Treating recurrent glioblastoma: an update

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Practice points

- Glioblastoma is the most common and aggressive primary brain tumor with a nearly universally fatal outcome.
- Basic and clinical research has led to better diagnostic techniques and therapeutics, but have only translated into a modest improvement in median survival due to a high rate of recurrence.
- More effective translation of our increasing understanding of molecular heterogeneity, tumor phenotype and tumor microenvironment into therapies is needed.
- Novel diagnostic techniques, treatments for recurrent disease and delivery of drugs are under investigation.

SUMMARY Glioblastoma, the most aggressive of the gliomas, has a high recurrence and mortality rate. The nature of this poor prognosis resides in the molecular heterogeneity and phenotypic features of this tumor. Despite research advances in understanding the molecular biology, it has been difficult to translate this knowledge into effective treatment. Nearly all will have tumor recurrence, yet to date very few therapies have established efficacy as salvage regimens. This challenge is further complicated by imaging confounders and to an even greater degree by the ever increasing molecular heterogeneity that is thought to be both sporadic and treatment-induced. The development of novel clinical trial designs to support the development and testing of novel treatment regimens and drug delivery strategies underscore the need for more precise techniques in imaging and better surrogate markers to help determine treatment response. This review summarizes recent approaches to treat patients with recurrent glioblastoma and considers future perspectives.

Glioblastoma is the most common primary malignant brain tumor in adults. Despite advanced diagnostic modalities and optimal multidisciplinary treatment that typically includes maximal surgical resection, conformal radiation and systemic chemotherapy, almost all patients experience tumor progression or recurrence with nearly universal mortality. The median survival for most patients from the time of diagnosis is less than 15 months, with a 2-year survival rate of 26–33% [1,2]. Molecular heterogeneity and inherent or acquired resistance to treatment are the greatest challenges in developing effective treatment for patients with recurrent glioblastoma. The following review summarizes the main recent advances in understanding the biology of glioblastoma, new diagnostic approaches and the results of recent therapeutic clinical trials finishing with future directions that should lead to an improved outcome for these patients

KEYWORDS

- bevacizumab
- glioblastoma
- immunotherapy
- lomustine • oncolytic viruses • pseudoprogression
- recurrent glioma
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Pseudoprogression

Testing novel therapies for patients with recurrent glioblastoma requires precise evaluation of tumor response. This evaluation of therapy response is typically highly dependent on MRI findings. The

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recognition that frontline treatment, particularly with the well established concurrent chemotherapy and radiation, may cause imaging changes that emulate tumor progression, now known as pseudoprogression (Figure 1). This is a transient postradiation treatment effect accounting for 50% of the cases of increased contrast enhancement and T2/FLAIR hyperintensity during the first month after radiation and 20–30% over the first 3 months as a consequence of increased vascular permeability [3]. This early and exaggerated MRI changes is typically observed in the setting of radiation therapy with temozolomide, and thought due to the radiosensitization effect of temozolomide [4]. Pseudoprogression may be associated with significantly improved survival, independently of its association with *MGMT* gene promoter methylation [5]. Advanced imaging techniques are being investigated and include MR spectroscopy, diffusion-weighted MRI, ^{18}F -fluorodeoxyglucose (FDG)-PET, MR perfusion imaging (with dynamic susceptibility contrast technique) and diffusion-tensor imaging. To date, there are no neuroradiological techniques to distinguish postradiation treatment effects from progressive disease. Pseudoprogression may confound the conduct of a clinical trial, as its characteristic spontaneous improvement may lead to the (false) determination of treatment response. Furthermore, bevacizumab, approved in the USA for recurrent glioblastoma, use may further complicate the MRI interpretation in both recurrent glioblastoma and pseudoprogression [6–8] due to normalization of tumor vasculature with an associated decrease of contrast leakage (T1 enhancement) and treating radiation necrosis, respectively (Figure 2).

