMZ and TMZ treatments [49]. These results require prospective validation and are met with skepticism because, despite a common genetic background, GBM cell states show considerable plasticity [50,51].

2.3. Genetic vulnerabilities and potential treatment targets in GBM

The landscape of genetic alterations in GBM is well known, and clinically annotated expression and mutation data are readily available from TCGA and other data repositories [46,52]. As shown in Fig. 1, genetic alterations in GBM frequently involve:

- 1 Alterations in growth factor receptors/receptor tyrosine kinases (RTK). The most frequent example is EGFR, altered by approximately 60% [52]. This includes amplification in about 40% [52,53], frequently associated with other alterations such as EGFR mutations or deletions, the most important being the EGFR variant III (EGFRvIII). While PDGFR is also frequently altered (10–15%), further alterations, including fibroblast growth factor receptor (FGFR, 2–5%), anaplastic lymphoma kinase (ALK), ROS-1, RET, c-Met, and neurotrophic

tropomyosin receptor kinase (NTRK) 1–3 alterations, are rare [52]. In the case of c-Met (1–4%; [54,55]), FGFR3 (3%; [52,55,56]), and NTRK1-3 (1–2%; [55,57]), alterations occur mostly in the form of gene fusions, which are also found for EGFR in 6–13% of patients [52,55,57].

- 2 Downstream signal transduction cascades induced by RTK activation also frequently bear alterations. This particularly applies to the PI3K/PTEN/AKT/mTOR (phosphatidylinositol 3-kinase/phosphatase and tensin homolog/AKT/mammalian target of rapamycin) pathway. Taking RTK, PI3K (25–30%, mainly PIK3CA or PIK3R1 alterations), and PTEN alterations (40%) together, at least one of these alterations is found in 90% of GBM [52]. Neurofibromin 1 (NF1) mutations activating the PI3K pathway by reducing its RAS-inhibiting effect have been found in 10% of cases [52].
- 3 Genes encoding cell cycle proteins are frequently altered, such as cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) deletion (60%) controlling cyclin-dependent kinase 4/6 (CDK4/6), p53 mutation (20–25%), p53-inhibiting amplifications of mouse double minute 2 homolog (MDM2) and MDM4 (15%), and RB1 mutation or deletion (8%; mutually exclusive with CDKN2A deletion). At least one of these genes is altered in 90% of GBM [52,58].
- 4 Further alterations include DNA repair mechanisms such as mismatch repair deficiency in about 10% of progressive GBM, mostly due to MSH6 loss, and alterations of homologous repair deficiency, DNA checkpoint, and base excision repair [49,59–61].

Illustrating the growing drug development pipeline in GBM, all of the aforementioned targets or their associated pathways are currently being investigated as potential treatment options [62,63]. Despite this well characterized landscape of actionable treatment targets, the identification of successful targeted treatments for GBM remains challenging. In contrast to IDH mutant astrocytoma, where IDH mutation is thought to occur early in gliomagenesis, there is no known early – and thus major – single-driver alteration in GBM [46,64,65]. Further, GBM displays significant cellular and spatial heterogeneity, and potential targets may not be present in most tumor cells [50,66]. The possibility of longitudinal heterogeneity represents another challenge; it was suggested that molecular targets profoundly change between the newly diagnosed and progressive disease [67,68], whereas prominent publications reported no substantial longitudinal changes in genetic alteration profiles [51,69,70]. Besides

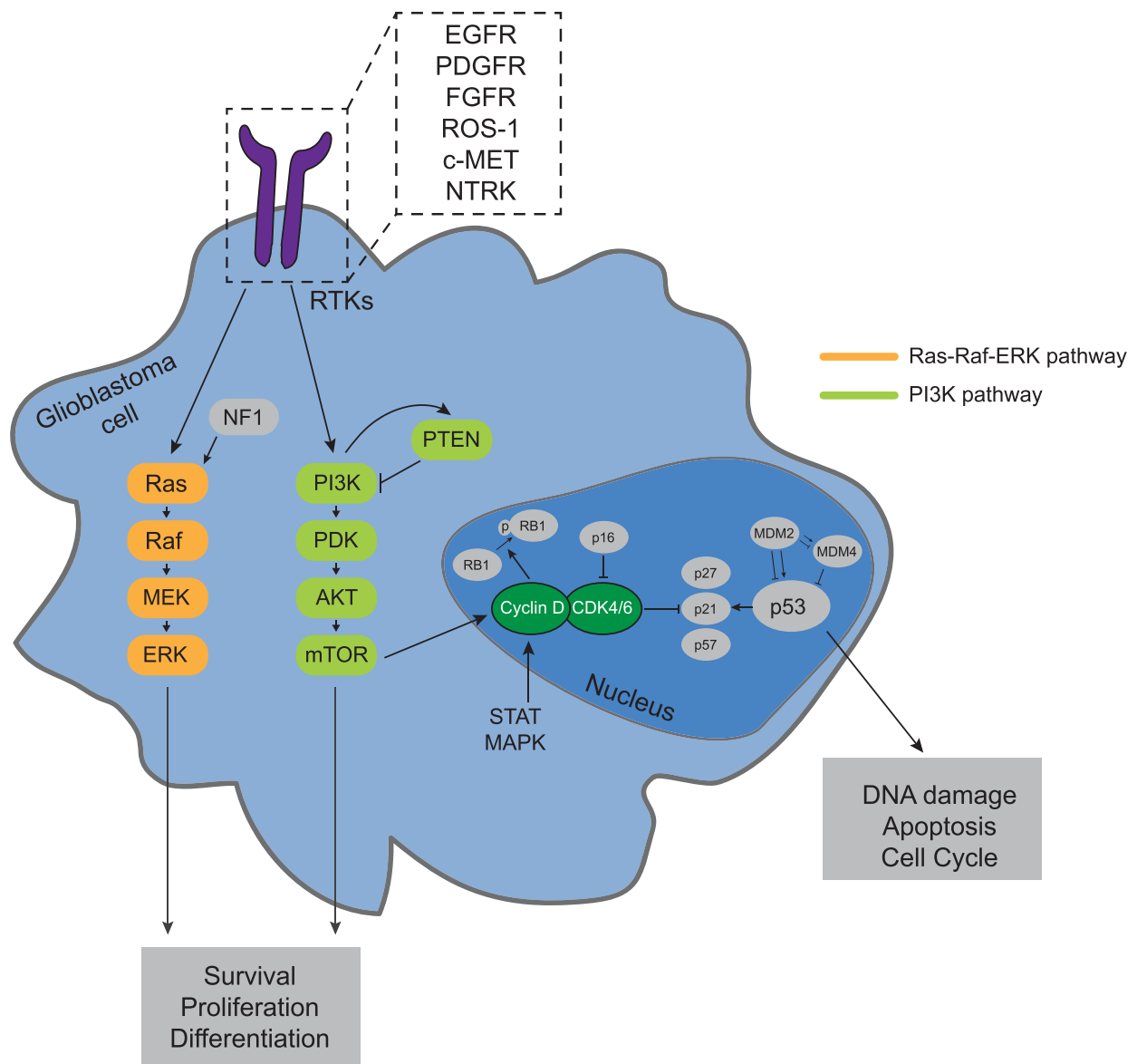


Fig. 1. Frequent targetable alterations in adult IDH wildtype glioblastoma. This figure illustrates a selection of currently targetable frequent molecular alterations in glioblastoma. Receptor tyrosine kinases (RTKs) are among the most frequent alterations. Following RTK activation, downstream signal transduction involves the phosphatidylinositol 3-kinase (PI3K) pathway with frequent PI3K or phosphatase and tensin homolog (PTEN) alterations, and the Ras pathway with frequent activation via neurofibromin 1 (NF1) mutations. Cell cycle dysregulation involving cyclin-dependent kinase pathway alterations occurs in 90% of glioblastoma, frequently involving p16 (encoded by cyclin-dependent kinase inhibitor 2A/B [CDKN2A/B]), cyclin-dependent kinase 4/6 (CDK4/6), p52, mouse double minute 2/4 homolog (MDM2/4), or RB1. For detailed explanations, refer to Section 2.3 in the main text. AKT, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; IDH, isocitrate dehydrogenase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NTRK, neurotropic tropomyosin receptor kinase; PDGFR, platelet-derived growth factor receptor; PDK, 3'-phosphoinositide-dependent kinase; STAT, signal transducer and activator of transcription.

the genetic profile, expression patterns of GBM (e.g. EGFR expression) and, in particular, cells of the tumor microenvironment may substantially change

over time and have a vast influence on the composition of the tumor tissue and amenability to therapy, further introducing complexity [51].

3. Targeted treatment in untargeted study populations

3.1. GBM trials with targeted agents

In the last 20 years, targeted drugs have been applied to GBM patients without individual prior target verification, and they have been mostly explored in patients with progressive or recurring tumors following one or more treatment lines. Table 1 provides an overview of drugs, targets, and observed outcomes, focusing on GBM hallmark alterations such as EGFR, PDGFR, FGFR, c-MET, and the PIK3CA/Akt/mTOR pathway (see also Fig. 1). Beyond this, many drugs directed at less GBM-specific targets involved in tumor cell growth and/or homeostasis have been evaluated, e.g. transforming growth factor β (TGF β)-directed galunisertib [71], CD95 ligand (CD95L)-directed APG101 [72], Src-directed dasatinib [73–75], phosphorylated signal transducer and activator of transcription 3 (pSTAT3)-directed therapy [76], hepatocyte growth factor/scatter factor (HGF/SF)-directed rilotumumab [77], and proteasome inhibitors such as marizomib [78]. In addition, there have been several approaches to target receptors thought to be mostly expressed in tumor cells (without individual confirmation beforehand) with locally applied ligand-toxin fusion proteins targeting, e.g. interleukin 13-receptor (IL13R), interleukin 4-receptor (IL4R), or transferrin receptors [79–81].

In summary, these approaches failed to achieve convincing results in adult malignant glioma cohorts. Still, some trials performed *post-hoc* secondary explorative analyses to identify biomarkers for treatment benefit [44,72], thus justifying the use of the mTOR inhibitor temsirolimus in the ongoing N2M2 trial and indicating a PFS-prolonging effect of the CD95L-inhibitor APG101 [72,82]. In retrospect, the failure of trials without prior target verification, and therefore without enrichment of tumors harboring the targeted alteration, comes as no surprise. Thus, targeted agents must be tested in cohorts preselected for the presence of the targeted genetic alterations.

The only exceptions are multi-kinase inhibitors with a broad spectrum of therapeutic targets, which enable studies in unselected cohorts. Regorafenib, a multi-kinase inhibitor targeting VEGFR1-3, TIE2, PDGFR- β , FGFR, KIT, RET, and RAF, increased OS in the progressive setting in the randomized phase II REGOMA trial [37]. While questions remain as to the extent to which these positive results rely on the VEGF-directed antiangiogenic effect [34,42] and the results have recently been challenged [83], some markers of therapeutic benefit have emerged in

explorative analyses. More specifically, the occurrence of a hand-foot reaction, a common side effect observed in approx. 30% of patients receiving regorafenib, was associated with an increased OS of 6.7 versus 2.6 months in a small retrospective bicentric cohort of patients with progressive glioblastoma receiving regorafenib, and a biomarker analysis of the REGOMA trial described the expression levels of several mRNAs and miRNAs to be associated with survival [84,85].

4. Targeted treatment with previous target verification

4.1. Successful trials with molecularly matched drugs in glioma patients

There are a few success stories emphasizing that targeted therapy may show efficacy in molecularly selected glioma subpopulations. In patients with tuberous sclerosis, treatment with the mTOR inhibitor everolimus for subependymal giant cell astrocytomas with alterations in the mTOR pathway is well established and leads to tumor reduction of $\geq 30\%$ in 75% of patients [86]. More recently, the IDH inhibitor vorasidenib increased PFS from 11.1 to 27.7 months in IDH-mutant grade 2 glioma and allowed for significantly delayed further interventions (likelihood of next treatment intervention or death by 24 months, 16.6% vs. 73%) [87].

In GBM, the only successful molecularly matched treatment to date is combined BRAF/MEK inhibition in patients with a constitutively activated MAPK pathway due to a BRAF V600E mutation. An interim analysis of the single-arm phase 2 ROAR basket trial exploring this approach in BRAF V600E-mutated progressive GBM with the BRAF inhibitor dabrafenib and the MEK1/2 (the downstream target of BRAF) inhibitor trametinib showed an objective response rate (ORR, complete or partial response according to RANO criteria) of 32% and a PFS of 2.8 and an OS of 13.7 months [24]. Consequently, EANO guidelines conclude that the clinical benefit in patients with BRAF V600E mutant progressive CNS tumors is sufficiently well established to consider it part of the standard of care [88].

