Research Article

Future Perspectives: A Review of Therapeutic Advances in Recurrent Glioblastoma

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Abstract

The outcomes for glioblastoma (GBM) patients remain dismal despite significant increases in our understanding of treatment-naïve disease biology and increased focus on clinical trials. Almost all patients experience disease recurrence. If we hope to develop more durable responses in this aggressive disease cohort, a better understanding of resistance mechanisms and drivers of GBM tumour recurrence will be needed. Here we review the findings to-date of current advances in therapeutic development and biology of recurrent GBM, highlighting recent research and clinical trials breakthroughs.

1. Introduction

Glioblastoma (GBM) is the most common and aggressive form of adult brain cancer. Prognosis is very poor with the majority of patients experiencing tumour recurrence within two years of diagnosis. The current standards of care add minimal survival benefit and include surgery followed by adjuvant radiotherapy and temozolomide (TMZ) chemotherapy [1]. It is now established that GBMs are highly heterogeneous tumours comprising significant numbers of cancer cells that reside in dynamic molecular cell states [2-4]. Among this cellular milieu, lie cancer cells with the ability to evade current therapies either through senescence, DNA repair mechanisms or inherent stem cell-like characteristics [5]. In addition, these brain cancer cells are typically highly invasive and migratory making complete resection practically impossible. Adding complexity, is the fact that radiation and chemotherapy add significant therapy-induced genetic alterations, ultimately increasing the aggressiveness of brain cancer cells and altering tumour evolution at recurrence [6]. During the past 30 years the neuro-oncology research community has largely focused their research efforts on understanding treatment naïve disease. This has left a knowledge gap with respect to resistance mechanism and drivers of GBM tumour recurrence. Recent efforts have sought to close this gap and increase our understanding of the biology of recurrent disease [7-9], better define resistance mechanisms [10] and develop and test new therapeutic approaches to treat this aggressive disease [11-13].

Historically, one of the biggest obstacles which has prevented clinical progress in the neuro-oncology field has been the blood brain barrier (BBB). The BBB works via endothelial cells restricting the passage of both small and large molecules into the brain [14]. This normal neuro-protective mechanism significantly reduces the ingress of almost all molecular and antibody-based therapeutics. Unfortunately, the majority of clinical trials to-date, both in upfront and recurrent disease, have relied largely on systemic routes of administration. This has meant, in most cases, that these trials were destined to fail. In rapidly growing solid tumours such as GBM, vascular hyper-proliferation is common and consequently blood vessel formation is not always tightly regulated [15]. Evidence also exists that tumour cells with stem cell-like properties can differentiate into tumour-derived pericytes and endothelial cells [16]. These abnormal processes lead to the formation of large irregular, often oedematous, blood vessels within recurrent tumours with compromised BBB dynamics. This so-named blood tumour barrier (BTB) may add benefit for therapeutic penetrance in the recurrent setting, although this is a topic of debate [17]. Alternate surgical approaches such as convection-enhanced delivery (CED) rely on intrathecal administration of drugs directly into the tumour site, circumventing the BTB [18]. CED has been used in recurrent GBM patients to administer a modified form of inactivated polio virus (PVS-RIPO) with encouraging results [12]. A trait common to aggressive solid tumours is the ability to evade the immune system, a mechanism now well established in brain cancer [19]. Adjuvant immuno-oncology (IO) has essentially failed in clinical testing in GBM. A recent breakthrough has shown that checkpoint inhibitors when given neo-adjuvantly (prior to tumour resection) greatly increases IO efficacy [13]. This seminal study paves