As described above, interpretation of conventional MRI may be confounded by nonspecific imaging changes; therefore there is increasing interest in developing new, more specific techniques. Novel tracers for positron emission tomography (PET) are under active investigation for evaluation of recurrent disease, including 3'-deoxy-3'- ^{18}F -fluoro-thymidine (^{18}F -FLT)-PET [9] and amino acid tracers such as ^{11}C -methionine (C-Met)-PET [10], O-(2-[^{18}F] fluoroethyl)-L-tyrosine (FET)-PET [11], and ^{18}F -labeled dopamine precursor (FDOPA)-PET [12]. These studies have demonstrated sensitivities close to 100% and specificity ranging from 60 to 93% for tumor recurrence. However, to date, no studies were designed to clearly distinguish between recurrent disease and postradiation treatment

effects, except for two small studies that demonstrated efficacy in distinguishing recurrent disease from such effects using ^{13}N - NH_3 -PET [13] or dynamic susceptibility contrast perfusion-MR with ferumoxytol [14]. To highlight this concern, the Response Assessment in Neuro-Oncology Working Group recognizing the difficulty of distinguishing tumor from pseudoprogression within the first 12 weeks after radiation treatment recommended exclusion of such patients from clinical trials, unless there is pathologic confirmation of progression or the radiological findings are clearly outside the radiation field (beyond the high-dose region or 80% isodose line) [3].

Imaging advances

MRI used with addition of contrast agents is the standard for detection, delineation and response assessment of brain tumors [15]. Thus, regions of hyperintensity on postcontrast T1-weighted images are thought to reflect the most aggressive portion of the tumor, which has been consequently been confirmed by biopsy [16,17]. In 2010, the Response Assessment in Neuro-Oncology criteria included the evaluation of nonenhancing tumor progression, definitions of progression for patients being considered for enrollment in clinical trials, pseudoprogression and response to treatment [3]. However, minor differences in hardware or in sequence timing may result in significant changes in image contrast and tumor measurements. Contrast-enhanced T1-weighted images show even higher variability, it will depend to the timing, dose and type of contrast agent used. This variability may be decreased with 3D volumetric contrast-enhancing tumor measurements [18]. A study comparing 1D, 2D and 3D tumor measurements in childhood brain tumors showed poor concordance between 3D and 1D/2D measurements of 61–66% [19]. This is likely due to difficulty to measure the exact margins and diameters as well as the tendency of 1D/2D measurements to overestimate tumor volume, leading to larger estimates of tumor burden at baseline with consequent longer progression-free survival (PFS). 3D T1-weighted MR images may have a significant impact on the evaluation of tumor progression and therapeutic benefit, allowing a better separation of responders and nonresponders on Kaplan–Meier curves [20]. Emerging techniques that helped physicians to image and guide treatment of brain tumors include diffusion MRI, perfusion MRI

and amino acid PET scans. Diffusion MRI has shown to predict the degree of malignancy [21], the response to cytotoxic therapy [22] and antiangiogenic therapy [23]. Perfusion MRI has also been investigated as potential biomarker for early response to radiation therapy [24] and to assess response to antiangiogenic therapy [25] while amino acid PET scan detects the accumulation of amino acid tracers in regions of active tumor [26] and treatment response based on coefficient of variance of tumor to brain [27]. As the infiltrative component of glioblastoma often represents the location of recurrence, improved visualization by fluorescence could change the surgical approach. Advances in imaging the border of the tumor include 5-ALA protoporphyrin IX (PpIX) fluorescence. A multicenter Phase III trial on 322 high-grade glioma patients in Germany showed an increase of gross total resection from 36 to 65% in favor of PpIX fluorescence, subsequently demonstrated better 6m-PFS of 41 (5-ALA) versus 21.1% (control) with no difference in severity and frequency of side-effects [28].

Treatment of recurrent disease

Tumor recurrence is nearly universal in glioblastoma [29]. Attempts to individualize treatment, as has been successfully done with non-small-cell lung cancer (targeting a specific *EGFR* mutation) or breast cancer (targeting HER-2 overexpression), has been unsuccessful to date. It is increasingly recognized that the initial tumor molecular profile may not represent the tumor at recurrence. Natural and treatment-induced genomic and epigenetic changes confound the use of early molecular profiles for treatment choice. Unfortunately, with few exceptions a repeat tumor biopsy for molecular analysis is not yet considered an essential component for clinical care.