4.2. Entity-agnostic drug registrations for patients with NTRK gene fusions or microsatellite instability/mismatch repair deficiency

While biomarker-specific drugs are usually marketed for specific cancer types, two drugs received an entity-

Table 1. Selection of clinical trials with targeted therapy in adult GBM without molecular pretesting.

Target	Drug	Trials	Major outcomes	Comments	Ref.
EGFR overexpression EGFR amplification EGFR mut EGFR vIII mut	Erlotinib	4 Phase (I)/II, (1 rand., n = 110)	rGBM: ORR 3.7–8.4%, PFS6 ≤ 20%, mOS 7.3–9.7 months First line (+RT/TMZ): mOS 19.3 months rGBM PFS6 13%, ORR 0%, mOS 9 months fGBM mOS 11.5 months rGBM: PF6 3–17.6%, ORR 0%	No survival signal in rand. trial No impact on downstream signaling in PD/PK trial Insufficient activity Target engagement but no impact on downstream signaling No sufficient activity, tumor concentration 497 nM Target engagement but no impact on downstream signaling	[110,185–187]
	Gefitinib	3 Phase (I)/rand. II			[109,188,189]
	Lapatinib	2 Phase II			[190,191]
	Neratinib	Phase II rand.	fGBM: mPFS 6.0 (vs. 4.7) months, mOS 13.8 (vs. 14.7) months	No significant survival effect; in subgroup analysis of EGFR-activated patients mPFS prolonged (6.3 vs. 4.6 months, P = 0.04) but no OS effect	[113,192]
	Afatinib (Afa)	Phase II rand. (Afa vs. TMZ vs. TMZ/Afa) Phase III rand. ^a (n = 142)	rGBM: ORR, mPFS, mOS in Afa arms not superior to TMZmono ndGBM: no significant difference for PFS12, mPFS and mOS	No sign of activity	[193]
	Nimotuzumab	Phase II (n = 55)	Relapsed high-grade glioma, mPFS 1.8–1.9 months, mOS 4.8–5 months ndGBM/rGBM: mPFS < 3 months, mOS 5–6.5 months	Limited activity, no correlation of survival with EGFR status No measurably activity	[194]
PDGFR overexpression PDGFR amplification	Cetuximab	Phase II rand, +/- hypofract RT (n = 59) Phase II (n = 231, all + Hydroxyurea; 1st relapse)	rGBM, PFS6 11%, mOS 20 weeks	No clinically meaningful activity	[195]
	Imatinib	Phase II (n = 231, all + Hydroxyurea; 1st relapse)			[196]
	Imatinib	Phase II (n = 231, all + Hydroxyurea; 1st relapse)			[197]
ALK/ROS1	Ceritinib	Phase 0 (n = 7)	rGBM, low drug levels without target engagement	CNS drug levels insufficient for target inhibition	[198]
FGFR c-MET	Nintedanib	Phase II (n = 25)	No CR/PR, mPFS 1 months	No clinically relevant activity	[199]
	Cabozantinib (also VEGFR2 inhibitor)	Phase II (n = 152) no prev. anti-VEGF-treatment (n = 70 with antiangiolo ther.) Phase Ib (n = 38 first-line)	ORR 17.6% ORR 4.3%	Statistical target not met Modest activity	[200] [201]
	Crizotinib (also ALK/ROS1 inhibitor)	Phase Ib (n = 38 first-line)	mPFS 10.7 months mOS 22.7 months	Regarded as promising, but CNS penetration inferior to other ALK inhibitors	[202]
mTOR	Temsirolimus	Phase II rand vs. TMZ (n = 257 MGMT unmethylated)	OS1y 72% vs 70% (ns)	Phospho-mTORSer2448 detected in 38%, associated with temsirolimus benefit	[44]

Table 1. (Continued).

Target	Drug	Trials	Major outcomes	Comments	Ref.
PIK3CA	Everolimus	Phase II rand, primary (171 RT +/- Ever)	ndGBM, mPFS similar, mOS worse (16.5 vs. 21.2 months)	No sign of activity	[203]
	Paxalisib	Phase II (n = 30)	rGBM, mPFS 8.4 months, mOS 15.7 months	Encouraging survival	[204]
PARP	Buparlisib	Phase II (n = 15)	PFS6 8%, mPFS 1.7 months	Insufficient activity ^b , incomplete pathway blockade despite BBB penetration	[205]
	Veliparib	Phase II, rando 2:1 (n = 125)	ndGBM, mOS 12.7 vs. 12.8 months	Insufficient clinical benefit	[206]
SMO	ABTC-0904	Phase 0/II (n = 40)	PFS6 2.4% mPFS 2.3 months mOS 7.8 months	Insufficient activity ^c	[207]

^aAdditional randomized trial comprising mainly anaplastic astrocytoma [208].

^bSimilar lack of activity in combination with chemotherapy [209].

^cSignificantly more CD133-producing neurospheres from tumors after ABTC-0904 than from tumors of untreated patients.

agnostic registration: larotrectinib, a tropomyosin kinase receptor inhibitor for tumors bearing NTRK alterations, and the programmed cell death protein 1 (PD-1) inhibitor pembrolizumab for tumors with microsatellite instability/mismatch repair deficiency. Data are accumulating that in childhood gliomas, larotrectinib addressing NTRK alterations (mostly NTRK fusion transcripts) may induce a high rate of responses [89], but reports on adult GBM patients remain anecdotal, with the largest series reporting disease stabilization (> 6 months) in 4 of 6 patients (Table 2) [90]. A larger series will hopefully provide more reliable information on the efficacy of larotrectinib in this setting. The case is even more challenging for pembrolizumab in patients with microsatellite instability/mismatch repair-deficient tumors. There are currently no strong data supporting this concept in GBM, as the clinical trial supporting pembrolizumab treatment did not include any GBM patients [91]. Of note, untargeted PD-1 immune checkpoint inhibition with nivolumab has been extensively studied in newly diagnosed glioblastoma, but failed to prolong survival in large phase 3 trials in *MGMT*-unmethylated as well as *MGMT*-methylated newly diagnosed GBM patients [41,92]. Also, the trial investigating nivolumab at the first relapse of GBM did not show any survival prolongation [93].

4.3. Lessons learned from unsuccessful trials with molecularly matched drugs

Further trials evaluating targeted treatments in GBM patients with target verification have shown no convincing efficacy data so far (Table 2). This applies to negative data in randomized phase 3 trials investigating depatuxizumab mafodotin, an antibody-drug conjugate composed of an anti-EGFR antibody conjugated to a tubulin inhibitor, which found no OS improvement in newly diagnosed GBM with confirmed EGFR-amplification (18.9 vs. 18.7 months, PFS 8.0 vs. 6.3 months), or rindopepimut, an EGFRvIII-specific peptide vaccine, which showed no benefit in newly diagnosed EGFRvIII-positive GBM (OS 20.1 vs. 20.0 months, PFS 7.1 vs. 5.6 months) [94,95]. For single-arm trials, an ORR of 25%, which translates to a median OS of 15 months [96], or surpassing a 6-month PFS rate of 16–20% as observed with CCNU [34,97], is generally expected for an effective second- or later-line treatment. Examples of single-arm trials not reaching this threshold are given in Table 2 and include targeting of EGFR, CDK4/6, and amplified c-MET.

The multifaceted problem of achieving efficacy in trials with molecularly matched treatments is

Table 2. Trials and case series with targeted therapy in molecularly pretested cohorts of GBM and high-grade glioma.

Drug	Required target	Trial/case series	Major outcome	Ref.
Dabrafenib/Trametinib	BRAF V600E	Phase II, $n = 45$ high-grade glioma (31 GBM)	12/45 PR 3/45 CR	[24]
Larotrectinib	NTRK gene fusions	$n = 33$ glioma (6 high-grade adult glioma)	4/6 with some tumor shrinkage 4/6 with treatment duration of 6 months or more (2/6 > 9 months)	[90]
EGFR-TKIs Dacomitinib	EGFR amp	Phase II trial, $n = 49$ rGBM (incl. 19 with EGFRvIII)	EGFRamp only: ORR 6.6%, PFS6 13.3%, mOS 7.8 months EGFRamp + EGFRvIII: ORR 5.3%, PFS6 5.9%, mOS 6.7 months	[210]
Osimertinib	EGFR amp, p53wt	Phase II trial, $n = 56$ rGBM, BEV naïve Phase II, $n = 12$ rGBM	ORR 3%, PFS6 17%, mOS 10 months ORR 0%, PFS6 0%, mOS 5.5 months	[211] [210] ^a
EGFR biologicals (selection) Depatuxizumab mafadotin (ABT414)	EGFR amp	Phase II, $n = 260$, rGBM, rand: TMZ, ABT414, TMZ + ABT414 Phase III, $n = 639$ rGBM, TMZ + ABT414 vs. TMZ + placebo	TMZ + ABT414 vs. TMZ HR 0.71, $P = 0.06$; ABT414 vs. TMZ HR 1.0, $P = 0.83$ No improvement of OS (HR 1.02; $P = 0.63$); PFS improved with ABT414 (8 vs. 6.3 months, $P = 0.029$)	[128] [94] ^b
EGFRvIII CAR T	EGFRvIII	Phase I, $n = 10$ rGBM Phase I, $n = 18$ rGBM Phase II, rand, $n = 73$ rGBM, BEV +/- CDX110	mOS ~ 7.1 months PFS6 28% vs. 16% ($P = 0.12$)	[212] [213] [214]
Rindopepimut (EGFRvIII vaccine CDX-110)	EGFRvIII	Phase III, rand, $n = 745$ ndGBM, Standard +/- CDX110	mOS 20.1 vs. 20.0 months	[95]
Palbociclib	CDK4/6, RB1 proficiency	Phase II, $n = 22$ rGBM	mPFS 5.1 weeks, mOS 15.4 weeks; stopped for futility	[116]
Capmatinib	PTEN loss/mut + c-Met amplification (FISH)	Phase II, $n = 10$ rGBM	No response; 3/10 stable disease for 16–20 weeks, stopped for futility	[215]

^aAdditional single cases and a retrospective case series of $n = 15$ rGBM treated with osimertinib and bevacizumab; pPFS 5.1 months; mOS 9 months; efficacy similar to BEVmono [216,217].

^bIn patients with EGFRvIII mutant (HR 0.72, $P = 0.002$) or *MGMT*-unmethylated tumors (HR 0.77, $P = 0.012$), PFS may be prolonged with ABT414, but no OS improvement.

highlighted by the largely unsuccessful therapy of EGFR-altered GBM despite previous target identification (EGFR amplification in 54%; [98]), which has taught many lessons in this regard:

1 Successful EGFR-directed therapy in NSCLC is applied in the context of mutations in the tyrosine kinase domain, which activate the receptor. In contrast, EGFR alterations in GBM mainly affect the extracellular domain, and multiple different oncogenic EGFR variants (mostly deletions and missense mutations) typically coexist and are not homogeneously distributed [26]. Mechanistically, EGFR alterations in GBM seem to alter ligand discrimination [99], suggesting alternative mechanisms in

response to EGFR-directed therapy in GBM compared to NSCLC (for review, see [26]).

2 EGFR alterations in GBM may not confer oncologic addiction, as they are considered late events in gliomagenesis and are subclonal rather than clonal [100–102]. This implies that the tumor is a mosaic of cells with different RTK alterations that may cooperate synergistically [103], increasing cellular fitness and resistance to therapy [104]. The resulting spatial heterogeneity renders biopsy sampling less reliable. Further, it implies temporal heterogeneity during further tumor growth, where relapsed GBM may show a loss of mutated targets [67,105]. This would require target verification in the progressive tumor rather than based on tissue from the primary

surgery. While EGFR amplification is usually conserved [106], some trials have shown that staining of the EGFR extracellular domain is changed or lost upon therapy and is not associated with a clinically relevant survival prolongation [95].

- 3 The treatment of GBM with at least some part of the tumor behind an intact blood–brain barrier represents a pharmacokinetic challenge, as many compounds may not sufficiently penetrate CNS tumors and have a reduced bioavailability, even in the case of in principle sufficient CNS penetration of, e.g., the EGFR inhibitors erlotinib or osimertinib [107,108]. This contributes to the observation that tumor tissue obtained after EGFR-directed therapy does not show sufficient target engagement and pathway alterations [109,110].

The difficulties of EGFR-directed therapies have been so substantial that new approaches for EGFR targeting, such as EGFR-directed chimeric antigen receptor (CAR) T cell therapy, are met with caution.

