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Therapy for glioblastoma: is it working?

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Glioblastoma (GBM) remains one of the most intransigent of cancers, with a median overall survival of only 15 months after diagnosis. Drug treatments have largely proven ineffective; it is thought that this is related to the heterogeneous nature and plasticity of GBM-initiating stem cell lineages. Although many combination drug therapies are being positioned to address tumour heterogeneity, the most promising therapeutic approaches for GBM to date appear to be those targeting GBM by vaccination or antibody- and cell-based immunotherapy. We review the most recent clinical trials for GBM and discuss the role of adaptive clinical trials in developing personalised treatment strategies to address intra- and inter-tumoral heterogeneity.

Introduction

Of the many types of brain tumour listed by the WHO [1], gliomas represent 81% of the malignant types – almost half of which are highly aggressive glioblastomas (GBM) [2,3]. GBM is particularly refractory to treatment, leading to a very poor prognosis in affected patients with median survival times of 15 months and 27% of patients surviving for 2 years [4]. Standard therapy for GBM involves surgery for tumour debulking, followed by radiotherapy, complemented by treatment with temozolomide (TMZ) if indicated by tumour histopathology – a treatment regime based on the results of a 5-year follow-up of participants in a 2004 clinical trial (EORTC-NCIC) conducted by European and Canadian groups [5].

Gliomas are rare, with 23 000 new cases being reported in the USA in 2017, compared with, for example, lung cancer with 243 000 cases over the same period (American Cancer Society; https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2018.html). The challenging nature of GBM is reflected in the scientific literature: we have identified 24 889 PubMed citations for the keywords 'glioblastoma and/or glioma' over the 5 years to March 2018; compared with 49 082 for lung cancer. From a drug discovery perspective; this enormous amount of information provides opportunities for identifying molecular mechanisms and interventions that improve survival times; however; translating effective laboratory treatments to clinical practice has so far proven elusive.

Here, we analyse therapies that are being tested in clinical trials to validate specific therapeutic strategies. We also discuss some promising molecular mechanisms and targets identified in preclinical studies, including repurposing of existing oncology drugs for GBM, as well as the development of new immunotherapies, complementing our previous analysis of the recent GBM patent literature [6]. Finally, we discuss the role of adaptive clinical trials to identify effective therapies for individual patients within the remit of personalised medicine.

Interventional clinical trials for glioma and glioblastoma

Most clinical trial data are curated by ClinicalTrials.gov, a resource provided by the National Library of Medicine in the USA (ClinicalTrials.gov; https://clinicaltrials.gov/ct2/home). Various aspects of clinical trial design and execution for GBM using ClinicalTrials.gov data have been described recently [7–9]. Cihoric *et al.* [7] analysed trials registered between 2005 and 2015, Paolillo *et al.* [8] analysed major trials completed between 2015 and 2017 and Vanderbeek *et al.* [9] accessed the data from 2005 to 2016 for trials associated with US centres only. Taken together, these reviews provide a useful overview of the state of GBM clinical

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trials along with analyses of parameters such as trial locations, phases, sponsors and development of new technologies.

To complete this analysis, we have specifically examined trial data from ClinicalTrials.gov for glioma or GBM and present the different therapeutic interventions used (or intended to be used) over a 3-year period up to March 2018. We recognise that, although this database has wide coverage of clinical trials in multiple territories, some important studies might not be registered. We have therefore searched PubMed for GBM trials in different clinical phases up to the time of writing (January 2019) to ensure that the coverage of published trial results in this area is comprehensive and up-to-date. Based on the results of these surveys, we create a forward-looking view of interventions that could improve outcomes over the existing standard of care. Out of 1805 interventional studies, 754 have been completed, 194 are active and 432 are either recruiting or invited, whereas 67 are not yet recruiting. These data are summarised in Table 1.

Most of the current trials for GBM are at early stages, up to Phase II, with relatively few (only 6.5%) in Phase III. The proportion of GBM Phase III trials is low compared with that seen for more prevalent cancers such as lung, colon and breast (Table S1, see supplementary material online), in which between 10% and 20% of trials enter Phase III. To put this in a wider context, in a survey by Hirsch *et al.* [10] of all 8942 oncology trials out of a total of 40 970 trials registered in ClinicalTrials.gov between 2007 and 2010, oncology represented the largest single discipline (21.85% of all trials) and trials were significantly more likely to be single arm (62.3% vs 23.8), open label (87.8% vs 47.3%) and nonrandomized (63.9% vs 22.7%). Oncology trials were also smaller, with more early-phase (I or II) trials than with other disciplines. Another important point from Table 1 is that small molecules are still the predominant drug class listed by intervention field in the database, with biologicals in second place (66.7% versus 13.1%).