There is no real standard of care for recurrent glioblastoma, and most studies report a limited response rate and when present, of short duration. As such, the median PFS and overall survival (OS) for recurrent glioblastoma are 10 weeks and 30 weeks, respectively [30]. The survival after resection of recurrent glioblastoma remains poor [31] and there is little prospective evidence suggesting that the addition of surgery provides additional benefit to re-irradiation and/or salvage chemotherapy. One retrospective study demonstrated an OS benefit (19 months) when the resection exceeds 80% of the volume of recurrent glioblastoma [32]. Only one prospective

Figure 1. MRI from a patient with pseudoprogression and resection confirmed radiation necrosis. (A) T1-weighted gadolinium-enhanced MRI and **(B)** T2-weighted and FLAIR postoperatively and 4 weeks postradiation showing large areas of contrast enhancement anterior to the surgical cavity (within the radiation field) **(C)** associated with vasogenic edema **(D)**. Surgery was performed and revealed extensive necrosis with no evidence of tumor. FLAIR: Fluid-attenuated inversion recovery; Gd: Gadolinium.

study demonstrated a higher median OS after undergoing multiple microsurgical resections for recurrent disease in patients with PFS greater than 3 months, compared to those who were treated nonsurgically (26 vs 16 months; $p = 0.052$) [33]. Other factors such as tumor volume and Karnofsky performance status may additionally influence outcomes following repeat surgery [34]. There may be additional benefits to consider regarding repeat tumor resection including the definitive diagnosis of tumor (vs treatment effect), obtaining a contemporary tumor sample for molecular analysis and potential introduction of therapy such as the introduction of the carmustine wafers (Gliadel, Eisai Inc., USA) [35]. Drug delivery remains an important limiting factor in treatment efficacy. Efforts to circumvent this issue include intratumoral implants, intra-arterial delivery of drugs, convection-enhanced delivery, nanoparticles and stem cell/mesenchymal cell-based deliveries.

Re-irradiation

Re-irradiation was initially believed to be contraindicated to treat recurrent disease because of

Figure 2. MRI from a patient with pseudoresponse during treatment with bevacizumab. A 33-year-old man with recurrent glioblastoma receiving bevacizumab. Axial T2 weighted and FLAIR sequence in the upper row (**A**, arrows) shows T2/FLAIR hyperintensity involving the region immediately adjacent to the resection cavity within the right frontal and parietal lobes, with no involvement of the corpus callosum (**C**). Six months after treatment with bevacizumab (lower row), there is extension of the abnormal signal to the right posterior parietal lobe as well as to the posterior body and splenium of the corpus callosum (**E & G**, arrows) with no contrast enhancement (**F & H**), suggesting nonenhancing tumor progression. Corresponding postcontrast images obtained at the same time points (**B, D, F & H**) indicate stable/unchanged enhancing component with a subtle foci posterior to the resection cavity (**B & F**). FLAIR: Fluid-attenuated inversion recovery; Gd: Gadolinium.

concern regarding toxicity to normal brain parenchyma. Recent data showed that re-irradiation is likely safe and may improve survival although this has not been verified in a randomized trial [36]. Based on retrospective series, re-irradiation with fractionated stereotactic radiosurgery can be beneficial, with 6-month OS of 79% and 1-year OS of 30% [37], and no significant treatment-related toxicity seen in follow-ups. Furthermore, there is increasing experience, albeit anecdotal that suggests that using bevacizumab with re-irradiation may provide some neuroprotection. In the retrospective series, the median radiation dose was 36 Gy in 18 fractions, delivered using 3D conformal radiotherapy (3D-CRT) or intensity-modulated radiation therapy. Patients who received bevacizumab showed improved postrecurrence survival (median: 8.6 vs 5.7 months; $p = 0.003$) and post-recurrence PFS (median: 5.6 vs 2.5 months; $p = 0.005$; 6-month PFS 42.1% for the bevacizumab group). The overall toxicity was not higher than the use of sole re-irradiation and bevacizumab alone, suggesting

that this regimen may be an effective salvage therapy; however, further investigation with randomized controlled trials are needed [38]. An ongoing randomized Phase II trial investigates the OS comparing concurrent bevacizumab and re-irradiation (35 Gy in 15 fractions) using intensity-modulated radiation therapy, 3D-CRT or proton beam radiation therapy with bevacizumab alone in patients with recurrent glioblastoma (RTOG 1205, ClinicalTrials.gov identifier: NCT01730950 [114]).

Chemotherapy

Chemotherapy options for recurrent glioblastoma are still limited. Chemotherapy drugs tested as salvage therapy continue to show disappointing results. These include alkylating agents such as temozolomide [39–42], nitrosoureas (carmustine/lomustine/fotemustine) [43,44] and procarbazine [45–47]; topoisomerase inhibitors (irinotecan/etoposide) [48,49], platinoids [50–54], vincristine, [55–57] and estrogen receptor antagonist [55].