The increasing number of available targeted drugs leads to the question of how to effectively scan for potentially successful target/drug combinations in GBM. Adaptive phase II trials in newly diagnosed *MGMT*-unmethylated GBM are promising tools to identify drugs for further analysis in confirmatory phase III trials. Examples of such trials are N2M2, INSIGHt, and the Adaptive Global Innovative Learning Environment for Glioblastoma (GBM AGILE), which investigate several targeted drugs in parallel with obligatory molecular testing and a common temozolomide standard arm [82,111,112]. These trials are even more intriguing because their results are continuously monitored, and a Bayesian approach is applied to guide the allocation of patients to more successful trial arms. The first results of the INSIGHt trial have already been published, showing superior PFS but similar OS for the CDK4/6 inhibitor abemaciclib (OS 15.3 vs. 14.8 months, PFS 6.2. vs. 4.7 months) and the EGFR/HER2 inhibitor neratinib (OS 14.2 vs. 14.8 months, PFS 6.0 vs. 4.7 months), both in addition to standard radio-chemotherapy [113]. However, preliminary results of GBM AGILE challenge the benefit of regorafenib, as mentioned above [83].

5. Experience with NGS screening and matched targeted therapy

5.1. Important points to consider for NGS-based individual GBM therapy

Instead of testing single or few drugs in cohorts selected by screening for a single molecular alteration,

next-generation sequencing (NGS) yields an array of genetic alterations for each patient, which may allow the selection of the most promising genetic alteration/targeted drug combination (matched therapy). This approach is an attractive way to evaluate the concept of precision oncology in GBM therapy. In cancer entity-agnostic case series of patients with metastatic cancer (without GBM patients) such as IMPACT [114,115], the ORR, 6-month stabilization rate, median PFS and OS, and 10-year survival rate of patients receiving matched therapy tended to be higher than those of patients receiving nonmatched therapies. The first steps are made to implement the approach for primary brain tumors in general and gliomas in particular (Table 3).

To extend this approach to GBM, three major problems have to be addressed:

- 1 Target identification. In GBM, it is not trivial to infer the most promising target constellation from a list of genetic alterations and whether single or combined alterations represent the best target. For example, it is unknown whether CDK4/6 inhibitors such as palbociclib or abemaciclib can be employed for RB1-proficient GBM or if CDKN2A/B and CDK4/6 status also need to be considered [116]. Dysregulation of the CDK4/6-p16-RB1 pathway is a hallmark of glioblastoma [52]. While CDK4/6 activation inhibits the tumor suppressor protein RB1, allowing cell cycle progression, CDK4/6 inhibitors cause reduced RB1 phosphorylation and apoptosis. Homozygous deletion of CDKN2A/B, encoding the CDK4/6 inhibitor p16, leads to CDK4/6 disinhibition, which might be required for sensitivity to pharmacological CDK4/6 inhibition [116]. CDK4 alterations or RB1 mutations were associated with resistance to CDK4/6 inhibition in patient-derived GBM xenografts [117]. Similarly, should application of EGFR block in patients with EGFR amplification or activating mutations be given on the base PTEN alterations, which are frequent in GBM and linked to reduced responsiveness to EGFR inhibitors [110]? Clear guidelines for these decisions are lacking. Of note, combined target selection inevitably narrows down treatment options for individual patients.
- 2 Treatment selection for precision oncology is challenging, irrespective of cancer type. In GBM, this problem may be accentuated as there has only been one successful molecularly guided trial thus far [24]. Therefore, treatment selection mostly has to rely on results from other tumor entities, e.g. breast cancer for DNA damage repair alterations, cholangiocarcinoma and

Table 3. Case series with targeted therapy based on NGS screening.

Series	Approach	Therapy decision criteria	Outcome parameters	Results/Signs of activity	Comment
Lazaridis et al. [126]	Retrospective, mostly 2nd relapse n = 41 gliomas screened, 36/41 with actionable target, 18/36 with targeted therapy	NGS panel, IHC panel, Seq (TERT), FISH (CCDKN2A/B) Known BBB penetration Molecular tumor board decision	PFS/OS of 16 rGBM with vs. 16 without targeted therapy	mPFS 3.8 vs. 2.0 months PFS6 25 vs. 20% mOS 13 vs. 4 months (P < 0.05) High PFS > 7 months and PFS2-PFS1 > 5 months in Dabrafenib/trametinib (n = 2) Cabozantinib (n = 2)	Reproduced the known activity of BRAF inhibitors Median 1 prior treatment Precision therapy aspect of Cabozantinib difficult to analyze due to the multikinase activity including VEGFR inhibition Some signs of activity with FGFR-fusion or mTOR-directed therapy > 2 lines of prior treatment Large cohort, also reporting on other CNS tumor entities
Renovanz et al. [122]	Prospective n = 262 GBM screened, 41 treated based on NGS results, 36 evaluable for response	NGS panel, RNA seq, IHC panel Biomarker-guided ranked molecular tumor board decision	PFS2/PFS1 ratio ≥ 1.3 MCBS score	mPFS 2.5 months 13/36 with PFS2/PFS1 > 1.3 3/6 mTOR-pathway/everolimus 3/4 FGFR-TACC fusion/erdafitinib 3/4 FLT missense or EGFR amp/regorafenib 1/5 EGFR/afatinib 1/5 TMB/nivolumab 2/8 EGFR amp + missense/ABT414 MCBS score 1 (PFS ≥ 6 months, or ORR > 60% or ORR 20–60% and DoR ≥ 9 months) in n = 5 (2 × Everolimus, 2 × Erdafitinib, 1 × ABT414)	No clear signs of activity, even with combination treatment Median 1 prior treatment Small and heterogeneous cohort Feasibility of approach shown Sign of activity in a single GBM patient
Blumenthal et al. [124]	Retrospective n = 43 (GBM, AA), 41/43 with actionable targets, 13/43 treated	NGS panel Treatment decision by physician's choice	Response, duration of treatment, mOS	mOS 4.6 months, longest OS 10 months (EGFR-directed afatinib) 15/16 recommendations within time frame 7/16 received recommended therapy PFS ~ 3 months n = 1 GBM with PFS > 12 months; PFS2/PFS1 = 3.7;	
Byron et al. [125]	Prospective 16/20 patients analyzed 13/16 with IDHwt GBM	WES RNA seq IHC panel Molecular tumor board decision	PFS, feasibility: molecularly based treatment decision within 35 days	Olaparib, trametinib and carboplatin mPFS 2.1 months 7/36 with PFS2/PFS1 > 1.3 4/9 dabrafenib/trametinib 2/4 FGFR3/erdafitinib 1/1 MET/capmatinib 0/1 ROS1/entrectinib 3 objective responses (2 dabrafenib/trametinib, 1 entrectinib)	Largest GBM cohort Reproduced known activity of BRAF inhibitors Starting form second-line treatment Median of targeted therapy lines was 3
Padovan et al. [123]	Retrospective N = 417 GBM screened, 343 with actionable targets, 36 with targeted therapy	NGS panel Clinical trials or off-label compassionate use, decision criteria not reported	PFS2/PFS1 ratio ≥ 1.3 Objective response rate (PR + CR)		

urothelial carcinoma for FGFR alterations, and NSCLC for EGFR alterations [5]. However, applicability may be reduced due to differences in mutation sites, activation of compensatory pathways, and tissue penetration. Treatment selection based on preclinical *in vitro/in vivo* results or on biological rationale is even less convincing and leads to a lower strength of recommendation according to current grading guidance [5,118]. In summary, the paucity of data requires the adoption of treatment strategies based on other tumor entities or preclinical data, both with reduced applicability for GBM.

- 3 Efficacy assessment. The assessment of treatment success by standard metrics such as ORR, PFS (both largely based on imaging parameters), or OS rates is limited in heterogeneous cohorts of GBM patients receiving precision oncology treatment. Further, interindividual comparison is impaired by the potential prognostic effect of the targeted molecular alterations and the treatment of patients at differing stages of their illness. Intraindividual comparison of PFS until first progression to PFS under matched therapy (PFS2/PFS1) is an elegant alternative, and a PFS2/PFS1 ratio of 1.3 or higher has been accepted as a marker of effective matched therapy in systemic cancers [119,120]. Considering the progression time scale with a median PFS of 7 months in newly diagnosed GBM and 2 months in progressive GBM, a PFS2/PFS1 ratio of 1.3 translates to a significant PFS increment if the evaluated treatment is initiated at first recurrence and to a numerically small PFS increment if initiated at further recurrence [30]. Accordingly, modifications have been suggested to adjust for very short PFS1 < 2 months to prevent overcalling and for significant PFS2 > 6 months to prevent undercalling of treatment responses in brain tumors, and the concept has been applied in brain tumors [121–123]. Further approaches include an adaptation of the ESMO Magnitude in Clinical Benefit Scale to brain tumors, combining PFS with imaging response duration (Neuro-MCBS, see Ref. [122]).

5.2. Case series with NGS-based molecular-guided GBM therapy in clinical routine

An increasing number of neuro-oncology centers offer NGS screening for actionable mutations to patients with progressive glioblastoma with no further standard treatment options. At these institutions, multidisciplinary molecular tumor boards are established, providing personalized recommendations for targeted therapies based on individual NGS results. So far, five publications

report on mono- or oligoinstitutional experience with NGS-informed personalized therapy [122–126]: Blumenthal et al. [124] reported the first retrospective cohort from five tertiary hospitals comprising 43 glioma patients (34 GBM), where a NGS panel detected actionable alterations in 95%, leading to targeted treatment in 30% (10/34), but without any treatment response. Byron et al. [125] performed a prospective monocentric trial to evaluate the feasibility of whole exome sequencing-informed treatment recommendations within 35 days of surgery. Among 16 GBM patients, a recommendation was possible in 94%, 44% (7/16) received a targeted treatment, and one patient (6%) achieved a treatment response. Lazaridis et al. [126] reported a retrospective monocentric cohort of 41 glioma (32 GBM) patients. Following NGS and further methods of genomic profiling, actionable targets were identified in 76% (24/32) and 16 GBM patients receiving targeted treatment achieved an increased PFS (3.8 vs. 2.0 months) and OS (13 vs. 4 months) compared to 16 GBM patients with unmatched empiric treatment. Renovanz et al. reported on their experience from the Center for Personalized Medicine Tübingen, which has an established certified clinical workflow for personalized medicine in the clinical routine for cancer patients without options for trial participation or further registered treatments. This heterogeneous and heavily pretreated cohort included 262 GBM [122]. Following comprehensive molecular profiling, molecularly instructed treatment recommendations were made in 93% (243/262) and 41 GBM patients were treated accordingly, resulting in a PFS2/PFS1 > 1.3 in 36% (13 of 36 evaluable patients). Padovan et al. [123] reported a retrospective, monocentric cohort of 417 GBM patients receiving NGS screening. While actionable targets were identified in 82%, 36 patients (8.6%) received a targeted treatment, of which 20% (7/36) achieved a PFS2/PFS1 > 1.3.

The five series are described in more detail in Table 3. Of note, only two of these studies were prospectively documented [122,125], and the number of evaluable patients with targeted therapy per cohort remains low, ranging from < 20 [124,125] to 36 patients [122,123,126]. The largest studies to date also highlight the current problem of precision therapy. As reported by Renovanz et al. [122], among 262 GBM patients receiving NGS screening, only 41 actually started matched therapy (about 14% of patients tested). While a high percentage of these (88%, $n = 36$) could be evaluated for efficacy and demonstrate the determination of the authors, the low rate of initiated therapies emphasizes the many obstacles for molecularly matched therapy, such as the rapid deterioration of patients with progressive GBM and the lack

of reimbursement by health insurance companies attributable to insufficient GBM-specific evidence of efficacy [122]. Padovan et al. [123] reported a similar experience, where only 36 (8.6%) of 417 GBM patients receiving NGS screening were able to initiate matched therapy. No treatment response was observed by Blumenthal et al., while Byron et al. report a single patient receiving a potentially successful treatment of olaparib/trametinib/carboplatin with a PFS2/PFS1 ratio of > 3 [124,125]. The larger studies provide some data on the efficacy of matched therapy in GBM [122,123,126].