TABLE 1

Breakdown of ClinicalTrials.gov interventional trials (excluding observational studies) up to March 2018 using search term 'glioma and/or glioblastoma'

Status	Number of studies	Percentage
All interventional trials	1805	100
Completed studies	754	41.8
Active studies	194	10.7
Recruiting and invited	432	23.9
Not yet recruiting	67	3.7
Terminated studies	161	8.9
Suspended studies	20	1.1
Withdrawn studies	65	3.6
Unknown status	112	6.2
Studies with results	239	13.2
Clinical phase		
Early Phase I	48	2.7
Phase I	518	28.7
Phase I/II	252	14
Phase II	656	56.3
Phase II/III	16	0.9
Phase III	117	6.5
Phase IV	9	0.5
Unannotated	189	10.5
Intervention		
Drug	1205	66.7
Biological	236	13.1
Radiation	120	6.6
Other	80	4.4
Procedure	75	4.2
Device	56	3.1
Behavioural	17	0.9
Dietary supplement	9	0.5
Diagnostic test	3	0.17
Genetic	3	0.17
Combination product	1	0.05

Overview of agents used in interventional trials

The 1805 interventional studies were subdivided into two main groups: 'completed' and a single grouping of 'active'; 'recruiting and invited' and 'not yet recruiting'. One-hundred-and-seventy-eight unique trials marked as completed by ClinicalTrials.gov were selected if their 'last update posted' date lay between 2016 and 2018; and 143 drug interventions ranging from small molecules to engineered cells were listed for each trial identifier and in the case of the completed trials plotted as a 2D map (Table S2, see Supplementary material online). This is summarised in Table 2 which provides a snapshot of clinical trial activity for GBM.

Our analysis confirms the extensive use of TMZ and radiation therapy, which is not surprising given that, together with surgery, they form the standard treatment for GBM [5]. The monoclonal antibody bevacizumab (Avastin®) also features prominently in these trials, often in combination with other agents. Bevacizumab appears to prolong progression-free survival but not overall survival, and adverse events are more frequent compared with placebo [11]. The above treatments are also prominently represented because they are used in control arms with other drug types, or are used in conjunction with biological therapies, for example valacyclovir and ganciclovir prodrugs used in concert with engineered viruses. The overall picture from these completed trials is broadly similar to that from those that are active or planned. As time moves on this picture will change to reflect a wider range of different drug types under evaluation.

Drug types by therapeutic class

The main target classes and drug types used in clinical trials are summarised in Table 3.

Clinical trial results published in PubMed

Although details of individual clinical trials are available on the ClinicalTrials.gov website, only a small number of those with results are published in the biomedical literature, which is a cause for concern [12,13]. This is reflected in our analysis of the trials described here using PubMed's facility for matching citations with ClinicalTrials.gov identifiers (NCT numbers). The trials studied up to March 2018 yielded 37 publications from 754 completed trials

TABLE 2

Overview of the number of trials in each clinical phase for different classes of intervention in glioma and glioblastoma

	Cytostatics, cytotoxics	Monoclonal antibodies and conjugates	Immunotherapy, peptides	Tyrosine kinase inhibitors	Immunomodulators, enzyme inhibitors, other small molecule
Unassigned	2	3	3	1	5
Phase I	17	9	22	7	22
Phase I/II	7	5	6	5	13
Phase II	41	21	10	15	17
Phase III	8	1	2	0	4

Trials completed and last updated between 2016 and 2018 are indicated. A more detailed view listing each intervention and trial separately is available in Table S2 (see Supplementary material online).