the way for future neo-adjuvant approaches and provides a therapeutic modality with general applicability without the need for specialist techniques such as CED. Here we review the research and clinical trial literature with respect to recent perspectives and developments in recurrent GBM. The scope of this review covers critical aspects of tumour evolution, heterogeneity, BBB penetrance, tumour immunology, and recent clinical trials efforts.
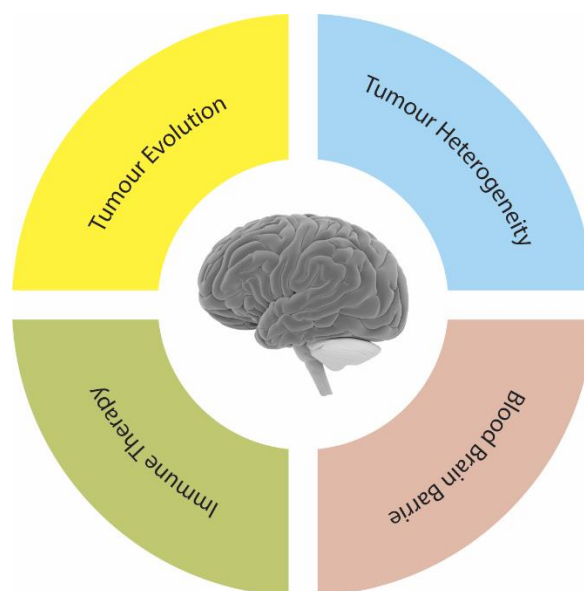

Figure 1: Central obstacles in the development of new therapeutic interventions in recurrent GBM.

2. Tumour Evolution

Understanding the genetic, epigenetic and transcriptomic differences that distinguish primary from recurrent GBM is critical to guide the development of successful targeted therapies. However, while primary GBM has been extensively characterised at the molecular level [2-4, 20-25], similar in-depth analysis is lacking in recurrent disease. Only recently have longitudinal studies begun to shed light on the spatiotemporal evolution of primary and recurrent tumours and studies with larger cohorts are finally beginning to emerge [5, 26].

2.1 Initiating events leading to tumour evolution

Despite significant intra- and inter-tumoural heterogeneity, a common initiating pathway to GBM tumourigenesis involving chromosome 7 (gain) and chromosome 10 (loss) has been established, with copy number variants remaining stable between disease initiation and recurrence [7, 26-31]. Single nucleotide variants (SNVs) occur later during tumour development, classified as either clonal mutations, occurring in all tumour cells, or sub-clonal, occurring in a subset of cells. A prominent example of sub-clonal mutation is the mosaicism of focal amplifications of receptor tyrosine kinases (RTKs) [8, 32-34]. Mutations in PIK3CA, TP53,

ATRX and the TERT promoter are mostly clonal, truncal events acquired early during tumour formation and are thought to be responsible for driving early cancer survival and growth [26, 28, 29, 35]. Alternatively, mutations in EGFR, PDGFRA, NF1 and PTEN have been found in both clonal and sub-clonal populations, suggesting these mutations are later events [5, 26, 28, 35]. The frequent sub-clonal occurrence of many known driver gene mutations in pre-treatment and recurrent disease suggests that these accumulate due to convergent evolution caused by common selection pressures rather than being tumour-initiating events. On a global level, the majority of mutations in the primary tumour are retained at recurrence [26, 36]. However, mutations in known driver genes, including EGFR, PDGFRA, the ARF/TP53 pathway, PTEN, NF1 and the INK/RB1 pathway, are disproportionally affected by mutational switching [5, 9, 26, 28, 35, 36]. Driver gene mutations can be lost, acquired, mutated at a different site, or show different amplification/deletion breakpoints. Importantly, Draaisma et al. found that approximately 20% of tumours show mutational changes in genes involved in cell cycle, the PI3K/AKT/mTOR and RTK signalling pathways at recurrence [26]. Mathematical modelling demonstrated that this has important implications for clinical trial design, particularly for therapies that target genes with lower mutation retention frequencies, such as EGFR [26]. Divergence of driver gene alterations was more pronounced in distant compared to local recurrences, suggesting that distant recurrences in particular should be reassessed to ascertain therapeutic vulnerabilities to targeted therapies [36]. Relapse-specific or -enriched alterations are rare [26, 29]. Examples include mutations in MSH6, a component of the DNA mismatch repair (MMR) pathway, LTBP4, a regulator of the TGF-beta pathway, the insulin-like growth factor 1 receptor (IGF1R) and TET2, DNMT3A and PRDM2, which are involved in DNA methylation [5, 26].