Lazaridis et al. [126], also considering CNS drug penetration in the decision-making process, presented 16 patients receiving matched therapy compared to 16 patients with unmatched therapy (Table 3), potentially introducing selection bias. The encouraging PFS results of 3.8 and 2.0 months with versus without matched therapy were mainly driven by the efficacy of BRAF V600E-directed therapy with dabrafenib/trametinib and c-Met-directed therapy with the tyrosine kinase inhibitor (TKI) cabozantinib. While the first observation is in line with the interim results from the ROAR trial discussed in Section 4.1 [24], the cabozantinib results warrant critical discussion as the drug targets not only c-Met but also VEGFR. Inhibition of the VEGF pathway, e.g. with the VEGF-A antibody bevacizumab, is well known to prolong PFS but not OS [34,42,127]. The intermingling of VEGF-directed therapy with therapy directed at other targets was also present in the study by Renovanz et al. [122]. As mentioned, 14% of GBM patients started matched therapy, and 13/36 evaluable patients (5% of screened patients or 36% of patients receiving treatment and being evaluable) were successfully treated according to a PFS2/PFS1 ratio > 1.3 , while the median PFS was 2.3 months [122]. Here, three of the four patients receiving regorafenib based on FMS-like tyrosine kinase (FLT) or EGFR alterations did benefit (PFS2/PFS1 > 1.3). As discussed above, regorafenib targets several tyrosine kinases, including VEGFR, raising the possibility that the observed PFS benefit might at least partially be attributable to VEGFR targeting. Apart from this, a signal for treatment benefit was only seen for everolimus in tumors with PTEN or PIK3CA alterations (3/6 with PFS2/PFS1 > 1.3), which is in line with results from the mTOR inhibitor temsirolimus trial in newly diagnosed *MGMT*-unmethylated GBM [44], and for tumors with FGFR fusion transcripts treated with the FGFR inhibitor erdafitinib (3/4 with PFS2/PFS1 > 1.3). Two patients had a positive PFS2/PFS1 signal with the EGFR antibody-toxin conjugate depatuxizumab mafodotin in the context of an early-access program,

illustrating its PFS prolongation in a previous trial [128], which did not translate into a longer OS in large phase III trials [94] (Table 3). Padovan et al. [123] confirm these observations: among the 36/417 patients receiving targeted treatment, 19% were treated successfully with a PFS2/PFS1 ratio > 1.3 , including three objective responses, again mainly driven by dabrafenib/trametinib in BRAF V600E-altered GBM and erdafitinib in FGFR3-altered GBM, while the median PFS was 2.1 months.

Other publications report the results of NGS screening and/or matched therapy allocation without including a substantial number of GBM patients evaluable for treatment efficacy [129,130]. Using a methodologically different approach, Luger et al. [131] retrospectively analyzed a cohort of 351 patients treated with off-label therapy and identified 15 patients with high-grade glioma (8 GBM) who received matched therapy. This series was dominated by the observation that 3/6 patients treated with BRAF V600E-directed therapy had disease stability for 5+ months. In summary, these reports highlight that few NGS-screened GBM patients receive targeted treatment, and even fewer may benefit from it.

6. Advancing GBM precision oncology: beyond tumor cell targets

While precision oncology heralds potentially great benefit in GBM, the clinical results achieved with targeted drugs remain underwhelming. There are several areas of ongoing research to overcome this. This includes (a) the identification of treatment targets beyond DNA sequencing, such as multi-omics-based exploitation of altered pathways, immunophenotyping, epigenetic profiling, metabolomics, and single-cell analyses [1,132,133]. Another area of current research focuses on (b) optimization of the method of target engagement, e.g. including CAR T- or NK-cells, tumor vaccination, oncolytic viruses, and antibody-drug conjugates (see [134] for review), despite the low frequency of currently addressable targets [135–137]. (c) The optimization of (sequential) target verification, e.g. via radiomic and liquid biopsy strategies [1,138,139], could also improve the extent of the therapeutic benefit/treatment response. (d) Understanding and overcoming molecular mechanisms of acquired therapy resistance is key to developing more potent therapeutic modalities [140], and (e) the optimization of clinical trial conductance could better inform future studies [141,142]. Here, we focus on discussing the advancement of precision oncology in GBM toward novel treatment targets beyond tumor cell-intrinsic targets, thus including targets in the

tumor-associated microenvironment (TME). The TME of GBM is increasingly well characterized [143], and changes in the TME may be crucial for tumor progression [51]. Interactions of tumor cells with vascular structures, immune cells, neurons, glial cells, and each other may provide new targets for therapy (Fig. 2).

In this context, extracellular vesicles (EV) are notable for their involvement at both a diagnostic and therapeutic level. EVs are membrane-bound vesicles secreted into the extracellular space that can cross the blood–brain barrier and carry a broad range of cargos, including nucleic acids, lipids, and proteins, together with markers reflecting their biogenesis [144]. GBM-derived EVs purified from blood or cerebrospinal fluid allow for tumor diagnosis and noninvasive longitudinal sampling for detection of tumor progression, treatment targets, and treatment response [144–149]. Further, EVs may be taken up by neighboring and distant cells in the TME as well as GBM cells, thus representing both an important means of GBM-TME communication and a possible therapeutic approach for targeted drug delivery [150–153].

6.1. Antiangiogenic therapy

The tumor-vascularization axis has been targeted with the application of the VEGF-A inhibitor bevacizumab, leading to prolonged PFS (potentially also due to anti-edematous effects) but not OS, both in newly diagnosed and progressive GBM [34,42,127,154]. Other anti-

angiogenic drugs, such as cediranib, a VEGFR inhibitor, had a similar PFS benefit in newly diagnosed GBM, but again, there was no OS benefit both in the newly diagnosed and progressive settings [155,156]. Cilengitide, an integrin inhibitor targeting angiogenesis, did not find its way into clinical application following a PFS- and OS-negative phase III trial [40,157]. However, anti-angiogenic therapy was applied in unselected cohorts without pretesting for the respective targeted angiogenic factors, and thus its potential might be higher in a precision oncology approach. Retrospective analyses aimed to identify molecular subgroups with an OS benefit from bevacizumab [158,159]. In a biomarker analysis of AVAglio, the ‘proneural’ gene expression subtype was associated with a significant OS advantage (17.1 vs. 12.8 months) in newly diagnosed GBM receiving bevacizumab, which seems counterintuitive as this subtype is associated with lower VEGF expression, and the results could not be confirmed in the GLARIUS trial [46,127,158,160]. In the progressive setting, NF1 mutation was associated with survival benefit from bevacizumab (OS approx. 17 vs. 8 months) in an exploratory biomarker analysis of EORTC-26101, but these results need further validation [159].

6.2. Immunological targets

The interaction of the immune system with GBM cells may provide further targets for precision oncology. To date, large clinical trials investigating immune checkpoint

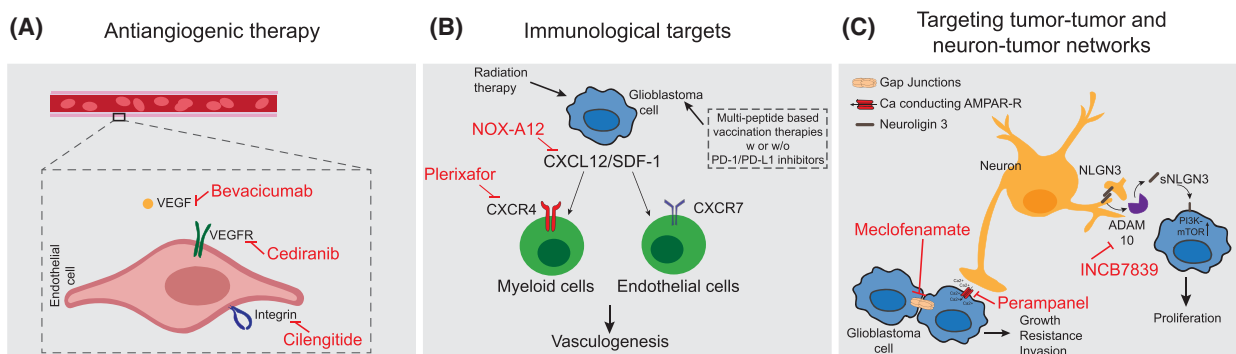


Fig. 2. Advancing precision oncology in glioblastoma: selection of potential microenvironmental targets. This figure illustrates potential treatment approaches targeting the tumor microenvironment. (A) Antiangiogenic therapy targeting the tumor-vascularization axis by vascular endothelial growth factor (VEGF) or integrin inhibition failed in unselected cohorts, but its potential in a precision oncology approach remains promising. (B) Immunological treatment strategies such as personalized vaccination using an individual peptide mix based on tumor tissue analysis have shown sustained immunological responses. Exclusion of myeloid cells by targeting the CXC chemokine ligand 12 (CXCL12)/CXC chemokine receptor 4 (CXCR4) axis reduces postirradiation tumor revascularization and is evaluated in ongoing clinical trials. (C) The disruption of tumor cell networks by gap junction inhibitors might increase radiochemotherapy sensitivity. Neuron-tumor signaling might be targeted by inhibition of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated synaptic input or by ADAMS10 sheddase inhibition to decrease neuroigin 3 (NLGN3) signaling, both mediators of activity-induced glioma proliferation. For a detailed explanation, refer to Section 6 in the main text. CXCR7, CXC chemokine receptor 7; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; sNLGN3, soluble NLGN3; VEGFR, VEGF receptor.

blockade in GBM have failed [41,92,93], and no predictive markers have been defined for matched therapy besides microsatellite instability (see Section 4.2). Alone or in combination with PD-1/programmed death-ligand 1 (PD-L1) inhibitors, some trials applied cell-based (e.g. DCVax [161]) or – more promisingly – multi-peptide-based vaccination therapy [162] after EGFRvIII-directed mono-peptide vaccination with rindopepimut failed as discussed above [95]. The peptide vaccination approach may be personalized using an individual peptide mix informed by tumor tissue analysis. First results from the phase I GAPVAC-101 trial, investigating highly individualized vaccinations against an individual selection of unmutated antigens and neoepitopes in 15 patients, document a sustained T-cell immune response, while meaningful clinical efficacy (e.g. prolongation of PFS and OS) has still not been demonstrated [163]. It remains a major challenge to identify immunomodulatory targets in the microenvironment that can overcome local immunosuppression and further enhance the immune reaction against tumor cells. Further, macrophages and microglia have been shown to interact with tumor cells and may even manipulate them to obtain a more aggressive phenotype [164]. Despite promising preclinical data, first approaches with colony stimulating factor 1 receptor (CSF-1R)-targeted inhibition of macrophages failed [165–167]. Macrophage exclusion from the tumor by inhibition of C-X-C motif chemokine receptor 4 (CXCR4) was shown to reduce post-irradiation tumor revascularization in a small phase I/II trial. Additionally, inhibition of CXCR4 by plerixafor or inhibition of its ligand C-X-C motif chemokine ligand 12 (CXCL12; formerly known as stromal cell derived factor-1, SDF-1) by NOX-A12 is being evaluated in several ongoing trials [168,169]. Macrophages and microglia provide several other markers that may be targeted to enable a stronger and more precise immune reaction in GBM immunotherapy [170]. Further, indirect targeting of immune cells with GBM-specific stromal protein-targeted immunostimulatory cytokines represents a novel approach. An antibody-cytokine conjugate targeting a tumor-associated fibronectin epitope to enable local distribution of tumor necrosis factor was associated with increasing tumor necrosis and local inflammation in a phase I study, with objective responses in 3/5 progressive GBM patients, and is currently being evaluated in further trials [171,172].

6.3. Targeting tumor-tumor and neuron-tumor networks

The interactions of tumor cells with each other and with neuronal or glial cells offer further opportunities for precision oncology. The rising field of cancer neuroscience has provided a host of landmark publications,

showing that GBM form tumor microtubule-based tumor cell networks that confer resistance to radiotherapy and chemotherapy [173–176] and promote tumor cell invasion by recapitulating developmental neuronal programs [177]. These observations may inform new targets for future therapeutic manipulation, e.g. or the disturbance of hub cells within the syncytium that dominate and organize the tumor cell network [178], or the disruption of tumor syncytia by gap junction inhibitors [174,179,180] – the latter being explored in an ongoing phase I/II trial [181].

Finally, several ways to modulate the neuronal input on tumor cell networks have been found and may be targeted. The synaptic protein neuroligin-3 (NLGN3) was identified as the leading mitogen mediating neuronal activity-induced glioma proliferation in patient-derived xenograft models, and reduction of the release of its soluble form (sNLGN3) by ADAMS10 sheddase inhibition with INCB7839 is explored in a phase I trial (NCT04295759) [173,182]. Similarly, neuronal activity was shown to mediate glioma invasion and growth via α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)-mediated synaptic input from neuroglial glutamatergic synapses in patient-derived xenograft models. In line with this, targeting the modulation of AMPAR synaptic transmission using the antiepileptic drug perampanel is explored in a phase II trial [176,183]. To further refine this as a precision therapy approach, predictive markers have yet to be defined.

7. Summary and further perspectives

Despite first successes with BRAF V600E-directed dabrafenib/trametinib, and signs of some efficacy in a low percentage of GBM patients receiving molecular-guided therapies, precision oncology has yet to find broad clinical application with proven efficacy in patients with GBM. Trials investigating targeted drugs in molecularly defined subgroups and treatment allocation based on broad NGS screening need further optimization, e.g. by taking into account the CNS penetration of drugs, more complex prediction models based on combinations of genetic vulnerabilities/interaction of pathways [184], and new targets beyond the tumor cell. New models of clinical trials are being conducted to allow efficient analysis of new substances and multi-omics approaches. The results of N2M2, GBM AGILE, and INSIGHT exploring multiple targeted treatments in comparison to a common standard of care will significantly advance the field. Until more efficacy data are available, matched personalized therapy may, with the exceptions mentioned above, be reserved for the experimental treatment of relapsed GBM.