TABLE 3

Summary of drug types used in clinical trials

Small molecules		
Enzyme and other protein modulators		Ser/Thr, Tyr, PI3K, mTOR kinase inhibitors
		Metabolic enzyme inhibitors
		Epigenetic modifiers
		Proteasome inhibitors
		Topoisomerase inhibitors
		Receptor modulators
		Transcription factor inhibitors
		Ion channel modulators
Cytotoxic and cytostatic agents		Alkylating agents
		Antimetabolites
		Microtubule-targeting agents
Apoptosis inducers		Thalidomide and analogues
		Radiation
Immunomodulators		Anti-inflammatories
		TLR agonists
Adjuvants		Radiation sensitisers
		Radiation enhancers
		Drug delivery enhancers
Biologicals		
Monoclonal antibodies	Tumour antigens	
	Checkpoint inhibitors	
	Angiogenesis inhibitors	
	Antibody–drug conjugates	
	Antibody–radiochemical conjugates	
Other proteins	Cytokines: IFNs, GM-CSF, IL-2	
	IL-4-toxin fusions	
	Peptides	
Engineered cells	CAR T cells	
	CAR NK cells	
	Autologous immune cells	
	Stem cells engineered to convert prodrugs *in situ*	
Gene therapy	Delivery of immunostimulatory molecules: IL-12, FLT3	
	Delivery of prodrug converters: TK	
Oncolytic viruses	HSV, measles, parvovirus, vaccinia, polio, adenovirus	
Vaccines	Dendritic cells pulsed with peptides or RNA: tumour antigens, whole cell lysates	
	Peptide vaccines	

Drug types broken down into small molecules and biologicals. A detailed list of drugs and targets is available in Table S3 (see supplementary material online). Abbreviations: HSV, herpes simplex virus; IL, interleukin; IFN, interferon; GM-CSF, granulocyte-macrophage colony stimulating factor; CAR, chimeric antigen receptor; NK, natural killer cells; FLT3, fms-like tyrosine kinase 3; TK, thymidine kinase.

and 36 from the 693 active and ongoing trials. To ensure that all trials of potential interest were captured, we also searched PubMed from 2018 to January 2019 for citations relating to GBM and clinical trial phase, independently of the ClinicalTrials.gov database. This resulted in 15 publications of potential interest. Some of the publications described modifications of existing therapies, for example [14], or were not specifically directed towards gliomas or glioblastomas, for example [15]. The key question is whether any of the clinical trials demonstrate a significant increase in progression-free survival or overall survival in at least a proportion of patients with GBM. After reviewing the published results, we observed that very few interventions fulfil these survival criteria, although some biological interventions show more promise, as reviewed below.

Chimaeric antigen receptor (CAR) T cells transfected with interleukin (IL)-13 receptor alpha 2 (IL-13Rα2)

CAR T cells are generated from the patient's white blood cells using lentiviral transfection to introduce specific genes that allow recognition of defined tumour antigens followed by cell killing. The success of this approach in treating refractory lymphomas [16] has prompted its use in other cancers, including GBM [17]. There are several ongoing studies with CAR T cells directed against glioma target antigens, including IL-13Rα which was used in the above trial. A report from this trial in which one patient experienced a prolonged remission from the disease appeared in 2016 [18].

Gene-mediated cytotoxic immunotherapy using aglatimagene besadenovec and valacyclovir

Aglatimagene besadenovec is an adenovirus vector that delivers the herpes simplex virus thymidine kinase gene to a tumour. This is followed by administration of the prodrug valacyclovir which is activated to a form that inhibits DNA replication and induces apoptosis [19]. The subsequent activation of the immune system is an important part of its antitumour action. In a cohort of 48 newly diagnosed GBM patients, the median overall survival of gene therapy plus standard of care (SOC) was 25 months versus 16.9 months for SOC alone (134 patients). One, two and three-year survival figures were 90%, 53% and 32% versus 64%, 28% and 6% for the SOC control group.

Autologous and allogeneic GBM antigens (Gliovac™) as a vaccine

Six-month survival for nine Gliovac™ advanced GBM patients, who had undergone standard radio- and chemo-therapy plus bevacizumab, was 100% versus 33% in the control group. Survival at 40 weeks was 77% compared with 10% of the untreated group. The vaccine has now entered Phase II trials [20].

Dendritic cell vaccine (DCVax®-L)

DCVax®-L is a preparation of the patient's own dendritic cells (DCs) that have been pulsed with proteins extracted from the brain tumour removed at surgical resection. The DCs process the tumour antigens and are reintroduced into the patient where they stimulate an antitumour immune response. Recently published interim results from a Phase III trial examined the effect of the DC vaccine on patients undergoing standard treatment for GBM (i.e., surgery, TMZ and radiation). Median overall survival of 40.5 months was seen in 100 patients (out of 331 intention-to-treat participants) which, along with relatively few adverse events, makes this a promising approach for further development [21].

