REVIEW

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What next for newly diagnosed glioblastoma?

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Glioblastoma is the most common primary brain tumor in adults. Despite current multimodality treatment including surgical resection and temozolomide-based chemoradiotherapy, median survival is only 14–16 months. Characterization of molecular alterations in glioblastoma has identified prognostic subgroups and therapeutic opportunities for clinical trials across glioblastoma subsets. Following a number of negative Phase III trials testing temozolomide dose intensification and angiogenesis inhibition, recent interim analysis data indicate survival prolongation with use of a device (Optune™) delivering alternating electrical field therapy in newly diagnosed glioblastoma patients. In this review, we present an overview of the data supporting the current standard of care and discuss novel experimental therapies in early and late phase clinical testing including devices, small molecule drugs, angiogenesis inhibitors, oncolytic virotherapy and immunotherapy.

Glioblastoma (grade IV astrocytoma) is the most common primary brain tumor in adults. Median survival is 14–16 months after diagnosis, and survival following progression is on average only 6–8 months [1]. The WHO classification of grade IV astrocytomas also includes gliosarcoma, which shares clinical and genetic similarities with glioblastoma, and giant cell glioblastoma, which represents a distinct subset with comparatively better prognosis [2–4]. A new version of WHO classification incorporating molecular markers is expected to be released in spring 2016.

In the past decade, there have been important gains in our understanding of genetic alterations associated with gliomagenesis, although this knowledge to date has not been translated into significant survival improvements. Better understanding of key pathways driving tumor growth and development and the role of the tumor microenvironment extends the promise of further improvements in therapy. Surgery, radiation and chemotherapy have established roles with some room for refinement, but new devices, small molecule cell cycle inhibitors, biologic and immune-based therapies have started yielding promising results in Phase II and early readings of Phase III trials and create optimism for future changes in the treatment landscape and standard of care.

Standard-of-care therapy

The standard backbone in the treatment of newly diagnosed glioblastoma is surgical resection, when feasible, followed by radiation therapy (RT) administered concurrently with oral temozolomide (TMZ), followed by six cycles of adjuvant TMZ therapy. RT for glioblastoma has formed the basis of postoperative therapy for decades [5]; developments in radiation delivery techniques have led to the current standard of involved field RT at a dose of 60 Gy delivered in 30 fractions. Increasing RT dose up to 90 Gy has been studied, and it provides no additional benefit regardless of the

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technique used, including intensity-modulated RT, brachytherapy or stereotactic radiosurgery [6,7]. The ongoing NRG-BN001 clinical trial (NCT02179086) is addressing the question if the addition of TMZ in conjunction with RT dose escalation (via intensity-modulated RT or proton beam) would result in enhanced benefit with acceptable safety.

The pivotal Phase III trial of the European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada Trials Group (EORTC/NCIC) demonstrated the superiority of chemoradiation with TMZ versus radiation alone and established TMZ as the new standard of care [8]. Other chemotherapy regimens in conjunction or following RT had previously been tested without convincing benefit, although a meta-analysis of randomized trials conducted in the pre-TMZ era had suggested improved survival with addition of chemotherapy to RT [9].

TMZ is a lipophilic alkylating agent with affinity for purine bases of DNA. TMZ methylates its primary target, O6-guanine, forming O6-methylguanine (O6-MeG); though N7-guanine and N3-adenine residues in DNA are also methylated [10]. The enzyme O6-methylguanine DNA methyltransferase (MGMT) normally repairs O6-MeG, so low expression due to promoter methylation causes decreased expression of the enzyme. At the absence of MGMT activity, O6-MeG aberrantly pairs with thymine (instead of cytosine) activating base excision repair mechanisms. When the base excision repair machinery is unable to effectively repair the lesion, unrepaired DNA strand breaks cause cell cycle arrest and apoptosis [11].

In the EORTC/NCIC-CE3 Phase III trial that established the benefit of TMZ-based chemoradiation, 60 Gy of radiation was delivered over 6 weeks concurrently with TMZ at a dose of 75 mg/m² daily. Adjuvant TMZ was restarted following a 4–6-week recovery time at a starting dose of 150 mg/m² on days 1–5 of a 28-day cycle for the first cycle, and 200 mg/m² on days 1–5 in subsequent cycles, if the first cycle was well tolerated. Median overall survival (OS) was 14.6 months in the TMZ-containing arm, a statistically significant improvement over the 12.1 months median OS in the radiation-only arm. In the 5-year analysis, combination therapy increased the 2-year OS from 10.9 to 27.2%, and 5-year survival improved from 1.9 to 9.8% [1]. While both patients with *MGMT* methylated