Acknowledgements

The review was written without external funding.

Conflict of interest

UH reports advisory and speaker honoraria from Bayer and speaker honoraria from Medac. The other authors report no conflicts of interest.

Author contributions

UH conceptualized and supervised the project. JW and UH researched the literature and wrote the first draft of the manuscript. A-LP designed the figures. All authors contributed to the writing and editing of the manuscript.

References

- Malone ER, Oliva M, Sabatini PJB, Stockley TL, Siu LL. Molecular profiling for precision cancer therapies. *Genome Med.* 2020;**12**(1):8.
- Le Tourneau C, Borcoman E, Kamal M. Molecular profiling in precision medicine oncology. *Nat Med.* 2019;**25**(5):711–2.
- Mateo J, Chakravarty D, Dienstmann R, Jezdic S, Gonzalez-Perez A, Lopez-Bigas N, et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO scale for clinical actionability of molecular targets (ESCAT). *Ann Oncol.* 2018;**29**(9):1895–902.
- Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn.* 2017;**19**(1):4–23.
- Horak P, Griffith M, Danos AM, Pitel BA, Madhavan S, Liu X, et al. Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC). *Genet Med.* 2022;**24**(5):986–98.
- Larkin J, Ascierto PA, Dréno B, Atkinson V, Liskay G, Maio M, et al. Combined vemurafenib and cobimetinib in *BRAF*-mutated melanoma. *N Engl J Med.* 2014;**371**(20):1867–76.
- Long GV, Stroyakovskiy D, Gogas H, Levchenko E, De Braud F, Larkin J, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med.* 2014;**371**(20):1877–88.
- Long GV, Hauschild A, Santinami M, Atkinson V, Mandalà M, Chiarion-Sileni V, et al. Adjuvant dabrafenib plus trametinib in stage III *BRAF*-mutated melanoma. *N Engl J Med.* 2017;**377**(19):1813–23.
- Robert C, Grob JJ, Stroyakovskiy D, Karaszewska B, Hauschild A, Levchenko E, et al. Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. *N Engl J Med.* 2019;**381**(7):626–36.
- Dummer R, Ascierto PA, Gogas HJ, Arance A, Mandalà M, Liskay G, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with *BRAF*-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* 2018;**19**(5):603–15.
- Hanna NH, Robinson AG, Temin S, Baker S, Brahmer JR, Ellis PM, et al. Therapy for stage IV non-small-cell lung cancer with driver alterations: ASCO and OH (CCO) joint guideline update. *J Clin Oncol.* 2021;**39**(9):1040–91.
- Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med.* 2006;**355**(26):2733–43.
- Martin M, Holmes FA, Ejlertsen B, Delaloge S, Moy B, Iwata H, et al. Neratinib after trastuzumab-based adjuvant therapy in HER2-positive breast cancer (ExteNET): 5-year analysis of a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol.* 2017;**18**(12):1688–700.
- Murthy RK, Loi S, Okines A, Paplomata E, Hamilton E, Hurvitz SA, et al. Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. *N Engl J Med.* 2020;**382**(7):597–609.
- Early Breast Cancer Trialists' Collaborative group (EBCTCG). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. *Lancet Oncol.* 2021;**22**(8):1139–50.
- Piccatt M, Procter M, Fumagalli D, de Azambuja E, Clark E, Ewer MS, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer in the APHINITY trial: 6 years' follow-up. *J Clin Oncol.* 2021;**39**(13):1448–57.
- Rugo HS, Im S-A, Cardoso F, Cortés J, Curigliano G, Musolino A, et al. Efficacy of margetuximab vs trastuzumab in patients with pretreated ERBB2-positive advanced breast cancer: a phase 3 randomized clinical trial. *JAMA Oncol.* 2021;**7**(4):573–84.
- Javle M, Lowery M, Shroff RT, Weiss KH, Springfield C, Borad MJ, et al. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. *J Clin Oncol.* 2018;**36**(3):276–82.

- 19 Abou-Alfa GK, Sahai V, Hollebecque A, Vaccaro G, Melisi D, Al-Rajabi R, et al. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol.* 2020;**21**(5):671–84.
- 20 Siefker-Radtke AO, Necchi A, Park SH, García-Donas J, Huddart RA, Burgess EF, et al. Efficacy and safety of erdafitinib in patients with locally advanced or metastatic urothelial carcinoma: long-term follow-up of a phase 2 study. *Lancet Oncol.* 2022;**23**(2):248–58.
- 21 Kelly CM, Gutierrez Sainz L, Chi P. The management of metastatic GIST: current standard and investigational therapeutics. *J Hematol Oncol.* 2021;**14**(1):2.
- 22 Desilets A, Repetto M, Yang S-R, Sherman EJ, Drilon A. RET-altered cancers—a tumor-agnostic review of biology, diagnosis and targeted therapy activity. *Cancers (Basel).* 2023;**15**(16):4146.
- 23 Sforza V, Palumbo G, Cascetta P, Carillio G, Manzo A, Montanino A, et al. BRAF inhibitors in non-small cell lung cancer. *Cancers (Basel).* 2022;**14**(19):4863.
- 24 Wen PY, Stein A, van den Bent M, De Greve J, Wick A, de Vos FYFL, et al. Dabrafenib plus trametinib in patients with BRAFV600E-mutant low-grade and high-grade glioma (ROAR): a multicentre, open-label, single-arm, phase 2, basket trial. *Lancet Oncol.* 2022;**23**(1):53–64.
- 25 Iannantuono GM, Riondino S, Sganga S, Rosenfeld R, Guerriero S, Carlucci M, et al. NTRK gene fusions in solid tumors and TRK inhibitors: a systematic review of case reports and case series. *J Pers Med.* 2022;**12**(11):1819.
- 26 Lin B, Ziebro J, Smithberger E, Skinner KR, Zhao E, Cloughesy TF, et al. EGFR, the Lazarus target for precision oncology in glioblastoma. *Neuro Oncol.* 2022;**24**(12):2035–62.
- 27 Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen H-J, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol.* 2006;**7**(5):392–401.
- 28 Walker MD, Alexander E, Hunt WE, MacCarty CS, Mahaley MS, Mealey J, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg.* 1978;**49**(3):333–43.
- 29 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;**352**(10):987–96.
- 30 Wen PY, Weller M, Lee EQ, Alexander BM, Barnholtz-Sloan JS, Barthel FP, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro Oncol.* 2020;**22**(8):1073–113.
- 31 Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA.* 2017;**318**(23):2306–16.
- 32 Weller M, Le Rhun E. How did lomustine become standard of care in recurrent glioblastoma? *Cancer Treat Rev.* 2020;**87**:102029.
- 33 Herrlinger U, Tzaridis T, Mack F, Steinbach JP, Schlegel U, Sabel M, et al. Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA-09): a randomised, open-label, phase 3 trial. *Lancet.* 2019;**393**(10172):678–88.
- 34 Wick W, Gorlia T, Bendszus M, Taphoorn M, Sahn F, Harting I, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med.* 2017;**377**(20):1954–63.
- 35 Karschnia P, Dono A, Young JS, Juenger ST, Teske N, Häni L, et al. Prognostic evaluation of re-resection for recurrent glioblastoma using the novel RANO classification for extent of resection: a report of the RANO resect group. *Neuro Oncol.* 2023;**25**(9):1672–85.
- 36 Tsien CI, Pugh SL, Dicker AP, Raizer JJ, Matuszak MM, Lallana EC, et al. NRG oncology/RTOG1205: a randomized phase II trial of concurrent bevacizumab and reirradiation versus bevacizumab alone as treatment for recurrent glioblastoma. *J Clin Oncol.* 2023;**41**(6):1285–95.
- 37 Lombardi G, De Salvo GL, Brandes AA, Eoli M, Rudà R, Faedi M, et al. Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol.* 2019;**20**(1):110–9.
- 38 Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med.* 2000;**343**(19):1350–4.
- 39 Hegi ME, Diserens A-C, Gorlia T, Hamou M-F, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005;**352**(10):997–1003.
- 40 Stupp R, Hegi ME, Gorlia T, Erridge SC, Perry J, Hong Y-K, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2014;**15**(10):1100–8.

- 41 Lim M, Weller M, Idubai A, Steinbach J, Finocchiaro G, Raval RR, et al. Phase 3 trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated MGMT promoter. *Neuro Oncol.* 2022;**24**:1935–49.
- 42 Herrlinger U, Schäfer N, Steinbach JP, Weyerbrock A, Hau P, Goldbrunner R, et al. Bevacizumab plus irinotecan versus temozolomide in newly diagnosed O6-methylguanine-DNA methyltransferase nonmethylated glioblastoma: the randomized GLARIUS trial. *J Clin Oncol.* 2016;**34**(14):1611–9.
- 43 Perry JR, Laperriere N, O'Callaghan CJ, Brandes AA, Menten J, Phillips C, et al. Short-course radiation plus temozolomide in elderly patients with glioblastoma. *N Engl J Med.* 2017;**376**(11):1027–37.
- 44 Wick W, Gorlia T, Bady P, Platten M, van den Bent MJ, Taphoorn MJB, et al. Phase II study of radiotherapy and temsirolimus versus radiochemotherapy with temozolomide in patients with newly diagnosed glioblastoma without MGMT promoter hypermethylation (EORTC 26082). *Clin Cancer Res.* 2016;**22**(19):4797–806.
- 45 Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. *Nature.* 2018;**555**(7697):469–74.
- 46 Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 2010;**17**(1):98–110.
- 47 Sturm D, Witt H, Hovestadt V, Khuong-Quang D-A, Jones DTW, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell.* 2012;**22**(4):425–37.
- 48 Wick A, Kessler T, Platten M, Meisner C, Bamberg M, Herrlinger U, et al. Superiority of temozolomide over radiotherapy for elderly patients with RTK II methylation class, MGMT promoter methylated malignant astrocytoma. *Neuro Oncology.* 2020;**22**(8):1162–72.
- 49 Castro M, Pampana A, Alam A, Parashar R, Rajagopalan S, Lala DA, et al. Combination chemotherapy versus temozolomide for patients with methylated MGMT (m-MGMT) glioblastoma: results of computational biological modeling to predict the magnitude of treatment benefit. *J Neurooncol.* 2021;**153**(3):393–402.
- 50 Neftel C, Laffy J, Filbin MG, Hara T, Shore ME, Rahme GJ, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell.* 2019;**178**(4):835–49.e21.
- 51 Hoogstrate Y, Draaisma K, Ghisai SA, van Hijfte L, Barin N, de Heer I, et al. Transcriptome analysis reveals tumor microenvironment changes in glioblastoma. *Cancer Cell.* 2023;**41**(4):678–92.e7.
- 52 Brennan CW, Verhaak RGW, McKenna A, Campos B, Noushmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. *Cell.* 2013;**155**(2):462–77.
- 53 Stichel D, Ebrahimi A, Reuss D, Schrimpf D, Ono T, Shirahata M, et al. Distribution of EGFR amplification, combined chromosome 7 gain and chromosome 10 loss, and TERT promoter mutation in brain tumors and their potential for the reclassification of IDHwt astrocytoma to glioblastoma. *Acta Neuropathol.* 2018;**136**(5):793–803.
- 54 Hu H, Mu Q, Bao Z, Chen Y, Liu Y, Chen J, et al. Mutational landscape of secondary glioblastoma guides MET-targeted trial in brain tumor. *Cell.* 2018;**175**(6):1665–78.e18.
- 55 Ferguson SD, Zhou S, Huse JT, de Groot JF, Xiu J, Subramaniam DS, et al. Targetable gene fusions associate with the IDH wild-type astrocytic lineage in adult gliomas. *J Neuropathol Exp Neurol.* 2018;**77**(6):437–42.
- 56 Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science.* 2012;**337**(6099):1231–5.
- 57 Frattini V, Trifonov V, Chan JM, Castano A, Lia M, Abate F, et al. The integrated landscape of driver genomic alterations in glioblastoma. *Nat Genet.* 2013;**45**(10):1141–9.
- 58 The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008;**455**(7216):1061–8.
- 59 Hodges TR, Ott M, Xiu J, Gatalica Z, Swensen J, Zhou S, et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: implications for immune checkpoint immunotherapy. *Neuro Oncol.* 2017;**19**(8):1047–57.
- 60 Indraccolo S, Lombardi G, Fassin M, Pasqualini L, Giunco S, Marcato R, et al. Genetic, epigenetic, and immunologic profiling of MMR-deficient relapsed glioblastoma. *Clin Cancer Res.* 2019;**25**(6):1828–37.
- 61 Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrsen CL, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature.* 2020;**580**(7804):517–23.
- 62 Johann P, Lenz D, Ries M. The drug development pipeline for glioblastoma – a cross sectional assessment of the FDA orphan drug product designation database. *PLoS One.* 2021;**16**(7):e0252924.
- 63 Majd NK, Yap TA, Koul D, Balasubramanian V, Li X, Khan S, et al. The promise of DNA damage