Alkylating agents

• Nitrosoureas

Nitrosoureas are routinely used as salvage therapy and still play an important role in the treatment of recurrent glioblastoma. In Phase II trials, nitrosoureas (carmustine, lomustine and fotemustine) as single-agents and in combination, such as procarbazine, lomustine and vincristine (PCV), have shown activity in recurrent glioblastoma with the 6-month PFS rate ranging between 18 and 52% [44,47,56]. A Phase III trial compared the targeted therapeutic agent enzastaurin (an oral serine threonine kinase inhibitor involved in angiogenesis) with lomustine established the efficacy of lomustine in the treatment of recurrent disease as it was superior to the experimental agent [57]. The REGAL study, a Phase III trial compared cediranib (an oral pan-VEGF receptor tyrosine kinase inhibitor) as monotherapy or in combination with lomustine. It did not meet the primary end point to prolong the PFS when comparing with lomustine alone [58]. Another Phase III trial compared PCV with temozolomide in 447 patients with recurrent glioblastoma and anaplastic astrocytoma following initial treatment with radiation therapy alone. There was no statistical benefit in PFS (3.6 vs 4.7 months) and OS (6.7 vs 7.2 months) with temozolomide [59].

• Temozolomide

Temozolomide is often used for rechallenge due to a good blood–brain barrier penetration and overall low toxicity profile. It has been speculated that further benefit from temozolomide therapy may depend on the presence of CpG island methylation in the *MGMT* gene promoter. *MGMT* is a DNA repair protein that reverses the damage induced by alkylating agents, representing the major mechanism of resistance to these drugs [60]. Different dosing regimens of temozolomide have been tested hypothesizing maximal suppression of *MGMT*. Dose-dense temozolomide regimen, 7-days on/7-days off, was evaluated in two Phase II studies and showed an overall response rate of 10%, 6-month PFS of 48% and median PFS of 21 weeks [61], while the other study showed a 6-month PFS of 44% and PFS of 24 weeks [62]. Metronomic-dose temozolomide at 50 mg/m²/day has been also tested and showed that long-term treatment with temozolomide is feasible and well tolerated [63]. Most recently, the Canadian RESCUE study found a 6-month PFS of 35.7% and 1-year survival

of 28.6% in patients treated with metronomic-dose temozolomide only if the tumor was previously stable and the patient was off adjuvant temozolomide for more than 2 months [64].

• Mutations induced by temozolomide therapy

The molecular heterogeneity of glioblastoma may be further complicated by continuous molecular changes that occur during the natural course of the tumor or when induced by treatment. There has been special interest had focused on mutations induced by temozolomide, the standard of care of newly diagnosed glioblastoma, in the hope of understanding and predicting post-treatment mutations in order to develop therapeutic strategies for recurrent disease. *MSH6* mutations were confirmed in post-treatment TCGA (The Cancer Genome Atlas) glioblastomas but were absent in matched pretreatment tumors. *MSH6* mutations are highly selective for glioblastomas during temozolomide therapy both *in vitro* and *in vivo* and are causally associated with temozolomide resistance [65]. Other recently reported mutations are driver mutations in the retinoblastoma (Rb) and Akt-mammalian target of rapamycin (Akt-mTOR) pathways. Driver mutations in the initial tumor such as those in *TP53*, *ATRX*, *SMARCA4* and *BRAF* were undetected at recurrence, suggesting that recurrent tumors are often seeded by cells derived from the initial tumor at a very early stage of their evolution [66]. Further investigation of glioblastoma genomics at initial diagnosis and recurrence would give us more clues to infer mutational changes and facilitate treatment.

Bevacizumab

In 2009, bevacizumab (Avastin, Genetech/Roche), an anti-VEGF inhibitor, was approved for the treatment of recurrent glioblastoma [4]. It has been administered as single-agent or in combination with cytotoxic therapy; however, neither regimen has been shown to prolong OS. Studies evaluating single-agent bevacizumab reported PFS, 6-month PFS and OS of 4–4.2 months, 29–42% and 7.8–9.2 months, respectively [67,68]. While there is no class 1 evidence to support the use of a bevacizumab-based combination regimen, the recent results from the randomized Phase II trial, BELOB provides support for this approach. The BELOB trial compared bevacizumab alone, lomustine alone with bevacizumab plus lomustine. The

9-month overall survival rate was 38, 43 and 63%, respectively [69]. These results are being validated in a Phase III trial that is currently accruing patients (ClinicalTrials.gov identifier: NCT01290939 [114]). Other bevacizumab-based regimens that have been tested include combinations with irinotecan [70], carboplatin [71,72] and etoposide [73]. However, there is no evidence that these combinations improve outcome, but clearly increase toxicity.