Depatuxizumab mafodotin (ABT-414) immunotoxin

Depatuxizumab mafodotin (Depatux-M) is an antibody–drug conjugate between the epidermal growth factor receptor (EGFR)-directed monoclonal antibody depatuxizumab directed to the EGFR and the potent antimicrotubule agent monomethyl auristatin F (mafodotin). EGFR amplification (as well as the EGFRvIII deletion variant) was found in ~50% of GBM cells, revealing a tumour-specific binding site for depatuxizumab [22]. Because the antibody has limited binding to EGFR in normal tissues, toxicity is limited, unlike other anti-EGFR treatments. The drug conjugate can enter the tumour via antibody targeting, leading to cell death. Results of a multinational clinical trial involving 66 EGFR-positive patients with GBM (pre-treated with standard of care) have been published recently [23]. Apart from ocular adverse events, as seen with other antibody–drug conjugates, 31 subjects responded with the remaining 34 showing progressive disease. This approach was considered sufficiently promising to warrant the creation of two randomised trials (INTELLANCE 1 and 2) [23].

It is noteworthy that these promising therapies are biologically based, which contrasts with more-conventional targeted oncology drugs such as small-molecule signalling inhibitors. These five examples could represent the tip of a larger iceberg that is yet to reveal itself, as the results of ongoing and future GBM trials become available. Improvements in early diagnosis, surgery and radiation therapy have not been highlighted in this review. Also, new medical devices might prove useful for GBM treatment; for example, tumour-treating fields (TTFs), 200 kHz alternating electrical fields that have antimitotic activity when transduced through the scalp, showed improved progression-free and overall survival in a trial published in 2017 [24].

Challenges of GBM drug development

Translational research in oncology has always been fraught with difficulty because drug candidates with impeccable profiles in preclinical models, including mouse xenografts, often provide no clinical benefit. A recent example is provided by the Phase III failure of Incyte's epacadostat, an inhibitor of indoleamine (2,3,)-dioxygenase (IDO) for treatment of various solid tumours, which is casting a shadow over related efforts in this area [25]. This example has implications for GBM therapy, because Incyte are sponsoring two trials for this condition (NCTs 02327078 and 03532295). There are several possible reasons for this disconnect between preclinical and clinical findings, among which target validation, drug resistance, tumour heterogeneity and clinical trial design are the most likely causes.

Choosing the right target

Preclinical target validation is essential before undertaking clinical development. This depends crucially upon the predictive quality of whichever cell-based and *in vivo* models are used to test a given hypothesis. These could bear no relation to the *in vivo* architecture of human tissues and, therefore, as suggested by Horvath *et al.*, more effort must be put into developing more-predictive preclinical models. These can include models based on pluripotent stem

cells, 3D co-culture and organ-on-a-chip systems, complemented by advances in single-cell imaging and gene editing technologies [26]. One practical example is provided by Miller *et al*. who used RNA interference screening technology to demonstrate that gene expression in primary explanted GBM cells is significantly different to that occurring in xenograft models when human cells are transplanted into mouse brains [27].

Drug resistance

The drugs covered in the clinical trials for GBM surveyed here and by others [7–9] mostly affect cell viability by acting on targets involved in cell division or regulation of apoptosis. It has been noted that targets with driver mutations in GBM such as platelet-derived growth factor receptor A (PDGFRA), phosphatase and tensin homologue (PTEN) and phosphoinositide 3 kinase (PI3K) subunits are poorly represented in clinical trials for the disease, except for EGFR, which is targeted in 11% of trials [28]. One reason for this low representation could be the poor brain penetration of many of these inhibitors, as described for the tyrosine kinase inhibitor sunitinib, which targets GBM driver mutations in PDGFRA and vascular endothelial growth factor receptor (VEGFR)1–3 tyrosine kinases. Despite having a good preclinical profile, this compound has not been successful in clinical trials, owing to activity of the efflux pumps P-gp (ABCG1) and Bcrp (ABCG2) in the blood–brain barrier [29]. Pharmacological inhibition of these efflux pumps at the same time as administering the therapeutic drug is clearly an attractive option; however, despite many years of research, no P-gp inhibitors are currently in clinical use, although promising leads are being generated [30].

Activation of signalling pathways that bypass the target of small-molecule inhibitors in tumour cells is another aspect of drug resistance during GBM therapy and argues for the use of combination therapies along the lines of those being evaluated in melanoma. For example, a Phase I clinical trial (NCT02097225) of a triple inhibitor combination, dabrafenib, trametinib and onalespib, that simultaneously targets B-Raf and MEK kinases and heat shock protein respectively, is near completion. This, and other combination therapies for several cancers (not specifically GBM) have been reviewed [31].