and unmethylated tumors benefited from combination therapy (Table 1), the magnitude of benefit from TMZ was greatest in patients with *MGMT* promoter methylation [1,12]. For patients with *MGMT* unmethylated tumors median OS was 11.8 months for RT-alone versus 12.6 months for chemoradiation, whereas for *MGMT* methylated tumors OS was 15.3 months for RT-alone versus 23.4 months for TMZ-based chemoradiation. *MGMT* promoter methylation was therefore identified as an important prognostic marker in glioblastoma and has been included as a stratification factor in all subsequent Phase III trials in newly diagnosed GBM. Similarly, *MGMT* promoter methylation appears to have predictive value in identifying TMZ responders, although, at least using the *MGMT* assay cutoff employed in the EORTC/NCIC study, *MGMT* unmethylated patients also derived some benefit from the addition of TMZ (Table 1). As such, TMZ represents the standard of care for all newly diagnosed GBM patients, independent of their *MGMT* methylation status. Nevertheless, omitting TMZ in unmethylated patients as part of clinical trials is gaining increased acceptance, especially when less strict *MGMT* methylation assay cut-offs limit eligibility to truly unmethylated patients [13].

The benefit of intensifying the dose density of adjuvant TMZ was evaluated in RTOG 0525; in this randomized Phase III trial, the standard TMZ schedule (150–200 mg/m² × 5 days) was compared with dose dense TMZ (75–100 mg/m² × 21 days) every 4 weeks for six to 12 cycles [14]. No statistically significant difference was observed between the two arms for median OS (16.6 vs 14.9 months; hazard ratio [HR]: 1.03; *p* = 0.63) or median progression-free survival (PFS), with 5.5 versus 6.7 months; HR: 0.87; *p* = 0.06. There was increased grade 3 toxicity associated with dose dense treatment (34 vs 53%; *p* < 0.001), mostly lymphopenia and fatigue. For patients discontinuing therapy after 6 months (*n* = 22), median OS was 24.9 months (95% CI: 19.2–36.2 months), compared with 30.2 months (95% CI: 25.5–35.4 months) for those continuing beyond 6 months (*n* = 239), although the small number of patients in the former group (*n* = 22) prevents definitive conclusions. *MGMT* methylated patients had a significantly better prognosis than *MGMT* unmethylated patients, with improved median OS (21.2 vs 14 months; HR: 1.74; *p* < 0.001) and PFS (8.7 vs 5.7 months; HR: 1.63; *p* < 0.001), confirming

Table 1. Kaplan–Meier overall survival in the EORTC-NCIC C3 trial, including subgroup analysis.

Treatment arm	Deaths/patients	Hazard ratio (95% CI)	Median (95% CI); months	2-year OS (95% CI); %	3-year OS (95% CI); %	4-year OS (95% CI); %	5-year OS (95% CI); %
MGMT unmethylated							
Radiotherapy	54/54	1.0	11.8 (10.0–14.4)	1.8 (0.1–8.6)	0	0	0
Combined	54/60	0.6 (0.4–0.8)	12.6 (11.6–14.4)	14.8 (7.2–25.0)	11.1 (4.7–20.7)	11.1 (4.7–20.7)	8.3 (2.7–18.0)
MGMT methylated^a							
Radiotherapy	43/46	0.5 (0.3–0.7)	15.3 (13.0–20.9)	23.9 (12.9–36.9)	7.8 (2.2–18.3)	7.8 (2.2–18.3)	5.2 (1.0–15.0)
Combined	37/46	0.3 (0.2–0.4)	23.4 (18.6–32.8)	48.9 (33.7–62.4)	27.6 (15.4–41.4)	22.1 (11.0–35.7)	13.8 (4.5–28.2)

^aHazard ratio relative to MGMT promotor unmethylated patients who received radiotherapy.
OS: Overall survival.
Data taken from [1].

the prognostic value of this alteration, although increased dose density of TMZ conferred no additional benefit.

Overall, TMZ is a well-tolerated oral chemotherapeutic, though nausea is common and prophylactic use of serotonin 5-HT₃ receptor antagonists, granisetron (Kytril) or ondansetron (Zofran), is routinely necessary. In the EORTC/NCIC study 7% of patients developed grade 3/4 hematologic toxicity during combined radiotherapy, and 14% of patients developed grade 3/4 toxicity in the adjuvant phase [8]. The most significant nonhematologic toxicity of TMZ is fatigue, with moderate-to-severe fatigue reported in 33% of patients treated with chemoradiotherapy versus 26% of patients treated with radiation alone. TMZ-induced lymphopenia increases the risk for *Pneumocystis jirovecii* pneumonia, and routine use of *Pneumocystis* prophylaxis, most commonly using trimethoprim/sulfamethoxazole is recommended [15]. Alternatives include atovaquone, dapsone and inhaled pentamidine in patients unable to receive trimethoprim/sulfamethoxazole due to allergic reactions.