Whole genome and exome sequencing of pair-matched primary and recurrent tumours showed that genetic evolution occurs mostly in an idiosyncratic manner [28]. This suggests that large population-based studies are required to characterise patient cohorts with shared evolutionary patterns of recurrence. Nonetheless, two overarching patterns were identified, supporting a model whereby recurrent tumours arises either from an early ancestral clone that branched off during tumour formation (divergent evolution) or from residual disease clones that persist through therapy (linear evolution) [36]. Branched evolution resulted in substantial genomic divergence with a low mutation retention frequency of approximately 25% between the primary and recurrent tumour. Linear evolution, on the other hand, gave rise to tumours with a high mutation retention frequency (~75%) [36]. Comparison of evolution patterns with clinical parameters found that local recurrences typically developed via linear evolution, whereas distant tumours via divergent evolution, accompanied with a small percentage of mutations from the primary tumour [36]. In support of this finding, Lee and colleagues also found greater genetic diversity between distant compared to local recurrences [35]. Together, these studies imply that spatially and/or temporally distant recurrent tumours arise from clones that diverged during early tumour development and underwent extensive clonal selection. However, even in the case of 'linear' evolution, Wang et al. reported that dominant clone(s) in recurrent disease are typically not linear descendants of the dominant clone(s) from the primary tumour, but rather are descendants of minor clones that persisted following therapy [5].

Several studies suggest that the clones responsible for tumour recurrence arise early during tumour development [5, 29, 31, 38]. Dominant clones at diagnosis and relapse had evolved separately from a common ancestor many years prior [5]. Early ancestral clones have been described to reside in the subventricular zone (SVZ) and within infiltrating margins [38-40]. Piccirillo et al. demonstrated the presence of ancestral cells with tumour-initiating capacity in the SVZ, a known stem cell niche [39]. The same group later extended this study, demonstrating that residual disease clones in the infiltrating tumour margins had diverged early during tumourigenesis [40]. Phylogenetic analysis of pair-matched samples suggested that these infiltrative sub-clones are/might be the 'missing link' between the primary and recurrent tumour [40].

2.2 The effect of temozolomide on tumour evolution and recurrence

Numerous studies have suggested that tumour heterogeneity contributes to treatment failure [31, 33, 34, 40, 41]. Muscat et al. provided evidence for chemoradiation-induced selection of resistant clones by comparing pair-matched primary and recurrent GBM. Therapy reduced the number of sub-clonal variants while expanding resistant sub-populations [6]. An example of this mechanism has been shown for EGFR-targeted therapies, where 82% of patients showed loss of EGFRvIII expression at relapse after receiving treatment that targeted the variant of this receptor [42]. TMZ is an oral alkylating agent that is the first-line chemotherapeutic agent for the treatment of GBM [1]. In the absence of a hypermutation phenotype, Muscat and colleagues found no enrichment of a TMZ-induced mutational signature (C$\rightarrow$T transitions at CpC and CpT dinucleotides) following TMZ treatment [6]. Similarly, Koerber et al. reported no enrichment of the TMZ signature

in recurrent tumours following TMZ therapy [29]. Together these findings show that TMZ has little influence on the generation of new mutations in recurrent disease in the absence of a hypermutation phenotype. In contrast, hypermutated tumours harbour defects in genes encoding proteins of the DNA mismatch repair (MMR) pathway and showed strong enrichment of the TMZ mutational signature [5, 26, 29]. Hypermutation was found to predominantly affect highly expressed genes, suggesting that TMZ and/or MMR preferentially target areas with open chromatin [5]. Also TMZ-associated hypermutation phenotype was found to be rare in IDH-WT disease suggesting that standard treatment poses little risk of developing hypermutation for patients with primary GBM [6, 27, 37]. This is in stark contrast to the observations made in the less common form of recurrent IDH1-mutant glioma [10]. Two possible explanations for this disparity between IDH1 wild-type and mutant gliomas has been proposed [36, 43]. The cumulative dose of TMZ given to patients with primary GBM is lower than IDH-mutant or low-grade glioma, raising the possibility that long-term exposure to TMZ increases the risk of hypermutation [5, 36]. Secondly, IDH1-mutation may predispose tumours to TMZ-induced hypermutation. IDH1 gain-of-function mutations predispose tumours to MGMT promoter methylation, thereby reducing the chance to repair TMZ-induced mutagenesis and as a result increasing the chance to acquire mutations in MMR pathway genes associated with hypermutation [5, 36]. Overall, longitudinal studies of GBM evolution have revealed a significant amount of evolutionary divergence in recurrent disease. As a corollary, several studies advocate for resampling of the recurrent tumour before selecting targeted therapies for treatment [7, 26, 36].