- response inhibitors for the treatment of glioblastoma. *Neurooncol Adv.* 2021;**3**(1):vdab015.
- 64 Juratli TA, Kirsch M, Robel K, Soucek S, Geiger K, von Kummer R, et al. IDH mutations as an early and consistent marker in low-grade astrocytomas WHO grade II and their consecutive secondary high-grade gliomas. *J Neurooncol.* 2012;**108**(3):403–10.
- 65 Parsons DW, Jones S, Zhang X, Lin JC-H, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science.* 2008;**321**(5897):1807–12.
- 66 Ravi VM, Will P, Kueckelhaus J, Sun N, Joseph K, Salié H, et al. Spatially resolved multi-omics deciphers bidirectional tumor-host interdependence in glioblastoma. *Cancer Cell.* 2022;**40**(6):639–55.e13.
- 67 Schäfer N, Gielen GH, Rauschenbach L, Kebir S, Till A, Reinartz R, et al. Longitudinal heterogeneity in glioblastoma: moving targets in recurrent versus primary tumors. *J Transl Med.* 2019;**17**(1):96.
- 68 Kim J, Lee I-H, Cho HJ, Park C-K, Jung Y-S, Kim Y, et al. Spatiotemporal evolution of the primary glioblastoma genome. *Cancer Cell.* 2015;**28**(3):318–28.
- 69 Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, et al. Longitudinal molecular trajectories of diffuse glioma in adults. *Nature.* 2019;**576**(7785):112–20.
- 70 Körber V, Yang J, Barah P, Wu Y, Stichel D, Gu Z, et al. Evolutionary trajectories of IDHWT glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. *Cancer Cell.* 2019;**35**(4):692–704.e12.
- 71 Brandes AA, Carpentier AF, Kesari S, Sepulveda-Sanchez JM, Wheeler HR, Chinot O, et al. A phase II randomized study of galunisertib monotherapy or galunisertib plus lomustine compared with lomustine monotherapy in patients with recurrent glioblastoma. *Neuro Oncol.* 2016;**18**(8):1146–56.
- 72 Wick W, Fricke H, Junge K, Kobayakov G, Martens T, Heese O, et al. A phase II, randomized, study of weekly APG101+reirradiation versus reirradiation in progressive glioblastoma. *Clin Cancer Res.* 2014;**20**(24):6304–13.
- 73 Franceschi E, Stupp R, van den Bent MJ, van Herpen C, Laigle Donadey F, Gorlia T, et al. EORTC 26083 phase I/II trial of dasatinib in combination with CCNU in patients with recurrent glioblastoma. *Neuro Oncol.* 2012;**14**(12):1503–10.
- 74 Galanis E, Anderson SK, Twohy EL, Carrero XW, Dixon JG, Tran DD, et al. A phase 1 and randomized, placebo-controlled phase 2 trial of bevacizumab plus dasatinib in patients with recurrent glioblastoma: Alliance/North Central Cancer Treatment Group N0872. *Cancer.* 2019;**125**(21):3790–800.
- 75 Lassman AB, Pugh SL, Gilbert MR, Aldape KD, Geinoz S, Beumer JH, et al. Phase 2 trial of dasatinib in target-selected patients with recurrent glioblastoma (RTOG 0627). *Neuro Oncol.* 2015;**17**(7):992–8.
- 76 de Groot J, Ott M, Wei J, Kassab C, Fang D, Najem H, et al. A first-in-human phase I trial of the oral p-STAT3 inhibitor WP1066 in patients with recurrent malignant glioma. *CNS Oncol.* 2022;**11**(2):CNS87.
- 77 Wen PY, Schiff D, Cloughesy TF, Raizer JJ, Laterra J, Smitt M, et al. A phase II study evaluating the efficacy and safety of AMG 102 (rilotumumab) in patients with recurrent glioblastoma. *Neuro Oncol.* 2011;**13**(4):437–46.
- 78 Bota DA, Mason W, Kesari S, Magge R, Winograd B, Elias I, et al. Marizomib alone or in combination with bevacizumab in patients with recurrent glioblastoma: phase I/II clinical trial data. *Neurooncol Adv.* 2021;**3**(1):vdab142.
- 79 Weaver M, Laske DW. Transferrin receptor ligand-targeted toxin conjugate (Tf-CRM107) for therapy of malignant gliomas. *J Neurooncol.* 2003;**65**(1):3–13.
- 80 Laske DW, Youle RJ, Oldfield EH. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat Med.* 1997;**3**(12):1362–8.
- 81 Sampson JH, Singh Achrol A, Aghi MK, Bankiewicz K, Bexon M, Brem S, et al. Targeting the IL4 receptor with MDNA55 in patients with recurrent glioblastoma: results of a phase IIb trial. *Neuro Oncol.* 2023;**25**(6):1085–97.
- 82 Wick W, Dettmer S, Berberich A, Kessler T, Karapanagiotou-Schenkel I, Wick A, et al. N2M2 (NOA-20) phase I/II trial of molecularly matched targeted therapies plus radiotherapy in patients with newly diagnosed non-MGMT hypermethylated glioblastoma. *Neuro Oncol.* 2019;**21**(1):95–105.
- 83 Wen P, Alexander B, Berry D, Buxton M, Cavenee W, Colman H, et al. CTNI-85. GBM agile platform trial for newly diagnosed and recurrent GBM: results of first experimental arm, regorafenib. *Neuro Oncol.* 2023;**25**(Suppl 5):v97–8.
- 84 Tzaridis T, Gepfner-Tuma I, Hirsch S, Skardelly M, Bender B, Paulsen F, et al. Regorafenib in advanced high-grade glioma: a retrospective bicentric analysis. *Neuro Oncol.* 2019;**21**(7):954–5.
- 85 Santangelo A, Rossato M, Lombardi G, Benfatto S, Lavezzari D, De Salvo GL, et al. A molecular signature associated with prolonged survival in glioblastoma patients treated with regorafenib. *Neuro Oncol.* 2021;**23**(2):264–76.
- 86 Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med.* 2010;**363**(19):1801–11.
- 87 Mellinghoff IK, van den Bent MJ, Blumenthal DT, Touat M, Peters KB, Clarke J, et al. Vorasidenib in IDH1- or IDH2-mutant low-grade glioma. *N Engl J Med.* 2023;**389**(7):589–601.

- 88 Capper D, Reifenberger G, French PJ, Schweizer L, Weller M, Touat M, et al. EANO guideline on rational molecular testing of gliomas, glioneuronal, and neuronal tumors in adults for targeted therapy selection. *Neuro Oncol.* 2023;**25**(5):813–26.
- 89 Laetsch TW, DuBois SG, Mascarenhas L, Turpin B, Federman N, Albert CM, et al. Larotrectinib for paediatric solid tumours harbouring NTRK gene fusions: phase 1 results from a multicentre, open-label, phase 1/2 study. *Lancet Oncol.* 2018;**19**(5):705–14.
- 90 Doz F, van Tilburg CM, Geoerger B, Højgaard M, Øra I, Boni V, et al. Efficacy and safety of larotrectinib in TRK fusion-positive primary central nervous system tumors. *Neuro Oncol.* 2022;**24**(6):997–1007.
- 91 Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord J-P, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J Clin Oncol.* 2020;**38**(1):1–10.
- 92 Omuro A, Brandes AA, Carpentier AF, Idbaih A, Reardon DA, Cloughesy T, et al. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: an international randomized phase III trial. *Neuro Oncol.* 2023;**25**(1):123–34.
- 93 Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol.* 2020;**6**(7):1003–10.
- 94 Lassman AB, Pugh SL, Wang TJC, Aldape K, Gan HK, Preusser M, et al. Depatuxizumab mafodotin in EGFR-amplified newly diagnosed glioblastoma: a phase III randomized clinical trial. *Neuro Oncol.* 2023;**25**(2):339–50.
- 95 Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol.* 2017;**18**(10):1373–85.
- 96 Ellingson BM, Wen PY, Chang SM, van den Bent M, Vogelbaum MA, Li G, et al. Objective response rate targets for recurrent glioblastoma clinical trials based on the historic association between objective response rate and median overall survival. *Neuro Oncol.* 2023;**25**(6):1017–28.
- 97 Lamborn KR, Yung WKA, Chang SM, Wen PY, Cloughesy TF, DeAngelis LM, et al. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. *Neuro Oncol.* 2008;**10**(2):162–70.
- 98 Lassman AB, Aldape KD, Ansell PJ, Bain E, Curran WJ, Eoli M, et al. Epidermal growth factor receptor (EGFR) amplification rates observed in screening patients for randomized trials in glioblastoma. *J Neurooncol.* 2019;**144**(1):205–10.
- 99 Hu C, Leche CA, Kiyatkin A, Yu Z, Stayrook SE, Ferguson KM, et al. Glioblastoma mutations alter EGFR dimer structure to prevent ligand bias. *Nature.* 2022;**602**(7897):518–22.
- 100 Mukasa A, Wykosky J, Ligon KL, Chin L, Cavenee WK, Furnari F. Mutant EGFR is required for maintenance of glioma growth in vivo, and its ablation leads to escape from receptor dependence. *Proc Natl Acad Sci USA.* 2010;**107**(6):2616–21.
- 101 Francis JM, Zhang C-Z, Maire CL, Jung J, Manzo VE, Adalsteinsson VA, et al. EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing. *Cancer Discov.* 2014;**4**(8):956–71.
- 102 Cheng Y-K, Beroukhim R, Levine RL, Mellinghoff IK, Holland EC, Michor F. A mathematical methodology for determining the temporal order of pathway alterations arising during gliomagenesis. *PLoS Comput Biol.* 2012;**8**(1):e1002337.
- 103 Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell.* 2011;**20**(6):810–7.
- 104 Inda M-M, Bonavia R, Mukasa A, Narita Y, Sah DWY, Vandenberg S, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes Dev.* 2010;**24**(16):1731–45.
- 105 Johnson BE, Mazar T, Hong C, Barnes M, Aihara K, McLean CY, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. *Science.* 2014;**343**(6167):189–93.
- 106 van den Bent MJ, Gao Y, Kerkhof M, Kros JM, Gorlia T, van Zwieten K, et al. Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas. *Neuro Oncol.* 2015;**17**(7):935–41.
- 107 Colclough N, Chen K, Johnström P, Strittmatter N, Yan Y, Wrigley GL, et al. Preclinical comparison of the blood–brain barrier permeability of osimertinib with other EGFR TKIs. *Clin Cancer Res.* 2021;**27**(1):189–201.
- 108 Tsang JE, Urner LM, Kim G, Chow K, Baufeld L, Faull K, et al. Development of a potent brain-penetrant EGFR tyrosine kinase inhibitor against malignant brain tumors. *ACS Med Chem Lett.* 2020;**11**(10):1799–809.
- 109 Hegi ME, Diserens A-C, Bady P, Kamoshima Y, Kouwenhoven MCM, Delorenzi M, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib – a phase II trial. *Mol Cancer Ther.* 2011;**10**(6):1102–12.