Angiogenesis inhibition in newly diagnosed GBM

Glioblastomas are highly vascular tumors, and therefore treatment with antiangiogenesis agents has been an attractive treatment strategy. Bevacizumab is a humanized monoclonal antibody to the soluble ligand VEGF-A, and it was approved for the treatment of recurrent GBM by the US FDA in 2010 on the basis of durable objective responses in this context. RTOG 0825 and AVAglio evaluated the addition of bevacizumab to standard chemoradiation: PFS and OS were co-primary end points [16,17]. Both trials demonstrated improvement in PFS with the addition of bevacizumab: 10.7 versus 7.3 months

($p = 0.007$) in RTOG 0825 and 10.6 versus 6.2 months ($p < 0.001$) in AVAglio. However, in neither trial did this improvement in PFS translate into an improvement in OS [16,17]. The AVAglio trial also analyzed corticosteroid use by treatment arm; patients in the bevacizumab containing arm had a higher rate of corticosteroid discontinuation and longer time to corticosteroid initiation (12.3 vs 3.7 months). Based on these data bevacizumab cannot be considered as the standard-of-care in newly diagnosed glioblastoma. Bevacizumab may be useful during and immediately after RT, however, especially for clinically significant radiation induced inflammation, refractory to corticosteroid therapy. Other agents with antiangiogenic activity such as the $\alpha v\beta 3/\alpha v\beta 5$ inhibitor cilengitide similarly failed to improve survival when added to standard of care treatment in newly diagnosed methylated GBM patients (CENTRIC trial) [18] and produced conflicting results when tested in unmethylated patients in a randomized Phase II trial (CORE trial) [19]. Table 2 summarizes results of Phase III trials for newly diagnosed GBM, reported in the post-TMZ era.

Several overlapping molecular pathways underlying glioblastoma growth signaling and tumorigenesis have been described and tools like next-generation sequencing have helped to paint a clearer picture of the genetic landscape of GBM [21–23]. Glioblastoma with mutated IDH1 is recognized as a distinct subtype with favorable prognosis, more closely related to lower grade gliomas though representing <5% of primary GBM [24]. Key molecular signatures defining specific subtypes of glioblastoma proposed and validated by The Cancer Genome Atlas (TCGA) project include Proneural, Neural, Classical and Mesenchymal, based on copy number alterations and expression characteristics of 840 genes, including *EGFR*, *TP53*, *PDGFRA*, *IDH1* and

Table 2. Outcome of newly diagnosed patients with glioblastoma treated in contemporary Phase III trials.

Study (year)	Treatment arm	n	OS (months)	Ref.
Stupp <i>et al.</i> (2005), EORTC/NCIC	RT control	286	12.1	[8]
	TMZ/RT	287	14.6	
Gilbert <i>et al.</i> (2013), RTOG 0525	TMZ/RT standard dose	411	18.9	[14]
	TMZ/RT dose dense	422	16.8	
Gilbert <i>et al.</i> (2014), RTOG 0825	TMZ/RT/Bev	312	15.7	[17]
	TMZ/RT/placebo	309	16.1	
Chinot <i>et al.</i> (2014), AVAglio	TMZ/RT/Bev	458	16.8	[16]
	TMZ/RT/placebo	463	16.7	
Stupp <i>et al.</i> (2015), EF14 [†]	TMZ/RT/Optune™	466	19.4	[20]
	TMZ/RT	229	16.6	
Stupp <i>et al.</i> (2014), CENTRIC [‡]	TMZ/RT/cilengitide	272	26.3	[18]
	TMZ/RT	273	26.3	

[†]Interim analysis.
[‡]MGMT methylated.
Bev: Bevacizumab; OS: Overall survival; RT: Radiation therapy; TMZ: Temozolomide.

NFI [25]. A recently reported analysis in patient tumors from the AVAglio trial showed a statistically significant PFS and OS benefit in patients with Proneural wild-type *IDH1* GBM, when bevacizumab was added to standard therapy (17.1 vs 12.8 months with placebo), thus identifying a predictive marker of response to bevacizumab in the newly diagnosed setting [26]. This finding will need to be further validated, ideally in samples from RTOG 0825, and it has practice changing potential.

Considerations for elderly patients

The standard of care setting EORTC/NCIC trial did not include patients over the age of 70 [8]. A Canadian prospective Phase II randomized trial of 100 patients age 60 years or older compared 6 weeks of radiotherapy (60 Gy/30 fractions) to 3 weeks (40 Gy/15 fractions) and found no significant difference in survival [27]. More recently the Nordic Clinical Brain Tumor Study Group randomized elderly patients with glioblastoma to receive standard radiation (60 Gy/30 fractions), hypofractionated radiation (34 Gy/15 fractions) or TMZ monotherapy. In patients over the age of 70 years, hypofractionated radiation (34 Gy/15 fractions) or TMZ monotherapy were superior to standard dose radiation; median OS was 8.3 versus 7.5 versus 6 months, respectively [28].