The optimal duration of bevacizumab therapy is not yet established. Most patients are treated until progression occurs, but in those without progression, it may be kept indefinitely. However, continuation may lead to the development of a more aggressive phenotype [74–77] while discontinuation may result in a rebound effect due to loss of anti-edema properties [78]. Some data suggest that continuation beyond initial progression modestly improves survival in recurrent glioblastoma patients [79]. Furthermore, those patients who progress despite a bevacizumab-containing regimen rarely responded to the second bevacizumab-containing chemotherapeutic regimen [80] demonstrating a median PFS of only 2 months, OS of 5.2 months and 6-month PFS of 0% [81]. However, sustained antitumor activity of bevacizumab in recurrent glioblastoma was recently evaluated by radiological findings. ‘Double-positive’, the term to describe hyperintense lesions in T1 and diffusion weighted restriction, was observed in 21 of 74 (28%) patients. OS for those with double-positive lesions was 13 months compared to those without any of these lesions, 6.6 months ($p < 0.005$) [82].

Other antiangiogenic agents were evaluated in Phase II trials including thalidomide in combination with irinotecan that showed limited antitumoral activity in recurrent glioblastoma [83,84]. Phase II trials testing small molecule antiangiogenic agents such as aflibercept, sunitinib and pazopanib have shown moderate toxicity and minimal evidence of single-agent activity in patients with recurrent glioblastoma [85–87]. Ongoing trials have been testing cedirinib, and sorafenib (ClinicalTrials.gov identifier: NCT00777153 and NCT01434602, respectively [114]).

Targeted therapy

Molecular-based targeted therapy initially emerged from cancer therapy for leukemia. Imatinib, an inhibitor of the Bcr-Abl tyrosine

kinase (necessary for its oncogenic activity), changes the natural progression of chronic myelogenous leukemia and has become the first-line treatment [88].

The molecular profiling in glioblastoma, one of the most characterized of all human cancers, has resulted in the identification of molecular prognostic factors but the potential ‘molecular vulnerabilities’ have yet to be translated into personalized treatments (Figure 3).

Alterations in the EGFR were among the first molecular abnormalities recognized in glioblastoma and are present in 40–50% of patients. Amplification and overexpression of EGFR are the most common alterations. *EGFRvIII* mutation, a partial deletion of the extracellular domain that leads to constitutive activation of the receptor of the ligand, is present in a relatively high percentage of patients as well [89]. The high frequency of EGFR amplification/overexpression and *EGFRvIII* mutations suggest that EGFR abnormalities could be drug targets. Unfortunately, EGFR tyrosine kinase inhibitors (erlotinib and gefitinib) and a monoclonal antibody against EGFR (cetuximab) have not been successful in glioblastoma [90–94]. Tumor resistance and response to EGFR inhibitors is determined by *PTEN* status. Loss of *PTEN* results in resistance to EGFR tyrosine inhibitors in glioblastoma patients who express the *EGFRvIII* mutation, maintaining the PI3K signaling pathway [95]. EGFR pathway inhibition of the intracellular domain of the receptor using drugs such as lapatinib may have greater efficacy [96]. Genetic lesions that activate the PI3K pathway occur in 90% of glioblastomas and may be changes other than alterations of *EGFR*, including RTKs, *PIK3CA* or *PTEN* loss [97]. Recognition of hyperactivation of PI3K and its key downstream effector Akt has led to targets for therapy in glioblastoma. New PI3K and Akt inhibitors are currently in early-phase clinical trials; prior agents proved to be too toxic for clinical use. Mammalian target of rapamycin (mTOR) inhibitors has become an attractive target for glioblastoma therapy. The mTOR protein, a key mediator of PI3K signaling pathway, forms two multimolecular complexes, termed mTORC1 and mTORC2 that are involved in several cell processes and are inhibited by rapamycin [98]. As mentioned above, certain mutations activating the Akt-mTORC1 signaling pathway are closely associated with temozolomide treatment, suggesting that mTORC1 hyperactivation in glioblastomas might represent a therapy-induced

Figure 3. Frequent genetic alterations in three critical signaling pathways. Significant copy number and mutation changes in (A) RAS/PI3K, (B) p53 and (C) RB signaling pathways. Red, activating genetic alterations; blue, inactivating mutations. Percentage of tumors affected are indicated next to each gene with boxes identifying the final percentage of patients with alterations within that specific pathway.