- 110 Mellingshoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med*. 2005;**353**(19):2012–24.
- 111 Alexander BM, Trippa L, Gaffey S, Arrillaga-Romany IC, Lee EQ, Rinne ML, et al. Individualized screening trial of innovative glioblastoma therapy (INSIGHt): a Bayesian adaptive platform trial to develop precision medicines for patients with glioblastoma. *JCO Precis Oncol*. 2019;**3**:1–13.
- 112 Alexander BM, Ba S, Berger MS, Berry DA, Cavenee WK, Chang SM, et al. Adaptive global innovative learning environment for glioblastoma: GBM AGILE. *Clin Cancer Res*. 2018;**24**(4):737–43.
- 113 Rahman R, Trippa L, Lee EQ, Arrillaga-Romany I, Fell G, Touat M, et al. Inaugural results of the individualized screening trial of innovative glioblastoma therapy: a phase II platform trial for newly diagnosed glioblastoma using Bayesian adaptive randomization. *J Clin Oncol*. 2023;**41**:5524–35.
- 114 Tsimberidou A-M, Hong DS, Wheler JJ, Falchook GS, Janku F, Naing A, et al. Long-term overall survival and prognostic score predicting survival: the IMPACT study in precision medicine. *J Hematol Oncol*. 2019;**12**(1):145.
- 115 Tsimberidou AM, Hong DS, Fu S, Karp DD, Piha-Paul S, Kies MS, et al. Precision medicine: preliminary results from the initiative for molecular profiling and advanced cancer therapy 2 (IMPACT2) study. *NPJ Precis Oncol*. 2021;**5**(1):21.
- 116 Taylor JW, Parikh M, Phillips JJ, James CD, Molinaro AM, Butowski NA, et al. Phase-2 trial of palbociclib in adult patients with recurrent RB1-positive glioblastoma. *J Neurooncol*. 2018;**140**(2):477–83.
- 117 Cen L, Carlson BL, Schroeder MA, Ostrem JL, Kitange GJ, Mladek AC, et al. p16-Cdk4-Rb axis controls sensitivity to a cyclin-dependent kinase inhibitor PD0332991 in glioblastoma xenograft cells. *Neuro Oncol*. 2012;**14**(7):870–81.
- 118 Leichsenring J, Horak P, Kreutzfeldt S, Heining C, Christopoulos P, Volckmar A-L, et al. Variant classification in precision oncology. *Int J Cancer*. 2019;**145**(11):2996–3010.
- 119 Horak P, Heining C, Kreutzfeldt S, Hutter B, Mock A, Hülle J, et al. Comprehensive genomic and transcriptomic analysis for guiding therapeutic decisions in patients with rare cancers. *Cancer Discov*. 2021;**11**(11):2780–95.
- 120 Von Hoff DD, Stephenson JJ, Rosen P, Loesch DM, Borad MJ, Anthony S, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol*. 2010;**28**(33):4877–83.
- 121 Mock A, Heilig CE, Kreutzfeldt S, Huebschmann D, Heining C, Schröck E, et al. Community-driven development of a modified progression-free survival ratio for precision oncology. *ESMO Open*. 2019;**4**(6):e000583.
- 122 Renovanz M, Kurz SC, Rieger J, Walter B, Becker H, Hille H, et al. Clinical outcome of biomarker-guided therapies in adult patients with tumors of the nervous system. *Neurooncol Adv*. 2023;**5**(1):vdad012.
- 123 Padovan M, Maccari M, Bosio A, De Toni C, Vizzaccaro S, Cestonaro I, et al. Actionable molecular alterations in newly diagnosed and recurrent IDH1/2 wild-type glioblastoma patients and therapeutic implications: a large mono-institutional experience using extensive next-generation sequencing analysis. *Eur J Cancer*. 2023;**191**:112959.
- 124 Blumenthal DT, Dvir A, Lossos A, Tzuk-Shina T, Lior T, Limon D, et al. Clinical utility and treatment outcome of comprehensive genomic profiling in high grade glioma patients. *J Neurooncol*. 2016;**130**(1):211–9.
- 125 Byron SA, Tran NL, Halperin RF, Phillips JJ, Kuhn JG, de Groot JF, et al. Prospective feasibility trial for genomics-informed treatment in recurrent and progressive glioblastoma. *Clin Cancer Res*. 2018;**24**(2):295–305.
- 126 Lazaridis L, Schmidt T, Oster C, Blau T, Pierscianek D, Siveke JT, et al. Precision neuro-oncology: a pilot analysis of personalized treatment in recurrent glioma. *J Cancer Res Clin Oncol*. 2023;**149**(7):3513–26.
- 127 Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med*. 2014;**370**(8):709–22.
- 128 Van Den Bent M, Eoli M, Sepulveda JM, Smits M, Walenkamp A, Frenel J-S, et al. INTELLANCE 2/EORTC 1410 randomized phase II study of Depatux-M alone and with temozolomide vs temozolomide or lomustine in recurrent EGFR amplified glioblastoma. *Neuro Oncol*. 2020;**22**(5):684–93.
- 129 Lorenz J, Rothhammer-Hampl T, Zoubaa S, Bumès E, Pukrop T, Kölbl O, et al. A comprehensive DNA panel next generation sequencing approach supporting diagnostics and therapy prediction in neurooncology. *Acta Neuropathol Commun*. 2020;**8**(1):124.
- 130 Kessler T, Berberich A, Casalini B, Drüschrer K, Ostermann H, Dormann A, et al. Molecular profiling-based decision for targeted therapies in IDH wild-type glioblastoma. *Neurooncol Adv*. 2020;**2**(1):vdz060.
- 131 Luger A-L, König S, Samp PF, Urban H, Divé I, Burger MC, et al. Molecular matched targeted therapies for primary brain tumors—a single center retrospective analysis. *J Neurooncol*. 2022;**159**(2):243–59.
- 132 Rodon J, Soria J-C, Berger R, Miller WH, Rubin E, Kugel A, et al. Genomic and transcriptomic profiling

- expands precision cancer medicine: the WINTHER trial. *Nat Med*. 2019;**25**(5):751–8.
- 133 Migliozi S, Oh YT, Hasanain M, Garofano L, D'Angelo F, Najac RD, et al. Integrative multi-omics networks identify PKC δ and DNA-PK as master kinases of glioblastoma subtypes and guide targeted cancer therapy. *Nat Cancer*. 2023;**4**(2):181–202.
- 134 Mair MJ, Bartsch R, Le Rhun E, Berghoff AS, Brastianos PK, Cortes J, et al. Understanding the activity of antibody–drug conjugates in primary and secondary brain tumours. *Nat Rev Clin Oncol*. 2023;**20**(6):372–89.
- 135 Karschnia P, Teske N, Thon N, Subklewe M, Tonn J-C, Dietrich J, et al. Chimeric antigen receptor T cells for glioblastoma: current concepts, challenges, and future perspectives. *Neurology*. 2021;**97**(5):218–30.
- 136 Burger MC, Forster M-T, Romanski A, Straßheimer F, Macas J, Zeiner PS, et al. Intracranial injection of NK cells engineered with a HER2-targeted chimeric antigen receptor in patients with recurrent glioblastoma. *Neuro Oncol*. 2023;**25**:2058–71.
- 137 Coy S, Lee JS, Chan SJ, Woo T, Jones J, Alexandrescu S, et al. Systematic characterization of antibody-drug conjugate targets in central nervous system tumors. *Neuro Oncol*. 2024;**26**:458–72.
- 138 Rothwell DG, Ayub M, Cook N, Thistlethwaite F, Carter L, Dean E, et al. Utility of ctDNA to support patient selection for early phase clinical trials: the TARGET study. *Nat Med*. 2019;**25**(5):738–43.
- 139 Zachariah MA, Oliveira-Costa JP, Carter BS, Stott SL, Nahed BV. Blood-based biomarkers for the diagnosis and monitoring of gliomas. *Neuro Oncol*. 2018;**20**(9):1155–61.
- 140 Aldea M, Andre F, Marabelle A, Dogan S, Barlesi F, Soria J-C. Overcoming resistance to tumor-targeted and immune-targeted therapies. *Cancer Discov*. 2021;**11**(4):874–99.
- 141 Kim Y, Armstrong TS, Gilbert MR, Celiku O. A critical analysis of neuro-oncology clinical trials. *Neuro Oncol*. 2023;**25**(9):1658–71.
- 142 Song K-W, Wen PY. Novel trial designs in neuro-oncology. *Curr Opin Neurol*. 2023;**36**:571–8. <https://doi.org/10.1097/WCO.0000000000001210>
- 143 Karimi E, Yu MW, Maritan SM, Perus LJM, Rezanejad M, Sorin M, et al. Single-cell spatial immune landscapes of primary and metastatic brain tumours. *Nature*. 2023;**614**(7948):555–63.
- 144 Soffietti R, Bettgowda C, Mellingshoff IK, Warren KE, Ahluwalia MS, De Groot JF, et al. Liquid biopsy in gliomas: a RANO review and proposals for clinical applications. *Neuro Oncol*. 2022;**24**(6):855–71.
- 145 Maire CL, Fuh MM, Kaulich K, Fita KD, Stevic I, Heiland DH, et al. Genome-wide methylation profiling of glioblastoma cell-derived extracellular vesicle DNA allows tumor classification. *Neuro Oncol*. 2021;**23**(7):1087–99.
- 146 Tzaridis T, Reiners KS, Weller J, Bachurski D, Schäfer N, Schaub C, et al. Analysis of serum miRNA in glioblastoma patients: CD44-based enrichment of extracellular vesicles enhances specificity for the prognostic signature. *Int J Mol Sci*. 2020;**21**(19):E7211.
- 147 Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, et al. Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. *Nat Commun*. 2015;**6**:6999.
- 148 Batool SM, Muralidharan K, Hsia T, Falotico S, Gambin AS, Rosenfeld YB, et al. Highly sensitive EGFRvIII detection in circulating extracellular vesicle RNA of glioma patients. *Clin Cancer Res*. 2022;**28**(18):4070–82.
- 149 Westphal M, Lamszus K. Circulating biomarkers for gliomas. *Nat Rev Neurol*. 2015;**11**(10):556–66.
- 150 Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM, Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. *Nat Rev Neurol*. 2018;**14**(8):482–95.
- 151 Yekula A, Yekula A, Muralidharan K, Kang K, Carter BS, Balaj L. Extracellular vesicles in glioblastoma tumor microenvironment. *Front Immunol*. 2019;**10**:3137.
- 152 Zhan Q, Yi K, Cui X, Li X, Yang S, Wang Q, et al. Blood exosomes-based targeted delivery of cPLA2 siRNA and metformin to modulate glioblastoma energy metabolism for tailoring personalized therapy. *Neuro Oncol*. 2022;**24**(11):1871–83.
- 153 Rehman FU, Liu Y, Zheng M, Shi B. Exosomes based strategies for brain drug delivery. *Biomaterials*. 2023;**293**:121949.
- 154 Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med*. 2014;**370**(8):699–708.
- 155 Batchelor TT, Mulholland P, Neyns B, Nabors LB, Campone M, Wick A, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol*. 2013;**31**(26):3212–8.
- 156 Batchelor TT, Won M, Chakravarti A, Hadjipanayis CG, Shi W, Ashby LS, et al. NRG/RTOG 0837: randomized, phase II, double-blind, placebo-controlled trial of chemoradiation with or without cediranib in newly diagnosed glioblastoma. *Neurooncol Adv*. 2023;**5**(1):vdad116.
- 157 Nabors LB, Fink KL, Mikkelsen T, Grujicic D, Tarnawski R, Nam DH, et al. Two cilengitide regimens in combination with standard treatment for patients with newly diagnosed glioblastoma and unmethylated MGMT gene promoter: results of the