One of the reasons for poor prognosis in GBM patients treated with the DNA alkylating agent TMZ is the emergence of acquired resistance to this drug and the outgrowth of malignant cells. TMZ methylates DNA at the O6 position in guanine leading to impaired DNA repair and ultimately apoptosis. The O6-methylguanine DNA methyltransferase (MGMT) removes the DNA adduct caused by this alkylating agent resulting in resistance to TMZ therapy, with DNA methylation on the MGMT promoter being a major predictor [32]. Another resistance mechanism involves loss of function in the MutS homolog 6 (MSH6) mismatch repair gene [33]. In addition to resistance to TMZ, the drug is itself mutagenic and can introduce new driver mutations during the initial successful phase of treatment, thereby leading to malignant progression [34]. Clearly, interventions that target TMZ-resistant cells are desirable. Through screening a range of GBM cell lines with FDA-approved chemotherapeutic drugs, Teng *et al*. have identified compounds that overcome resistance to TMZ by interfering with specific resistance pathways [35]. One of these, the ribonucleotide reductase M2 (RRM2) inhibitor hydroxyurea, was particularly effective in overcoming TMZ resistance and a Phase I trial for GBM (NCT03463733) is currently at the recruiting stage.

Finally, the issue of drug resistance and cancer stem cells (CSCs) must be considered. GBMs contain self-renewing CSCs that contribute to tumour initiation and therapeutic resistance (for a review, see [36]). Resistance to TMZ is due in part to CSCs hijacking the activation of DNA repair by normal progenitor cells that maintain tissue homeostasis. Increased DNA repair leads to resistance to therapy, including radiation [37,38]. However, the picture could be more complicated. As noted by Chumakova and Lathia [37], differentiated cells as well as CSCs are still very resistant to TMZ at clinically relevant concentrations. The failure of an MGMT inhibitor (O6-benzylguanine) along with an alkylating agent (carmustine) and radiation to improve clinical outcomes implies that TMZ resistance might not be caused by higher levels of MGMT expression in susceptible patients [39]. The mechanisms of resistance to TMZ and similar agents are therefore not fully resolved.

Tumour heterogeneity

Another aspect of GBM that could contribute to clinical trial failure is the heterogeneity of tumour cells within and between patients. Technical advances have helped to reveal this heterogeneity at the single-cell level using DNA and RNA sequence analysis [40,41]. On a larger scale, The Cancer Genome Atlas (TCGA) and Repository for Molecular Brain Neoplasia Data (REMBRANDT) contain gene expression data on GBM tumours from different patients and therefore can be used to assess interpatient heterogeneity [42,43].

A more recent initiative by the Allen Institute for Brain Science has taken this a step further by creating the 'Ivy Glioblastoma Atlas' from anatomical features in tumours taken from 41 patients and identified by *in situ* hybridisation [44]. Four features [leading edge (LE), cellular tumour (CT), palisading cells around necrosis (PAN) and microvascular proliferation (MVP)] were isolated by laser capture microdissection and profiled for mRNA expression. A detailed analysis of inter- and intra-tumour heterogeneity is also included in the Ivy Atlas [44], as well as some published applications. For example, Atlas data indicate that brain-tumour-initiating cells (BTICs) are found predominantly in the bulk of cellular tumours; this led Yu *et al*. [45] to direct delivery of specific inhibitors (in this case RNA encapsulated in lipopolymeric nanoparticles) to BTICs in the tumour bulk. This approach circumvents problems with blood–brain barrier penetration when using systemic delivery. In xenograft models with encapsulated RNAis against a combination of four key transcription factors, via an implanted osmotic pump, median survival of the mice was extended by 19 days, representing 50% increased survival.

We have already mentioned the lack of success in clinical trials using inhibitors of GBM targets affected by driver mutations. Apart from poor pharmacokinetics or the induction of resistance pathways, a given tumour can contain a mixture of cells with distinct vulnerabilities. For example, GBM contains a mosaic of EGFR-amplified and PDGFRA-amplified cells and can express different variants of these in the same tumour [46] such that a single kinase inhibitor is unable to eradicate all cells [47]. This, alongside the need to inhibit multiple resistance pathways, is another argument for the use of combination therapies. Encouragingly, new techni-

ques for identifying synergistic drug combinations are now entering the laboratory (e.g., L1000 gene perturbation profiling) [48], which makes combinatorial drug testing faster and more efficient. With >500 drug candidates already known to modulate GBM behaviour, including many FDA-approved agents [49], effective combinatorial treatment strategies, based upon our existing drug armamentarium as well as future discoveries, are very likely to emerge.