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oncogenic transformation [66]. The development of rapamycin analogs (rapalogs) has provided new opportunities for clinical trials, they include temsirolimus (Pfizer), everolimus (Novartis) and ridaforolimus (Ariad).

The recent discovery that a small subset of glioblastomas (3%) harbors an oncogenic chromosomal translocation that fuses in-frame the tyrosine kinase coding domains of *FGFR* genes (*FGFR1* or *FGFR3*) to the transforming acidic coiled-coil (TACC) coding domains of *TACC1* or *TACC3*, respectively has generated excitement

that this fusion may be 'targetable.' Oral administration of FGFR inhibitors prolonged OS in mice with FGFR3-TACC3-initiated glioma supports moving this approach [99] and clinical trials targeting multiple pathways including this fusion protein are in development for glioblastoma (Table 1).

Immunotherapy

Modulation of the immune system has emerged as a promising treatment modality in many cancers including gliomas. Glioblastoma is known

Table 1. Ongoing clinical trials of targeted therapy for recurrent glioblastoma.

Therapy	Target	Trial Phase	Trial identifier	Number of patients	Completion date	Sponsor
AMG 102 + bevacizumab	HGF/SF + VEGF	II	NCT01113398	36	02/2016	Sanofi
AMG 386	Ang 1/2	II	NCT01290263	86	12/2016	Dana-Farber Cancer Institute
AMG 386 + bevacizumab	Ang 1/2 + VEGF	II	NCT01609790	141	07/2015	National Cancer Institute
AMG 595	EGFRvIII	I	NCT01475006	60	10/2015	Amgen
APG101 + radiation therapy	CD95	II	NCT01071837	83	06/2015	Apogenix GmbH
Axitinib	VEGFR/PDGF	II	NCT01562197	52	05/2016	Bart Neyns
BKM120	PI3K	II	NCT01339052	65	04/2015	Dana-Farber Cancer Institute
BKM120 + bevacizumab	PI3K + VEGF	I/II	NCT01349660	93	12/2016	SCRI Development Innovations, LLC
BKM120 + carboplatin vs BKM120 + lomustine	PI3K	I/II	NCT01934361	176	03/2017	Novartis Pharmaceuticals
BKM120 + LDE225	PI3K + Hh	I	NCT01576666	118	04/2015	Novartis Pharmaceuticals
BKM120 + INC280	PI3K + c-Met	I/II	NCT01870726	74	08/2015	Novartis Pharmaceuticals
BGJ398	pan-FGFR	II	NCT01975701	34	05/2016	Novartis Pharmaceuticals
BIBW2992 + temozolomide	HER2/EGFR	I/II	NCT00727506	151	03/2015	Boehringer Ingelheim
Bortezomib + bevacizumab + temozolomide	26S proteasome	I	NCT01435395	18	12/2015	Emory University
Bevacizumab + dasatinib	VEGF + Src	I/II	NCT00892177	183	11/2014	Alliance for Clinical Trials in Oncology
Cediranib + lomustine	pan-VEGFR	III	NCT00777153	423	12/2014	AstraZeneca
Crizotinib	ALK/ROS1	I/II	NCT00939770	'96	12/2014	Children's Oncology Group
Dacomitinib	pan-HER	II	NCT01520870	64	07/2015	Grupo Español de Investigación en Neurooncología
Dacomitinib	pan-HER	II	NCT01112527	56	12/2014	Massachusetts General Hospital
Dasatinib + bevacizumab	Src + VEGF	II	NCT00892177	183	11/2014	National Cancer Institute
Dovitinib	FGFR	II	NCT01753713	55	12/2014	Case Comprehensive Cancer Center
Erlotinib	EGFR	N/A	NCT01257594	22	12/2014	Columbia University
GDC-0084	PI3K	I	NCT01547546	68	08/2016	Genentech
Indoximod + temozolomide	IDO	I/II	NCT02052648	18	03/2015	NewLink Genetics Corporation
INK128	mTORC 1/2	Pilot	NCT02133183	40	12/2015	National Cancer Institute
INK128 + bevacizumab	mTORC 1/2 + VEGF	I	NCT02142803	58	11/2014	National Cancer Institute
Lonafarnib + temozolomide	FPTase	I	NCT00102648	35	12/2015	MD Anderson Cancer Center
LY2157299 + lomustine vs lomustine	HGF/SF	II	NCT01582269	180	12/2014	Eli Lilly and Company
Macitentan + temozolomide	ET-1	I	NCT01499251	48	12/2014	Actelion
MK-1775 + temozolomide	WEE1	I	NCT01849146	114	12/2014	National Cancer Institute
Pazopanib + topotecan	c-Kit/VEGF/PDGR	II	NCT01931098	66	11/2020	MD Anderson Cancer Center
PF-00299804	pan-ERBB	II	NCT01112527	56	12/2014	Massachusetts General Hospital
Olaparib + temozolomide	PARP	I	NCT01390571	34	09/2015	Cancer Research UK
Onartuzumab + bevacizumab vs onartuzumab vs bevacizumab	c-Met + VEGF	II	NCT01632228	135	03/2015	Hoffmann-La Roche
Ridaforolimus + MK-2206 or Ridaforolimus + MK-0752	mTOR + Akt or mTOR + Notch	I	NCT01295632	124	12/2014	Merck Sharp & Dohme Corp.
RO4929097 + cediranib	Notch	I	NCT01131234	50	09/2014	National Cancer Institute
Selinexor	Exportin 1 (XPO1/CRM1)	II	NCT01986348	30	08/2015	Karyopharm Therapeutics, Inc
Sorafenib + everolimus	VEGFR/Raf1 + mTOR	I/II	NCT01434602	118	10/2018	MD Anderson Cancer Center
Sorafenib + valproic acid + sildenafil	VEGFR/Raf1	II	NCT01817751	66	12/2017	Virginia Commonwealth University