In the NOA-08 trial, patients were randomized to dose-dense TMZ (100 mg/m² days 1–7 every 2 weeks) or standard RT (60 Gy/30 fractions) with similar outcomes between the two groups [29]. In both the Nordic and NOA-08

trials patients with *MGMT* promoter methylation fared better when treated with TMZ. These trials support use of TMZ monotherapy in the elderly patients with MGMT methylated tumors when performance status precludes combination chemoradiation. Hypofractionated radiotherapy can be considered for nonmethylated patients unable to tolerate standard chemoradiation. Combination of TMZ and hypofractionated radiation is being studied in CAN-NCIC-CE6, a recently completed international Phase III trial comparing 3 weeks of radiation with or without concurrent and adjuvant TMZ (75 mg/m²/day) in newly diagnosed elderly GBM patients [30].

Tumor treatment fields therapy

Optune™ is a portable, battery-powered device that delivers low-intensity, intermediate-frequency alternating electrical fields to supratentorial glioblastoma tumors via transducer arrays applied directly to the scalp [31]. The device’s mechanism of action is disruption of mitotic spindle formation during cell division. Stupp and colleagues conducted an international, multicenter, prospective, randomized Phase III trial in newly diagnosed GBM patients following completion of concomitant TMZ/RT. Patients were randomized (2:1) to receive adjuvant TMZ along with Optune use or adjuvant TMZ alone. In a prespecified interim analysis median PFS was 7.1 versus 4.0 months; HR: 0.63 (p = 0.001), OS was 19.4 versus 16.6 months; HR: 0.75 (p = 0.02), and 24-month survival was 43% (CI: 36–50%) versus 29% (CI: 21–39%) for the Optune/TMZ and TMZ alone arms, respectively [20]. On the basis of this data, the US FDA approved Optune for newly diagnosed GBM patients in October 2015. Use of the device was well tolerated with the most common side effect being skin irritation.

Approaches aiming to optimize TMZ benefit in newly diagnosed GBM

PARP is a DNA repair enzyme involved in repairing DNA strand breaks induced by TMZ methylation and RT. PARP inhibition in addition to TMZ/RT may therefore facilitate tumor cell-cycle arrest and apoptosis following treatment induced DNA damage. Several PARP inhibitors have shown preclinical activity in the treatment of GBM including iniparib (BSI 201), olaparib (AZD-2281), veliparib (ABT-888) and niraparib (MK-4827). In a Phase I trial, the addition of the oral PARP inhibitor veliparib to

TMZ/RT was associated with significant dose limiting hematologic toxicity [32]. Preclinical data in patient derived orthotopic xenografts have convincingly demonstrated that the combination of TMZ with veliparib can significantly prolong survival when added to TMZ in *MGMT* methylated but not unmethylated tumors [33]. Based on this data, the Alliance for Clinical Trials in Oncology is currently conducting a randomized Phase II/III trial of veliparib versus placebo in combination with adjuvant TMZ in newly diagnosed GBM with *MGMT* promoter hypermethylation (A071102, NCT02152982).

MGMT protein inhibitors such as the small nucleoside inhibitor, O⁶-benzylguanine (O⁶BG), have been developed as another means of augmenting TMZ potency. O⁶BG binds to the *MGMT* protein and causes structural changes marking the protein-nucleotide complex for degradation, thus potentiating TMZ effect. Combination therapy with *MGMT* inhibitors such as O⁶BG, though, has been associated with excessive hematologic toxicity [34]. A follow-up prospective Phase I/II clinical trial in newly diagnosed *MGMT* nonmethylated GBM patients showed autologous *MGMT* *P140K* gene-modified hematopoietic stem cell transplant can prevent dose limiting hematopoietic toxicity during combination O⁶BG/TMZ chemotherapy [35]; it is unclear, however, if the benefit materialized can justify further testing of this higher risk approach.

Carmustine (BCNU) loaded gliadel wafers have also been investigated as local therapy; these are biodegradable polymer wafers containing the chemotherapy drug BCNU placed in the surgical resection cavity at the time of initial surgery [36]. The FDA-approved BCNU wafers for newly diagnosed glioblastoma based on Phase III trial results in 240 newly diagnosed high grade glioma patients randomized to placement of carmustine wafers versus placebo followed by RT alone [36]. The subset of GBM patients receiving carmustine loaded wafers had no statistically significant increase in OS ($p = 0.10$) in unadjusted analysis, but did reach statistical significance ($p = 0.04$) when adjusted for baseline prognostic factors (age and Karnofsky performance status). Following approval of TMZ for newly diagnosed GBM several nonrandomized studies have since demonstrated safety of combining Gliadel wafer implantation with TMZ-based chemoradiation in patients with newly diagnosed glioblastoma, however, there are no data

demonstrating superiority over chemoradiation alone, so their routine use cannot currently be recommended [37–40].