Clinical trial design

GBM trials completed between 2005 and 2016 showed deficiencies in patient recruitment and a disconnect between trial design for Phase II and Phase III, often leading to failure in the latter as highlighted by Vanderbeek *et al*. [9] who also pointed out that many drugs are trialled in combination with the standard of care (TMZ and/or radiation), thus adding an extra level of complexity. Another significant problem lies in the primary endpoint used in many studies. Increasing overall survival time is an obvious goal, but many trials show that progression-free survival times can significantly differ from this. An example of this is the failure of bevacizumab and TMZ/radiation to increase overall survival in a Phase III trial, as discussed previously [11]. This failure to translate Phase II success to Phase III is due in part to the complexity of the cellular and molecular responses occurring during therapy. Furthermore, progression-free survival at 6 months (PFS6) depends on MRI measurements that can show pseudoprogression or pseudoresponses depending on the type of therapy [50] and even tumour type. For example, patients with MGMT promoter methylation in their tumours have a higher likelihood of showing pseudoprogression but have a better prognosis with respect to overall survival [32].

To address this situation, there is a burgeoning interest in adaptive clinical trial design, described below. According to the FDA guidance, an adaptive clinical trial is defined as 'a study that includes a prospectively planned opportunity for modification of one or more specified aspects of the study design and hypotheses based on analysis of data (usually interim data) from subjects in the study' (FDA Guidance for Industry: Adaptive design clinical trials for drugs and biologics; http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm201790.pdf). Analyses of the accumulating study data are performed at prospectively planned timepoints within the study. In adaptive clinical trials, investigators can allocate subjects to experimental arms as required and drop ineffective treatments at early stages.

Alexander *et al*. discussed the application of Bayesian statistics and biomarker selection methodology to adaptive GBM trials based on lessons learned from the successful I-SPY-2 trial for breast cancer [51,52]. The latter was designed as a multi-arm Phase II randomized trial using experimental agents from five different pharmaceutical companies. The primary decision criterion was the Bayesian predictive probability of being successful in a subsequent confirmatory Phase III study, for each drug–biomarker signature pair. Drugs that were found during the trial to have a sufficiently low probability of success were dropped from the study, allowing new treatment arms to take their place, thereby creating a dynamic and flexible framework. However, this adaptive breast cancer clinical trial had access to a range of well-validated biomarkers and matching therapeutics (e.g., HER2 expression and trastuzumab), which is not currently the case for GBM. Here, the options are more limited, with a few possible biomarker–therapeutic pairs such as MGMT methylation status/TMZ and EGFRvIII expression or targeted biologicals, as well as mutant isocitrate dehydrogenases (IDH1 and 2) and their inhibitors currently in development [53]. The search for biomarkers and matching drugs continues using genomic technologies and stratified patient groups, reviewed in Ref. [54], reinforcing the point (discussed in Ref. [52]) that adaptive clinical trials require careful integration of therapeutic and diagnostic modalities for effective deployment.

Recognizing the pressing therapeutic need, adaptive clinical trials for GBM are currently being planned by the Global Coalition for Adaptive Research in the form of the GBM AGILE platform (https://www.gcaresearch.org/gbm-agile/) with Bayer's regorafenib being the first drug to be trialled. Regorafenib (Stivarga) is a multikinase inhibitor with antiangiogenic activity that shows some improvement in overall survival in a Phase II trial using lomustine as comparator [55].

Emerging therapeutic strategies

GBM does not have the same response rate to chemotherapy as, for example, testicular cancer which has a >90% cure rate using platinum-based drugs [56]. GBM drug therapies must also overcome the serious obstacles of tumour heterogeneity, CNS penetration and drug resistance, summarised in Fig. 1. These important issues are being addressed in second-generation GBM drugs (see for example the development of brain-penetrant PI3K inhibitors [57]). The use of optimised drugs in combination also holds considerable promise for addressing the inter- and intra-patient tumour heterogeneity seen in GBM, especially where this can be included in adaptive clinical trials designed to optimise individual patient outcomes. For such adaptive approaches to become a practical reality, the development of a range of new, noninvasive biomarkers, capable of rapid deployment within a clinical setting, will be needed.