- open-label, controlled, randomized phase II CORE study. *Neuro Oncol.* 2015;**17**(5):708–17.
- 158 Sandmann T, Bourgon R, Garcia J, Li C, Cloughesy T, Chinot OL, et al. Patients with proneural glioblastoma may derive overall survival benefit from the addition of bevacizumab to first-line radiotherapy and temozolomide: retrospective analysis of the AVAglio trial. *J Clin Oncol.* 2015;**33**(25):2735–44.
- 159 Kessler T, Schrimpf D, Doerner L, Hai L, Kaulen LD, Ito J, et al. Prognostic markers of DNA methylation and NGS sequencing in progressive glioblastoma from the EORTC-26101 trial. *Clin Cancer Res.* 2023;**29**:3892–900.
- 160 Weller J, Zeyen T, Schäfer N, Schaub C, Potthoff A-L, Steinbach JP, et al. The proneural subtype is not associated with survival benefit from bevacizumab in newly diagnosed glioblastoma: a secondary analysis of the GLARIUS trial. *J Neurooncol.* 2023;**164**(3):749–55.
- 161 Liao LM, Ashkan K, Brem S, Campian JL, Trusheim JE, Iwamoto FM, et al. Association of autologous tumor lysate-loaded dendritic cell vaccination with extension of survival among patients with newly diagnosed and recurrent glioblastoma: a phase 3 prospective externally controlled cohort trial. *JAMA Oncol.* 2022;**9**:112–21.
- 162 Ahluwalia MS, Reardon DA, Abad AP, Curry WT, Wong ET, Figel SA, et al. Phase IIa study of SurVaxM plus adjuvant temozolomide for newly diagnosed glioblastoma. *J Clin Oncol.* 2023;**41**(7):1453–65.
- 163 Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanović S, Gouttefangeas C, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature.* 2019;**565**(7738):240–5.
- 164 Schmitt MJ, Company C, Dramaretska Y, Barozzi I, Göhrig A, Kertalli S, et al. Phenotypic mapping of pathologic cross-talk between glioblastoma and innate immune cells by synthetic genetic tracing. *Cancer Discov.* 2021;**11**(3):754–77.
- 165 Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med.* 2013;**19**(10):1264–72.
- 166 Butowski N, Colman H, De Groot JF, Omuro AM, Nayak L, Wen PY, et al. Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study. *Neuro Oncol.* 2016;**18**(4):557–64.
- 167 Akkari L, Bowman RL, Tessier J, Klemm F, Handgraaf SM, de Groot M, et al. Dynamic changes in glioma macrophage populations after radiotherapy reveal CSF-1R inhibition as a strategy to overcome resistance. *Sci Transl Med.* 2020;**12**(552):eaaw7843.
- 168 Giordano FA, Link B, Glas M, Herrlinger U, Wenz F, Umansky V, et al. Targeting the post-irradiation tumor microenvironment in glioblastoma via inhibition of CXCL12. *Cancers (Basel).* 2019;**11**(3):272.
- 169 Thomas RP, Nagpal S, Iv M, Soltys SG, Bertrand S, Pelpola JS, et al. Macrophage exclusion after radiation therapy (MERT): a first in human phase I/II trial using a CXCR4 inhibitor in glioblastoma. *Clin Cancer Res.* 2019;**25**(23):6948–57.
- 170 Lin Y-J, Wu CY-J, Wu JY, Lim M. The role of myeloid cells in GBM immunosuppression. *Front Immunol.* 2022;**13**:887781.
- 171 Weiss T, Puca E, Silgner M, Hemmerle T, Pazahr S, Bink A, et al. Immunocytokines are a promising immunotherapeutic approach against glioblastoma. *Sci Transl Med.* 2020;**12**(564):eabb2311.
- 172 Look T, Puca E, Bühler M, Kirschenbaum D, De Luca R, Stucchi R, et al. Targeted delivery of tumor necrosis factor in combination with CCNU induces a T cell-dependent regression of glioblastoma. *Sci Transl Med.* 2023;**15**(697):eadf2281.
- 173 Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, et al. Neuronal activity promotes glioma growth through neuropilin-3 secretion. *Cell.* 2015;**161**(4):803–16.
- 174 Osswald M, Jung E, Sahn F, Solecki G, Venkataramani V, Blaes J, et al. Brain tumour cells interconnect to a functional and resistant network. *Nature.* 2015;**528**(7580):93–8.
- 175 Weil S, Osswald M, Solecki G, Grosch J, Jung E, Lemke D, et al. Tumor microtubules convey resistance to surgical lesions and chemotherapy in gliomas. *Neuro Oncol.* 2017;**19**(10):1316–26.
- 176 Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature.* 2019;**573**(7775):532–8.
- 177 Venkataramani V, Yang Y, Schubert MC, Reyhan E, Tetzlaff SK, Wißmann N, et al. Glioblastoma hijacks neuronal mechanisms for brain invasion. *Cell.* 2022;**185**(16):2899–2917.e31.
- 178 Hausmann D, Hoffmann DC, Venkataramani V, Jung E, Horschitz S, Tetzlaff SK, et al. Autonomous rhythmic activity in glioma networks drives brain tumour growth. *Nature.* 2023;**613**(7942):179–86.
- 179 Potthoff A-L, Heiland DH, Evert BO, Almeida FR, Behringer SP, Dolf A, et al. Inhibition of gap junctions sensitizes primary glioblastoma cells for temozolomide. *Cancers (Basel).* 2019;**11**(6):858.
- 180 Schneider M, Vollmer L, Potthoff A-L, Ravi VM, Evert BO, Rahman MA, et al. Meclofenamate causes loss of cellular tethering and decoupling of functional

- networks in glioblastoma. *Neuro Oncol.* 2021;**23**(11):1885–97.
- 181 Zeyen T, Potthoff A-L, Nemeth R, Heiland DH, Burger MC, Steinbach JP, et al. Phase I/II trial of meclofenamate in progressive MGMT-methylated glioblastoma under temozolomide second-line therapy—the MecMeth/NOA-24 trial. *Trials.* 2022;**23**(1):57.
- 182 Venkatesh HS, Tam LT, Woo PJ, Lennon J, Nagaraja S, Gillespie SM, et al. Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. *Nature.* 2017;**549**(7673):533–7.
- 183 Heuer S, Burghaus I, Gose M, Kessler T, Sahn F, Vollmuth P, et al. PerSurge (NOA-30) phase II trial of perampanel treatment around surgery in patients with progressive glioblastoma. *BMC Cancer.* 2024;**24**(1):135.
- 184 Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med.* 2019;**25**(5):744–50.
- 185 van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MCM, Kros JM, Carpentier AF, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol.* 2009;**27**(8):1268–74.
- 186 Lassman AB, Rossi MR, Raizer JJ, Abrey LE, Lieberman FS, Greife CN, et al. Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from North American Brain Tumor Consortium Trials 01-03 and 00-01. *Clin Cancer Res.* 2005;**11**(21):7841–50.
- 187 Prados MD, Chang SM, Butowski N, DeBoer R, Parvataneni R, Carliner H, et al. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J Clin Oncol.* 2009;**27**(4):579–84.
- 188 Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol.* 2004;**22**(1):133–42.
- 189 Chakravarti A, Wang M, Robins HI, Lautenschlaeger T, Curran WJ, Brachman DG, et al. RTOG 0211: a phase 1/2 study of radiation therapy with concurrent gefitinib for newly diagnosed glioblastoma patients. *Int J Radiat Oncol Biol Phys.* 2013;**85**(5):1206–11.
- 190 Vivanco I, Robins HI, Rohle D, Campos C, Grommes C, Nghiemphu PL, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. *Cancer Discov.* 2012;**2**(5):458–71.
- 191 Thiessen B, Stewart C, Tsao M, Kamel-Reid S, Schaiquevich P, Mason W, et al. A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: clinical outcomes, pharmacokinetics and molecular correlation. *Cancer Chemother Pharmacol.* 2010;**65**(2):353–61.
- 192 Arrillaga-Romany I, Trippa L, Fell G, Lee EQ, Rahman R, Touat M, et al. CTNI-05. Preliminary results of the neratinib arm in the individualized screening trial of innovative glioblastoma therapy (insight): a phase II platform trial using Bayesian adaptive randomization. *Neuro Oncol.* 2021;**23**(Suppl 6):vi59.
- 193 Reardon DA, Nabors LB, Mason WP, Perry JR, Shapiro W, Kavan P, et al. Phase I/randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma. *Neuro Oncol.* 2015;**17**(3):430–9.
- 194 Westphal M, Heese O, Steinbach JP, Schnell O, Schackert G, Mehdorn M, et al. A randomised, open label phase III trial with nimotuzumab, an anti-epidermal growth factor receptor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma. *Eur J Cancer.* 2015;**51**(4):522–32.
- 195 Neyns B, Sadones J, Joosens E, Bouttens F, Verbeke L, Baurain J-F, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. *Ann Oncol.* 2009;**20**(9):1596–603.
- 196 Sautter L, Hofheinz R, Tuettenberg J, Grimm M, Vajkoczy P, Groden C, et al. Open-label phase II evaluation of imatinib in primary inoperable or incompletely resected and recurrent glioblastoma. *Oncology.* 2020;**98**(1):16–22.
- 197 Reardon DA, Dresemann G, Taillibert S, Campone M, van den Bent M, Clement P, et al. Multicentre phase II studies evaluating imatinib plus hydroxyurea in patients with progressive glioblastoma. *Br J Cancer.* 2009;**101**(12):1995–2004.
- 198 Mehta S, Fiorelli R, Bao X, Pennington-Krygier C, Derogatis A, Kim S, et al. A phase 0 trial of ceritinib in patients with brain metastases and recurrent glioblastoma. *Clin Cancer Res.* 2022;**28**(2):289–97.
- 199 Muhic A, Poulsen HS, Sorensen M, Grunnet K, Lassen U. Phase II open-label study of nintedanib in patients with recurrent glioblastoma multiforme. *J Neurooncol.* 2013;**111**(2):205–12.
- 200 Wen PY, Drappatz J, de Groot J, Prados MD, Reardon DA, Schiff D, et al. Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients naive to antiangiogenic therapy. *Neuro Oncol.* 2018;**20**(2):249–58.
- 201 Cloughesy TF, Drappatz J, de Groot J, Prados MD, Reardon DA, Schiff D, et al. Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients with prior antiangiogenic therapy. *Neuro Oncol.* 2018;**20**(2):259–67.

- 202 Martínez-García M, Velasco G, Pineda E, Gil-Gil M, Alameda F, Capellades J, et al. Safety and efficacy of Crizotinib in combination with temozolomide and radiotherapy in patients with newly diagnosed glioblastoma: phase Ib GEINO 1402 trial. *Cancers (Basel)*. 2022;**14**(10):2393.
- 203 Chinnaiyan P, Won M, Wen PY, Rojiani AM, Werner-Wasik M, Shih HA, et al. A randomized phase II study of everolimus in combination with chemoradiation in newly diagnosed glioblastoma: results of NRG oncology RTOG 0913. *Neuro Oncol*. 2018;**20**(5):666–73.
- 204 Wen PY, De Groot JF, Battiste J, Goldlust SA, Garner JS, Friend J, et al. Paxalisib in patients with newly diagnosed glioblastoma with unmethylated MGMT promoter status: final phase 2 study results. *J Clin Oncol*. 2022;**40**(16 Suppl):2047.
- 205 Wen PY, Touat M, Alexander BM, Mellinghoff IK, Ramkissoon S, McCluskey CS, et al. Buparlisib in patients with recurrent glioblastoma harboring phosphatidylinositol 3-kinase pathway activation: an open-label, multicenter, multi-arm, phase II trial. *J Clin Oncol*. 2019;**37**(9):741–50.
- 206 Sim H-W, McDonald KL, Lwin Z, Barnes EH, Rosenthal M, Foote MC, et al. A randomized phase II trial of veliparib, radiotherapy, and temozolomide in patients with unmethylated MGMT glioblastoma: the VERTU study. *Neuro Oncol*. 2021;**23**(10):1736–49.
- 207 Sloan AE, Nock CJ, Ye X, Buerki R, Chang S, Lesser G, et al. ABTC-0904: targeting glioma stem cells in GBM: a phase 0/II study of hedgehog pathway inhibitor GDC-0449. *J Neurooncol*. 2023;**161**(1):33–43.
- 208 Solomón MT, Selva JC, Figueredo J, Vaquer J, Toledo C, Quintanal N, et al. Radiotherapy plus nimotuzumab or placebo in the treatment of high grade glioma patients: results from a randomized, double blind trial. *BMC Cancer*. 2013;**13**(1):299.
- 209 Rosenthal M, Clement PM, Campone M, Gil-Gil MJ, DeGroot J, Chinot O, et al. Buparlisib plus carboplatin or lomustine in patients with recurrent glioblastoma: a phase Ib/II, open-label, multicentre, randomised study. *ESMO Open*. 2020;**5**(4):e000672.
- 210 Cloughesy T, Ellingson B, Gafita A, Chun S, Filka E, Nghiemphu P, et al. CTNI-14. Evaluating metabolic alterations in patients with EGFR activated recurrent glioblastoma (RGBM) by inhibiting EGFR with osimertinib. *Neuro Oncol*. 2021;**23**(Suppl 6):vi62.
- 211 Chi AS, Cahill DP, Reardon DA, Wen PY, Mikkelsen T, Peereboom DM, et al. Exploring predictors of response to dacomitinib in EGFR-amplified recurrent glioblastoma. *JCO Precis Oncol*. 2020;**4**:593–613.
- 212 O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrisette JJD, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med*. 2017;**9**(399):eaaa0984.
- 213 Goff SL, Morgan RA, Yang JC, Sherry RM, Robbins PF, Restifo NP, et al. Pilot trial of adoptive transfer of chimeric antigen receptor-transduced T cells targeting EGFRvIII in patients with glioblastoma. *J Immunother*. 2019;**42**(4):126–35.
- 214 Reardon DA, Desjardins A, Vredenburgh JJ, O'Rourke DM, Tran DD, Fink KL, et al. Rindopepimut with bevacizumab for patients with relapsed EGFRvIII-expressing glioblastoma (ReACT): results of a double-blind randomized phase II trial. *Clin Cancer Res*. 2020;**26**(7):1586–94.
- 215 van den Bent M, Azaro A, De Vos F, Sepulveda J, Yung WKA, Wen PY, et al. A phase Ib/II, open-label, multicenter study of INC280 (capmatinib) alone and in combination with buparlisib (BKM120) in adult patients with recurrent glioblastoma. *J Neurooncol*. 2020;**146**(1):79–89.
- 216 Cardona AF, Jaramillo-Velásquez D, Ruiz-Patiño A, Polo C, Jiménez E, Hakim F, et al. Efficacy of osimertinib plus bevacizumab in glioblastoma patients with simultaneous EGFR amplification and EGFRvIII mutation. *J Neurooncol*. 2021;**154**(3):353–64.
- 217 Makhlin I, Salinas RD, Zhang D, Jacob F, Ming G-L, Song H, et al. Clinical activity of the EGFR tyrosine kinase inhibitor osimertinib in EGFR-mutant glioblastoma. *CNS Oncol*. 2019;**8**(3):CNS43.