Table 1. Ongoing clinical trials of targeted therapy for recurrent glioblastoma (cont.).

Therapy	Target	Trial Phase	Trial identifier	Number of patients	Completion date	Sponsor
Tivozanib	VEGF	II	NCT01846871	21	06/2015	Massachusetts General Hospital
TRC105 + bevacizumab	Endoglin (CD105) + VEGF	II	NCT01564914	22	06/2015	Tracon Pharmaceuticals Inc.
TRC105 + bevacizumab vs bevacizumab	Endoglin (CD105) + VEGF	I/II	NCT01648348	128	12/2014	National Cancer Institute
Vandetanib + carboplatin vs Vandetanib → carboplatin	VEGFR/EGFR	II	NCT00995007	112	07/2016	National Cancer Institute
Vandetanib + bevacizumab vs Bevacizumab	VEGFR/EGFR + VEGF	I/II	NCT01266031	108	07/2016	MD Anderson Cancer Center
Vorinostat + bevacizumab	HDAC + VEGF	II	NCT01738646	40	12/2015	Duke University
Vorinostat + isotretinoin + temozolomide	HDAC	I/II	NCT00555399	189	11/2015	MD Anderson Cancer Center
Vorinostat + radiation therapy	HDAC	I	NCT01378481	30	07/2016	National Cancer Institute

to cause host immunosuppression through a variety of mechanisms. Several approaches are being studied and include the use of autologous stimulated lymphocytes, cytokines and dendritic cells and tumor- or peptide-based vaccines. Rindopepimut (PEPvIII-KLH, CDX-110; Celldex Therapeutics, NJ, USA), a single peptide-based vaccine, targets the 13-amino acid sequence EGFRvIII receptor antigen in tumors expressing EGFRvIII. It has showed encouraging results in two Phase II clinical trials that evaluated rindopepimut in patients with newly diagnosed glioblastoma [100,101]. Based on this, the ReACT trial, an ongoing study, was designed to find out whether adding rindopepimut to the commonly used bevacizumab can improve PFS of patients with first or second recurrence of glioblastoma expressing EGFRvIII (ClinicalTrials.gov identifier: NCT01498328 [114]).

An alternative vaccine approach utilizes dendritic cells that play an important role in antigen presentation to immune effector cells. In these clinical trials, dendritic cells are matured and loaded with tumor antigens (such as EGFRvIII, MHC class I and autologous tumor lysate) *ex vivo* and then infused back into the patient to stimulate a tumor-specific T-cell response. Preliminary results of a Phase II trial using a dendritic cell vaccine (DCVax-Brain, Northwest Biotherapeutics) in recurrent glioblastoma showed it to be effective in inducing antitumor immunity with low toxicity [102].