3. Tumour Heterogeneity

Intratumoural heterogeneity describes a phenomenon where individual cells or compartments within a single tumour mass are associated with different subgroups or molecular characteristics, (reviewed in [44]). Surgical multisampling of spatially separated GBM tumours has identified both unique and common genetic, epigenetic, and transcriptional alterations [31, 45]. On a transcriptional level GBM heterogeneity is characterised by three transcriptional subtypes, namely classical, proneural and mesenchymal [3, 4]. Each subtype is enriched for specific genetic alterations, classical GBM shows a high frequency in EGFR alterations and 95% of classical GBM exhibit a homozygous deletion of *CDKN2A* (INK4a/ARF) [4]. The proneural subtype is associated with IDH1 mutation, and TP53, PDGFRA amplification or mutations. IDH1 mutations are relatively rare in primary *de novo* GBM and even more so in recurrent GBM [46, 47]. Mesenchymal GBM shows a high level of heterogeneity and alterations in tumour suppressor genes such as Neurofibromatosis 1 (NF1), TP53 and loss of phosphatase and tensin homolog (PTEN); 30-49% of GBM tissues can be classified as mesenchymal [4, 24]. This is of importance, since patients with this subtype, both at the primary and recurrent state of the disease, tend to have a worse survival rate compared to classical and proneural subtypes [48]. Several studies have elegantly stratified GBM into molecular subtypes [3, 4, 24]. This concept has recently been extended by Suva and colleagues using single cell RNASeq. In this study they defined four dynamic cell states with the ability to recapitulate the tumour mass post-therapy [2]. Among these classifications, the mesenchymal subtype has been associated with a more stem cell-like phenotype and has been linked to radioresistance and GBM recurrence [48]. Moreover, two-thirds of primary GBMs classified as a proneural or classical switch towards the mesenchymal subtypes at tumour recurrence [5]. Several well-known markers have been associated with a mesenchymal phenotype in GBM such as vimentin and CD44. More recently other receptors such as EphA3 and dystroglycan have been shown to be enriched in mesenchymal GBM tissue and linked to tumour recurrence [49-52]. Phase I EphA3-targeting trials have shown promise with significant levels of EphA3-positive tumour tissue detected in recurrent GBM patients [53].

Glioma stem cells (GSCs) comprise a small population of cancer cells that are present in tumour tissue and are characterised by high tumorigenicity and self-renewal capacity [54]. It is now well-described that GSCs, harbour the capacity to differentiate into other tumour cell types giving rise to the diverse and dynamic heterogeneity observed within GBM [2, 5, 23, 31, 55]. In addition, GSCs have been associated with resistance to therapy and tumour recurrence [56]. Seminal studies conducted by Rich and colleagues showed that CD133+ GSCs could effectively promote radioresistance [56, 57]. More recently, GSCs have been shown to promote chemoresistance at nearly every pharmacologic level [58]. Secretion of exosomes, activation of autophagy [59], cell metabolism [60], ROS production [61] drug efflux [62], and microRNA expression [63, 64] are also altered in GSCs and can further enhance therapeutic resistance. Significant effort has been leveraged to tackle GBM heterogeneity by defining therapeutic strategies to target the GSC pool in effect targeting the tumour at its roots. An alternate strategy, could be approaches which induce differentiation of the entire tumour cell population delaying growth [65]. Hence, constraining GSCs to a limited transcriptional program as cells differentiate and reduce available escape routes from therapy [66]. Differentiation