Investigational molecularly targeted therapy approaches

Several ongoing trials are evaluating combinatorial approaches in the newly diagnosed setting. Lapatinib, an EGFR kinase inhibitor, in combination with TMZ/radiation is being evaluated in a Phase II open-label trial (NCT01591577), an appealing approach especially for EGFR overexpressing patients [25]. Early phase trials evaluating combination TMZ/radiation with the oral antiangiogenic tyrosine kinase inhibitor, pazopanib (PAZAGLIO) and the ALK and ROS-1 inhibitor crizotinib (NCT02270034) are also currently underway. Carboxyamidotriazole orotate, an inhibitor of calcium-dependent intracellular and extracellular signal transduction pathways affecting VEGF and PI3K signaling, is being evaluated as monotherapy, in combination with TMZ or in combination with TMZ/radiation in a Phase I clinical trial of newly diagnosed GBM patients (NCT01107522). Another Phase I study, conducted through ABTC, is evaluating the WEE1 inhibitor MK-1775 in combination with radiation and TMZ (NCT01849146); WEE1 is involved in progression from G2 to mitosis, and its inhibition prevents cell division. With all targeted agents, lack of satisfactory blood–brain barrier penetration can hamper efficacy and needs to be carefully assessed as part of the early stage development.

Immunotherapeutic approaches

Different immunotherapeutic approaches are currently being explored in the treatment of glioblastoma including immunomodulatory approaches, using immune checkpoint inhibitors, dendritic cells, tumor vaccines, as well as chimeric antigen receptor (CAR)-modified T-cell therapy. The immunosuppressive microenvironment created by GBM cells and infiltrating immunosuppressive immune cell subsets such as myeloid-derived suppressor cells and Tregs make GBM particularly challenging to treat with immune-based therapies. The first biologic immunomodulatory treatment used in the management of glioblastoma was interferon (IFN). Several clinical trials of IFN either alone or in combination with cytotoxic chemotherapy have been conducted, but treatment is associated

with significant side effects related to therapy, including fatigue and flu-like symptoms, without convincing evidence of efficacy [41–43].

Recently developed immune checkpoint inhibitors, however, such as anti-CTLA-4 and PD-1/PDL-1 antibodies have been shown to augment effector T-cell function and have demonstrated clinical efficacy in several solid tumors, which has culminated in FDA approval of anti-PD1 antibodies for melanoma and non-small-cell lung cancer. In an ongoing open-label, nonrandomized, multicenter Phase II trial using the anti-PD-L1 antibody MEDI4736, newly diagnosed unmethylated MGMT GBM patients are receiving anti-PD-L1 directed therapy every 2 weeks in combination with standard radiotherapy (NCT02336165). Another Phase I trial is testing the combination of TMZ with dual check-point inhibition employing the anti-CTLA4 antibody ipilimumab and/or the anti-PD1 antibody nivolumab (NCT02311920) in newly diagnosed GBM patients. Trials with the anti-PD1 antibody pembrolizumab are in planning stages. Several studies evaluating this approach in the recurrent setting are already currently underway, such as CheckMate143 (NCT02017717), which is evaluating the safety of nivolumab versus different doses of the nivolumab/ipilimumab combination in recurrent glioblastoma and it is in parallel comparing efficacy of nivolumab versus bevacizumab as first line treatment in this context. This study also includes Phase I/II cohorts for newly diagnosed GBM patients.

Dendritic cell and peptide vaccines are also under clinical investigation for treatment of GBM patients. DCs obtained from peripheral blood or bone marrow are normally created using *ex vivo* autologous tumor cell lysate loading. Alternatively, pooled tumor lysates from multiple patients or synthetic tumor peptides may be used to activate dendritic cells. A Phase III trial evaluating the efficacy of the autologous dendritic cell vaccine DCVax-L versus placebo in patients with newly diagnosed glioblastoma is ongoing (NCT00045968) [44,45]. Early phase trials are evaluating the ability of DCs pulsed with cytomegalovirus (CMV) phosphoprotein 65 (pp65) RNA to generate an anti-GBM immune response, as CMV pp65 is known to be expressed in more than 90% of GBM clinical isolates. The impact of DC vaccine site preconditioning in GBM patients was assessed in another pilot study [46]. Patients with glioblastoma

received either preconditioning with mature DCs or tetanus toxoid (Td) prior to vaccination with DCs pulsed with CMV pp65 RNA. Preconditioning with Td led to enhanced DC migration and improved survival in vaccinated patients, suggesting that preconditioning with a potent recall antigen may improve antitumor immunotherapy.