Ipilimumab (Yervoy, Bristol-Myers Squibb), a human IgG1 monoclonal antibody to cytotoxic T-lymphocyte antigen 4 (CTLA-4) has been used in metastatic or unresectable melanoma

and demonstrated improved OS compared with the nonipilimumab treatment arms in randomized trials [103,104], and tumor reduction was also observed in patients with brain metastases treated with ipilimumab as monotherapy [105,106]. Nivolumab (MDX1106; BMS-936558, Bristol-Myers Squibb), a fully humanized IgG4 monoclonal antibody to the immune checkpoint molecule programmed cell death-1 (PD-1), also induced an antitumor response in patients with metastatic melanoma, reversing the immunosuppression mediated by PD-1, a complementary checkpoint protein to CTLA-4.

There are no data available for ipilimumab or nivolumab in patients with glioblastoma at present, but there is an ongoing randomized Phase II trial investigating nivolumab or nivolumab combined with ipilimumab versus bevacizumab in adults with recurrent glioblastoma (ClinicalTrials.gov identifier: NCT02017717 [114]) that has generated increasing interest in immunotherapy.

Gene therapy, oncolytic viruses & targeted toxins

The use of gene therapy for malignant gliomas in clinical studies was first reported in 1994, with the intratumoral implantation of retroviral vector-producing cells [107] and the incorporation of transgenes in the tumoral DNA to activate prodrugs into toxic forms provoking cell death. Other gene therapy approaches are the delivery of tumor-suppressor genes such as the *TP53* gene or antitumor immune modulation by expression of cytokines and lymphokines such as interferons and interleukins [108]. More recently, replication

competent oncolytic viruses have been developed and are undergoing clinical evaluation. These viruses can selectively infect active tumoral cells during mitosis, replicate, infect adjacent cells and provoke cytolysis [109]. Other approaches include direct infusion of targeted toxins that are recombinant proteins consisting of a tumor cell-specific ligand conjugated with a toxic functional group (*Pseudomonas* exotoxin or diphtheria toxin) [110]. To date, none of these approaches has shown a clear therapeutic activity or survival benefit compared with the standard treatment but delivery issues remain a factor.

Electrical fields

In 2011, NovoTTF-100A system (Novocure Ltd, Haifa, Israel) was approved by the FDA for the treatment of recurrent glioblastoma. It is a novel portable device that delivers low intensity, intermediate frequency, alternating electric fields through scalp electrodes, interfering with cell division and assembly of organelles. In 2012, a randomized Phase III trial, investigators found that the tumor treating fields (TTF) provided comparable efficacy to salvage chemotherapy in recurrent glioblastoma, although OS benefit was not demonstrated. However, the toxicity profile and quality of life favored the TTF [111]. NovoTTF in combination with temozolomide is also being evaluated for newly diagnosed glioblastoma (ClinicalTrials.gov identifier: NCT00916409 [114]) based on the mentioned results and data indicating that TTF may increase the efficacy of chemotherapy without additional toxicity [112].

Conclusion & future perspective

The management of recurrent glioblastoma is challenging, and despite recent advances in the understanding of the molecular heterogeneity, tumor phenotype and tumor microenvironment that provide insight into potential targets in the

development of targeted therapies, personalized therapy is still not feasible. A variety of challenges remain starting with the need for imaging technologies that accurately distinguish tumor from treatment effect and assess the full extent of the tumor. Tumor tissue collection should be mandatory at the time of recurrence, as these pertinent tumor tissues are needed to direct treatment in hypothesis-based clinical trials. Surgery may be also useful in selected cases to distinguish between tumor recurrence and radiation necrosis, or to provide symptom palliation. Antiangiogenic agents provide relief of symptoms improving the quality of life and extend PFS, but further investigation is necessary to determine its optimal utilization. Aside from bevacizumab and in selected patients, carmustine-wafers, no standard of care exists for recurrent glioblastoma. Recent studies suggest that focal re-irradiation may be safe and may help with disease control. Systemic administration of nitrosoureas or rechallenge with temozolomide are often used as salvage therapy. Nevertheless, significant advances in treatment and improvement in outcomes will require detailed understanding of tumor genetics and microenvironment, innovative clinical trials, novel therapies and multi-institutional collaboration.

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