therapy has shown clinical promise in acute promyelocytic leukemia (APL) with the use of all trans retinoic acid (ATRA) [67]. Bone morphogenetic protein (BMP) signalling triggers cell-cycle exit and astrocyte differentiation of GSCs and might therefore be useful as a differentiation therapy [68]. A BMP-mimicking peptide GBMP1a, induces astroglial differentiation of GSCs *in vitro* [69]. However, these cells show limited differentiation commitment and remain vulnerable to cell-cycle re-entry, retaining stem cell-like DNA methylation patterns [70]. Moreover, Blocking WNT and SHH signalling in combination with BMP treatment has been shown to supress GSC self-renewal capacity and extended survival of tumour-bearing mice [71]. Clinical translation of differentiation therapies in brain cancer still appears difficult and further investigation into the mechanisms by which tumour cells evade differentiation commitment is needed.

4. Blood Brain Barrier (BBB)

The BBB is a brain-specific complex architecture comprising endothelial cells, pericytes, astrocytes, neurons, and extracellular matrix components. Tight junctions between endothelial cells and pericytes form the BBB to restrict the diffusion of larger molecules (>180 Daltons) from entering the brain [14]. This naturally occurring protective mechanism prevents or significantly reduces the ingress of many therapeutic agents. In addition, the BBB expresses high levels of drug efflux pumps posing a further problem. BBB integrity is partially compromised in brain tumours, commonly referred to as the blood–tumour barrier (BTB) [72]. The BTB can be detected during magnetic resonance imaging (MRI) by measuring the diffusion of gadolinium. Despite being leakier than the BBB, the BTB is heterogeneously permeable to most chemotherapeutic agents and is the rate-limiting factor in clinically effective therapy.

The BTB is often more disrupted in recurrent disease and is likely the main reason why novel therapies that fail in the upfront setting show promise in a recurrent cohort [73, 74]. A recent example is Depatux-M (ABT-414) monotherapy that showed improved progression-free survival in the recurrent setting but then failed in newly diagnosed patients and the trial was subsequently ceased [11].

Numerous efforts have been made to bypass the BBB to improve drug uptake. Targeting low-density lipoprotein receptor-related protein 1 (LRP1) on the cell surface of BTB cells has shown some efficacy in recurrent GBM studies [75]. An interesting report in the recurrent disease showed that Bevacizumab globally reduced permeability, but had a positive effect in leaky regions allowing better delivery of TMZ [76]. Success of studies combining chemotherapeutic agents with drugs that inhibit efflux pumps suggest that more potent inhibitors could increase drug penetration across the BTB [77]. Physical approaches to breach the BTB are garnering recent renewed interest. Magnetic resonance-guided focused ultrasound (MRgFUS) is at the forefront of these technologies and has demonstrated great success in clinical studies. MRgFUS employs short ultrasound pulses with circulating microbubbles to transiently disrupt the BTB thus increasing permeability for 6-8 hours, allowing a window for increased drug uptake [15, 78]. Phase II clinical studies have not only confirmed preclinical findings but also demonstrated the safety and efficacy of this novel technology in recurrent disease [79, 80]. Nanotechnology has also shown promise as an emerging technology. A recent study showed the successful transport of TMZ and an anticancer drug JQ1 packaged in a liposomal nanoparticle using mouse orthotopic glioma models [81]. The study reported stable drug circulation in the bloodstream when encapsulated by nanoparticles with higher drug accumulation in xenografted

tumours and improved animal survival. Nanoparticles have also been successfully delivered directly to the brain via the intranasal route, bypassing systemic circulation [82].

A recent phase II clinical trial showed very promising results in a small cohort of recurrent GBM patients [12]. The trial was based on the expression of the poliovirus receptor CD115 in GBM that was recognised by a recombinant poliovirus. The polio vaccine PVS-RIPO was infused directly into the patient's tumour via convection- enhanced delivery (CED). CED employs a syringe pump connected to a catheter implanted at the tumour site to exert a constant pressure differential to drive drug-laden fluid throughout the tumour [18]. The success of this trial has driven renewed interest in CED and similar approaches as it enables a platform to test novel approaches and to re-test numerous intravenously administered drugs that have failed in clinical trial. A recent study used MR imaging to monitor CED in real time using iron oxide nanoparticles, showing broader distribution of a glioma-specific targeting therapy [83]. Many other CED and FUS based trials are currently in progress in recurrent disease and will reveal if these approaches will bring more durable responses in the future.