Heat shock proteins (HSPs) are intracellular chaperones, which when bound to tumor associated antigens can stimulate uptake by antigen presenting cells for cross-presentation on MHCI or MHCII molecules. A recent Phase II single-arm study tested an autologous heat shock protein peptide vaccine in addition to standard therapy in the newly diagnosed setting [47]. In that study 46 patients received weekly HSP vaccination within 5 weeks of completing radiotherapy followed by adjuvant TMZ with monthly vaccinations. Median PFS was a promising 17.8 months and median OS was 23.8 months. Interestingly, median OS for patients with low PD-L1 expression on peripheral monocytes was 44.7 months as compared with 18.0 months for high PD-L1 expressors (HR for death 3.35; 95% CI: 1.36–8.23; $p = 0.003$). These data suggest that HSP vaccination may improve survival compared with standard therapy alone, and raises the possibility that vaccine efficacy may be impacted by peripheral monocyte PD-L1 expression, an important finding that merits prospective validation. A Phase III trial with this approach in newly diagnosed GBM patients is in the planning stages, while a randomized Phase II trial testing this approach in recurrent GBM patients following resection is ongoing (Alliance A071101, NCT01814813).

In antigen peptide vaccination approaches, synthetic peptides representing tumor-specific antigens are used to stimulate the immune system. The use of synthetic peptides has the advantage of not requiring custom vaccine preparation, but also has the drawback of targeting a smaller repertoire of tumor antigens. As an example, the mutated, constitutively active EGFRvIII results from an in-frame deletion of exons 2–7, and it is a common target in antigen peptide vaccination because of its unique amino acid sequence at the fusion junction, extracellular epitope location and specificity for brain tumors. These vaccines can only be used in the subset of patients with the same antigenic determinant; however, ACT III, a Phase II, single-arm, multicenter trial assessed the immunogenicity of rindopepimut,

a 13-mer EGFRvIII- peptide Ag vaccine and evaluated PFS and OS in newly diagnosed GBM patients with EGFRvIII+ expressing tumors following gross total resection. Median PFS in ACT III was 9.2 months and median OS was 21.8 months from study entry [48]. The development of specific antibodies against EGFRvIII increased greater than fourfold in 85% of patients, and antibody titers increased with duration of treatment. Rindopepimut immunization also led to elimination of EGFRvIII expression in 67% of tumor samples obtained after >3 months of therapy. On the heels of ACT III, the international, double blind, randomized Phase III trial ACT IV has recently completed accrual and will conclusively evaluate the efficacy of intradermal rindopepimut (CDX-110), added to standard therapy in newly diagnosed EGFRvIII expressing glioblastoma. These data are awaited with interest, especially in the aftermath of the ReACT trial, a randomized Phase II study, that showed a survival improvement when rindopepimut was combined with bevacizumab versus bevacizumab alone in recurrent GBM patients [49]. Ongoing trials against other glioblastoma associated antigens include the Phase I trial evaluating WT2725 vaccination against Wilms tumor (WT1) protein, overexpressed in many glioblastoma tumors (NCT01621542). In another Phase I/II trial, SL-701, a multivalent glioma antigen vaccine is administered with the immunostimulant poly-ICLC and combined with intravenous bevacizumab therapy for recurrent GBM (NCT02078648). Although these trials target recurrent GBM patients, their findings may justify testing of these vaccination approaches in the context of newly diagnosed GBM.

Engineered CAR T cells represent a promising novel therapeutic strategy against glioblastoma. CAR T cells are *ex vivo* modified T cells with a chimeric single chain Fv variable region domain of an antibody, a linker region, and an intracellular signaling portion with several costimulatory domains. After reinfusion of the modified T cells, binding of tumor cell antigen initiates intracellular activation signals that promote T-cell activation and cytotoxicity. CAR mediated binding is MHC-independent, thereby allowing for direct targeting without the need for antigen processing and MHC loading. A recent pilot study has demonstrated safety of targeting tumors in recurrent GBM patients through IL13R α 2, a monomeric IL-13 receptor

overexpressed by more than 50% of GBM and frequently expressed in the mesenchymal subtype of glioblastoma [50]. Another trial of anti-EGFRvIII directed CAR T cells in recurrent GBM patients with EGFRvIII-positive tumors is ongoing (NCT02209376). Convincing demonstration of the safety and biologic activity of CAR-T cell-based approaches in the recurrent GBM setting is expected to also open up opportunities for testing in newly diagnosed GBM patients.