Among emerging therapies, immunotherapy currently seems to hold the most promise, reviewed recently by Khansur *et al*. [58], and treatments based on checkpoint inhibitor antibodies [59] and antibody–toxin immunoconjugates [60] are currently in clinical trials. Considerable attention is also being paid to cell-based immunotherapy approaches, based on the observation that elimination of target-antigen-positive GBM cells (tumour editing) occurs in patients with GBMs expressing IL-13Rα2 and EGFRvIII, supporting the idea that a tumour-antigen-specific immune response occurs *in vivo* [18]. Although antigen escape and interpatient tumour heterogeneity are major issues yet to be tackled, multiple antigens (including CD133 and EphA2) could be targeted simultaneously using, for example, bi- and tri-specific CAR T cells [61,62]. Further encouragement for a T cell approach is provided by the recent finding that just a single (clonally expanded) CAR T cell might be sufficient to cause remission in other cancers such as chronic lymphocytic leukaemia [63].

There are, however, caveats for immunotherapy in GBM, as for other solid tumours, where success rates have not yet matched those for B cell malignancies. GBM is seen as a 'cold tumour' (i.e., one with limited immune cell infiltration and a low tumour mutation burden compared with other cancers) [64]. However,

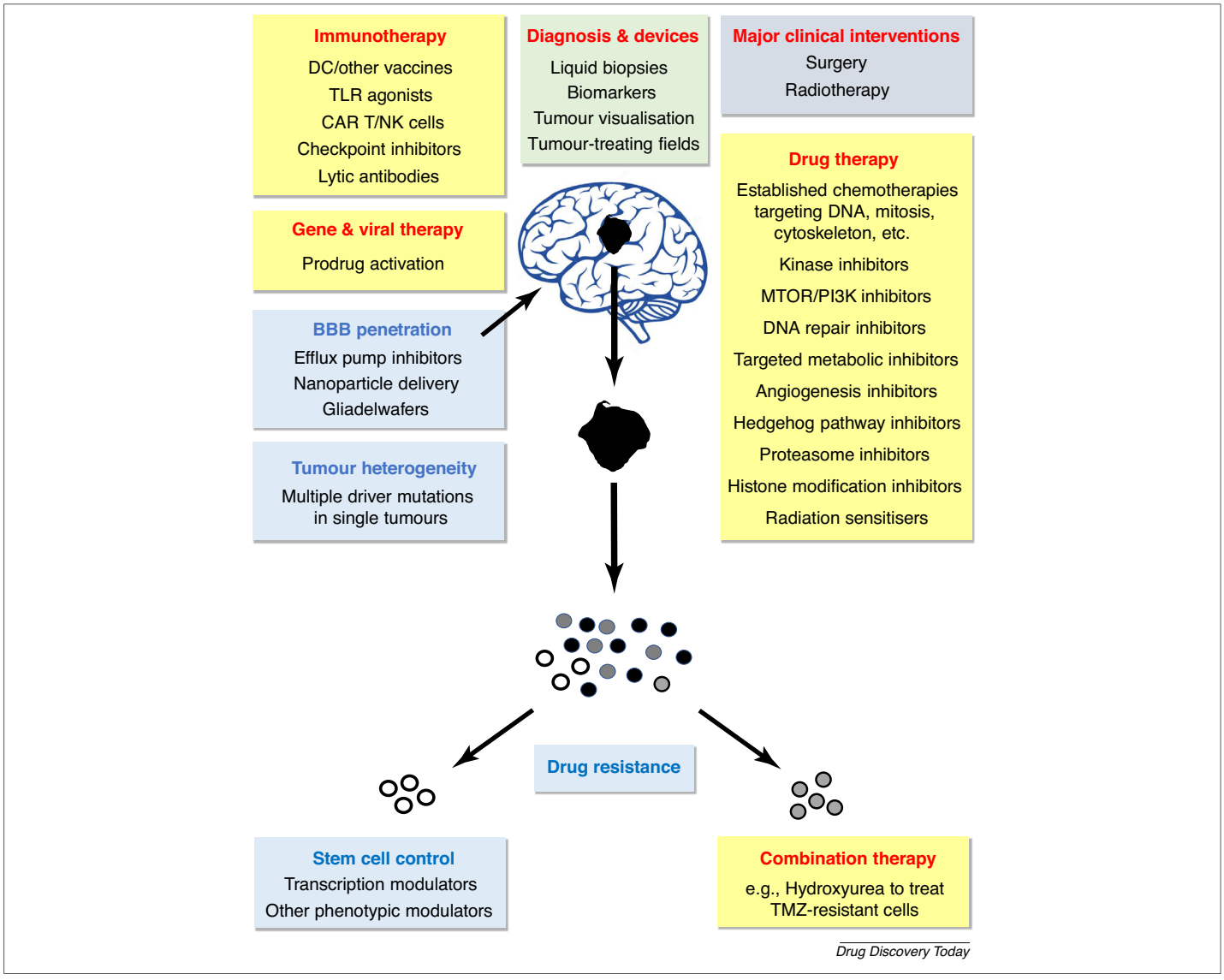

FIGURE 1

Summary of points of intervention in glioblastoma (GBM) treatment. A wide variety of therapies are currently being trialled in GBM to complement the standard clinical interventions of surgery and radiotherapy (grey panel). These range from conventional drug therapy to new combination therapies, as well as biological approaches including immunotherapy and gene therapy (yellow panels). The potential impacts of new diagnostics such as liquid biopsies and devices such as tumour-treating fields have yet to be determined, although there is a clear need for new biomarkers to define patient response (green panel). Drug quality issues, such as blood–brain barrier penetration, combine with biological challenges, such as tumour heterogeneity, drug resistance and stem cell control (blue panels), to make GBM a particularly intransigent disease.

despite this, there are cell-surface molecules on GBM that can be recognised by the immune system for vaccines and engineered killer cells, as highlighted above. This cautious optimism is reinforced by recent findings from two Phase I trials of peptide vaccines which stimulated robust T cell responses to neoantigens expressed in GBM tumours [65,66]. Interestingly, in one of the studies [66], these responses were enhanced in patients who were not prescribed anti-inflammatory steroids as part of their standard of care, offering a pointer to future investigations. Although these two studies demonstrated the feasibility of creating immune responses to GBM antigens, the patients failed to demonstrate any survival advantage in these trials, possibly owing to clonal