5. Immune Therapy

The immune system in the brain follows different principles from the immune system elsewhere. Most apparent reasons are the limited access to the tumour facilitated by the BBB, and the substantial endogenous and treatment-induced immunosuppression of the host. The CNS was once considered an immune-privileged site on the basis that non-syngeneic tissues are not rejected when implanted into the brains of mice; a perception that has only recently been disproved [84-87]. It is now clear that the brain is accessible

to the afferent and efferent arms of the immune system, and thus to immune therapy, reviewed in [88].

5.1 Tumour microenvironment

Relative to other solid tumours, CNS tumours display low numbers of tumour-infiltrating lymphocytes (TILs) and other immune effector cells [89]. This 'cold tumour' phenotype is associated with inadequate responses to immune checkpoint inhibitors (ICIs) [90]. Also, TILs that are present frequently display an exhausted phenotype [91]. Unlike in peripheral organs, unrestrained inflammation and increased intracranial pressure pose a threat to the brain. For this reason, both inflammatory and adaptive immune responses are tightly regulated in the CNS. This regulation involves a variety of immunosuppressive mechanisms at both the molecular and cellular levels [92]. In response to inflammation, brain stromal cells produce high levels of immunosuppressive cytokines, transforming growth factor β (TGFβ) and interleukin-10 (IL-10), thereby counteracting inflammatory cytokines to maintain homeostasis [93, 94]. Glioma cells regulate tryptophan levels in the microenvironment by the expression of indolamine 2,3-dioxygenase (IDO). Which in turns leads to the accumulation of regulatory T (Treg) cells and the suppression of T cell activity [95, 96]. Both microglia and tumour-infiltrating macrophages produce high levels of arginase, thereby depleting arginine in the tumour tissue. Low arginine levels have an inhibitory effect on T cell proliferation and function [97]. Specific inhibition of immunosuppressive factors in combination with other therapies are currently under investigation in patients with brain tumours. Targeting TGFβ with antisense oligonucleotides [98] or blocking antibodies [99], as well as kinase inhibitors targeting the TGFβ receptor 1 (TGFβR1) [85] have failed. Several ongoing studies are examining the use of IDO inhibitors in brain tumours. Clinical trials of

arginase inhibitors in solid tumours are also underway, but none is specific for brain tumours. Targeting the immunosuppressive cells within the tumour microenvironment represents an alternative strategy. Up to 30% of a GBM tumour is composed of tumour-associated macrophages (TAMs) [87]. Data from mouse models and human samples show that the vast majority of TAMs in GBM arise from circulating monocytes (85%), with a minor portion being of microglial (<15%) origin [100-102]. The anti-inflammatory M2 macrophage phenotype and the number of infiltrating TAMs positively correlate with GBM grade and negatively with tumour prognosis [103, 104]. Both TAMs and GSCs are enriched in perivascular regions and hypoxic niches in GBM [37, 105, 106], suggesting a close interaction between these cell types [107]. Moreover, GSCs facilitate a pro-tumour microenvironment by promoting the survival of TAMs [108]. Interestingly, both GSC and TAM populations are elevated in recurrent tumours after irradiation [56, 109]. The close association between TAMs and GSCs strongly suggests a reciprocal molecular crosstalk that is crucial for GBM malignant progression. Although macrophages are important in modulating the immune system, targeting TAMs alone is not sufficient to elicit an effective immune response. Additionally, many existing treatment modalities affect and are affected by the myeloid compartment, therefore, emphasising the need for combination with myeloid targeting to prevent myeloid-mediated therapy resistance.