Other studies are also underway assessing inhibition of Tregs after tumor resection and TMZ/radiation. Basiliximab, directed against the α -chain of the IL-2R (CD25) on T cells, inhibits Tregs and in the setting of DC vaccination with CMV pp65-lysosomal-associated membrane protein mRNA-loaded DCs and GM-CSF administration could augment cytotoxic T-cell-mediated antitumor responses. Treg inhibition could prove particularly useful following TMZ-induced lymphopenia when T-cell subsets are being reconstituted *in vivo* (NCT02366728).

Viral & gene therapy approaches

Oncolytic virotherapy represents a novel approach in GBM management. Viruses lack cross resistance with chemotherapy, and they can be synergistic with RT and eliminate cells with stem cell-like properties. Furthermore, oncolytic cell death has shown to initiate antitumor immune responses [51], which paves the way for combinatorial strategies with immunomodulatory drugs. Several oncolytic viruses are currently being tested for recurrent glioblastoma and some are expected to transition their way into the newly diagnosed setting. Examples of viruses currently being tested in recurrent GBM patients include the replication competent adenovirus DNX-2401 (Delta-24-RGD) [52], which is now being studied in combination with TMZ (NCT01956734) or IFN- γ (NCT02197169) treatment, Ad-RTS-hIL-12, an adenovirus expressing IL-12 under the control of the activator ligand Veldimex (NCT02026271), an HSV-1 (M032) replicating strain expressing hIL-12 (NCT02062827), vaccine strains of measles virus (NCT00390299), an attenuated, oral (SABIN) serotype 1 poliovirus vaccine (NCT01491893) and the replicating retrovirus TOCA511 (NCT01985256, NCT01470794).

A disproportionately smaller number of trials have been completed or are ongoing in the

newly diagnosed GBM setting. GTI-115 is the first Phase III study of a gene therapy approach for newly diagnosed GBM patients. In that study, mouse fibroblasts were used as retroviral vector producing cells injected into the walls of the resection cavity at the time of initial surgical resection [53]. No statistically significant difference was seen in PFS (180 vs 183 days), median OS (365 vs 354 days) or 12-month survival rates (50 vs 55%) in the gene therapy and control groups, respectively. However, the excellent biosafety profile of the treatment supported additional development of similar approaches.

More recently in the ASPECT trial Westphal and colleagues used adenovirus (Ad5)-mediated gene therapy in patients with operable high-grade glioma [54]. Sitimagene ceradenovec is a first-generation replication-deficient Ad5 containing E1 and partial E3 deletions and insertion of the *HSV-TK* (thymidine kinase) gene: 250 patients randomized into either standard therapy (n = 126) or experimental therapy (n = 124) arms. Surgery followed by RT (60 Gy in 30 fractions) was the protocol-prescribed standard therapy, with the TMZ/RT approach being added later. In addition to standard therapy, patients in the experimental arm received one-time treatment with sitimagene ceradenovec given as injections of 1×10^{10} viral particles into the walls of the resection cavity following surgical resection. Viral transduction was allowed to proceed for 5 days and then intravenous ganciclovir 5 mg/kg twice daily was given on days 5 through 19. In both groups, RT was to be started within 8 weeks of surgery. The prespecified composite primary end point was time to death or re-intervention, defined as any kind of treatment (including surgery, radiotherapy or chemotherapy) given to extend survival after tumor recurrence or progression and this was longer in the experimental versus the control group (308 vs 268 days; HR: 1.5; p = 0.006). There was no difference in OS, however: 497 days in the experimental group vs 452 days in the control group (HR: 1.40; 0.92–2.12; p = 0.112). Interestingly in an analysis of treatment effect by baseline antibody status, the effect of sitimagene ceradenovec seemed to be greater in patients with higher titers of neutralizing antibodies at baseline, suggesting a possible mechanistic role for antibody-dependent cell-mediated cytotoxicity. In an ongoing Phase I study, Ad-hCMV-TK and Ad-hCMV-Flt3L are both administered at the time of resection in newly diagnosed patients followed by systemic

oral administration of valacyclovir in addition to standard of care treatment with TMZ/RT. HSV1-TK encoded by Ad-hCMV-TK is expected to kill transduced tumor cells, following administration of valacyclovir, thus exposing tumor antigens. Treatment with Flt3L, a cytokine known to cause proliferation of DCs, is intended to cause migration of DCs into involved peritumoral brain to mediate specific antitumor immune responses.