exhaustion of the T cells. These clinical results are similar to those from a recent Phase III vaccine trial [67], indicating that more work has to be done to build on the successful induction of T cell responses in 'cold tumours'. This might include combination therapy with checkpoint inhibitors, as suggested by Zaidi and Jaffee [64]. This approach appears to work in an animal model of pancreatic cancer at least, where coadministration of a vaccine with anti-PD-1 and OX40 agonist antibodies increased survival benefit and reduced T cell markers of clonal exhaustion [68].

Clearly, many practical hurdles have yet to be overcome for successful immunotherapy, not least the problem of providing a 'one size fits all' cell or vaccine therapy instead of having to isolate and modify cells from each patient. The pharmaceutical industry in the past has been unwilling and unable to provide the resources for individualised therapies, instead focusing on small-molecule and (mostly) protein drugs that can be manufactured at scale. However, the marketing approval for Novartis's Kymriah® (tisagenlecleucel) CAR T cell therapy against comparatively rare B cell

lymphomas has changed this landscape (https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm574058.htm) and encouraged others, such as Gilead Sciences, to develop similar products (https:// ash.confex.com/ash/2018/webprogram/Paper111368.html).

Although formidable manufacturing and logistical problems accompany personalised cell therapies, efforts are now being made to generate T cells that can be used 'off the shelf'. For example, allogeneic cells can be engineered to remove the endogenous T cell receptor responsible for provoking graft versus host disease in recipients and it can be expected [69] that a new generation of cell therapies will emerge in due course to replace those currently based on patient-specific T cells.

Concluding remarks

In this review, we pose the question: is GBM therapy working? To answer this question, we surveyed the clinical literature in some detail to gain an understanding of the types of intervention that are showing promise. Our conclusion is that small-molecule interventions have not significantly improved the standard of care to date, although the vast majority of new clinical trials continue to focus on small-molecule therapy. With new molecules and targets in hand, together with smarter combination therapy selection and adaptive clinical trial design, this recipe could still deliver success. Moreover, a detailed examination of the emerging clinical literature indicates that developments in modulation of T cell function and specificity, coupled with an unprecedented interest in novel immunotherapies specifically targeted at GBM, provide considerable promise for increasing the overall survival and quality of life of GBM patients – a great leap forward from today's dismal outlook.

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Appendix A. Supplementary data

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