Conclusion

Although chemoradiation with TMZ/RT for newly diagnosed GBM patients has led to modest outcome improvement as compared with RT alone, the median survival of GBM patients remains dismal at only 14–16 months, and further improvements are urgently needed. This is especially the case for *MGMT* unmethylated patients for whom the addition of TMZ results in minimal or no benefit. Characterization of glioblastoma subtypes has also begun to inform design of clinical trials, although with the exception of the possible predictive value of the proneural subtype for patients treated with bevacizumab, which merits additional prospective validation, no conclusive association between molecular subtypes and treatment outcomes has been demonstrated.

Several therapies are poised to enter standard management practice in the treatment of newly diagnosed GBM. Based on interim analysis results, the Optune (tumor treatment fields) device has shown efficacy when added to standard TMZ/RT, with statistical prolongation of median PFS and OS. Although unblinding of study patients in late 2014 and allowing crossover may impact the final survival results, the very recent (October 2015) approval of this device for newly diagnosed GBM patients is expected to change the standard of care.

As immune system manipulations are increasingly incorporated with success in cancer treatment, they are expected to also play a larger role in GBM management. Phase III immunotherapy trials such as ACT IV (testing the addition of an EGFR VIII vaccine to standard chemoradiation) might further inform standard of care approaches for the subgroup of patients with EGFRVIII-positive tumors. Although safety data in the recurrent disease setting support feasibility, how to best incorporate immune checkpoint inhibitor therapy in the management of GBM remains to be determined. As our knowledge of molecular pathogenesis of gliomas, tumor micro-environment and tumor immunology expands,

there is increased optimism regarding successful incorporation of novel therapeutics including cell cycle small molecular inhibitors, virotherapy, immunotherapy and devices into the treatment paradigm. In addition to efficacy and safety, the new treatment's impact on the patient's quality of life and pharmacoeconomics considerations need to be increasingly factored in, especially when deciding between treatments with comparable efficacy or borderline clinical benefit.

Future perspective

Treatment of newly diagnosed glioblastoma is likely to continue to capitalize on gains made from a deeper understanding of molecular mechanism of gliomagenesis and tumor immunology. The current standard of care of TMZ/RT with the recent addition of Optune is expected to continue being refined as novel effective therapeutic approaches become available.

In the long term, cytotoxic chemotherapy and/or radiotherapy approaches may be supplanted by immune-based therapies. Combination of immune and viral therapies is expected to continue evolving. Selective activation of key effector pathways and functions may be sequenced to allow for the desired predicted antitumor effects,

while limiting off-target side effects. As more effective treatments for patients with unmethylated tumors develop, the role of TMZ, a drug with borderline benefit in this context, will likely be revisited.

Biomarker driven study designs allowing rational selection of candidates for specific treatments are expected to allow us to capitalize on advances in molecular mechanisms of gliomagenesis and the development of novel, target specific compounds. Implementation of other novel clinical trial designs [55] could help optimize the likelihood of detecting a signal worthy of further investigation with the least number of patients, foster a seamless and effective transition to Phase III trials and expedite new drug approval.

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EXECUTIVE SUMMARY

- The standard backbone in the treatment of newly diagnosed glioblastoma (GBM) based on the results of the EORTC-NCIC-CE3 trial is surgery, when feasible, followed by radiation therapy administered concurrently with oral temozolomide (TMZ), and six cycles of adjuvant TMZ therapy. The *MGMT* status of the tumor represents a prognostic and possibly predictive factor.
- Interim analysis results from the EF14 Phase III trial, demonstrated that use of Optune™ (tumor treatment fields) device in conjunction with adjuvant TMZ therapy was associated with improvement in progression-free survival and overall survival in newly diagnosed GBM patients. Recently approved by the US FDA for newly diagnosed GBM, this device now represents an additional standard of care option.
- Elderly patients with *MGMT* methylated GBM tumors may be treated with TMZ monotherapy when performance status precludes combination chemoradiation, whereas hypofractionated radiotherapy can be considered for nonmethylated patients unable to tolerate standard chemoradiation.
- In AVAGLIO and RTOG 0825, no improvement in overall survival was observed with the incorporation of bevacizumab to the standard-of-care treatment in newly diagnosed GBM patients, despite an approximate 4-month improvement in progression-free survival. The proneural molecular subtype was identified as a possible predictive factor of response to bevacizumab in the AVAGLIO trial.
- Immune-based therapy is likely to play an increasingly prominent role: immunotherapeutic approaches currently being explored include immune checkpoint inhibitors (anti-PD1/PDL1), tumor vaccines, oncolytic virotherapy, chimeric antigen receptor-modified T-cell therapy and combinatorial strategies. Safety has been satisfactory; efficacy assessment is ongoing with randomized Phase II trials and one Phase III trial ongoing or recently completed.
- Imaging protocols allowing more accurate assessment of response, progression and pseudoprogression, and incorporation of innovative clinical trial designs including treatment arm assignment based on molecular signatures, represent important future research opportunities.

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