5.2 Checkpoint inhibitors

Checkpoint inhibitors work by releasing a natural brake on the immune system allowing anticancer CD8+ T cells to recognise and eliminate tumours. Targeting PD1 (programmed death 1), its ligand PD1L1 (PD1 ligand 1) or cytotoxic T lymphocyte-associated antigen 4 (CTLA4) have demonstrated activity in a variety of solid tumours [110, 111]. Although numerous preclinical studies reported positive results [89, 112, 113], a phase III clinical trial (CheckMate-143) comparing anti-PD1 therapy nivolumab with bevacizumab (anti-vascular endothelial growth factor A (VEGFA)) in the treatment of recurrent GBM did not show a benefit over bevacizumab? [114]. CheckMate-489 a phase III study in newly diagnosed patients with O6-methylguanine-DNA-methytransferase (MGMT) promoter-unmethylated GBM showed similarly disappointing results (NCT02617589). In this study, nivolumab in combination with radiation was compared to standard-of-care. A third study (CheckMate-548) evaluating nivolumab in combination with TMZ in patients with MGMT-methylated GBM, is still pending (NCT02667587).

More promising are the results of an early phase clinical trial evaluating immune response and survival following neoadjuvant and/or adjuvant therapy with pembrolizumab in 35 patients with recurrent, surgically resectable GBM. Patients receiving neoadjuvant pembrolizumab, with continued adjuvant therapy showed a significant increase in overall survival compared to adjuvant PD-1 blockade alone. This result was associated with the T cell-mediated interferon response, which resulted in a downregulation of cell-cycle-related genes within the tumour cells. The study also showed that pre-surgical checkpoint inhibition resulted in a systemic expansion of tumour-specific T lymphocytes resulting in a greater initial T cell diversity, which in turn potentiated responsiveness to PD-1 blockade [13]. Although no obvious clinical benefit was observed in a single-arm phase II clinical trial using neoadjuvant nivolumab treatment, enhanced expression of chemokine transcripts, higher immune cell infiltration and increased clonal T cell receptor diversity among tumour-infiltrating T cells indicate a local

immunomodulating effect of treatment [92]. Neoadjuvant administration/treatment promises significant improvements in patient's outcome with minimal changes to treatment modality.

5.3 Vaccines

Cancer vaccine therapy aims to activate the patient's immune system to recognise tumour-associated antigens and destroy the tumour. Vaccines encompass a range of treatments, including systemic exposure to autologous or allogeneic antigens as well as the induction of a tumour-specific immune response by dendritic cell (DC) vaccination. The advantage of this approach is the potential for eliciting a widespread and durable response. GBM-specific antigens are rare, and some of these antigens are restricted by HLA types, limiting the patient population in which these vaccines may be considered. Peptide vaccines targeting a single tumour antigen, such as EGFRvIII, IDHR132H and Wilms tumour 1 (WT1) led to substantial increase in survival in an uncontrolled phase II trial, but no benefit was observed in a randomised phase III trial [115]. Single-peptide vaccinations harbour the potential for tumour immune escape. An EGFRvIII vaccine study revealed that the majority of patients with recurrence lost EGFRvIII expression [42]. Multi-peptide vaccines are considered to resolve the problem of antigen loss, but none of these clinical trials investigating multi-peptide vaccines have given a clear indication of efficacy [116, 117]. SL701 a vaccine consisting of short synthetic peptides targeting IL-13Rα2, EphA2, and Survivin, showed a median overall survival of 12 month in a phase II study in recurrent GBM. The study reported that 8/28 patients mounted a target-specific CD8 response, which was associated with longer survival [118]. Given that GBM-specific antigens are rare a number of tumour-associated antigens are being studied in GBM. Although being not specific to tumour cells, limited expression elsewhere makes these safe targets to exploit [119]. Immunisation with whole GBM tissue lysate was lethal when studied in animal models [120]; however, vaccines formulated from heat shock proteins (HSP) and DC vaccines, have been well-tolerated with promising early results. A detailed review of completed vaccine trials in GBM was recently published [119].

5.4 Oncolytic viral therapy

Oncolytic viruses (OVs) are viruses that are naturally cancer-selective or can be genetically modified to reduce pathogenicity, increase lytic potential, as well as induce innate and adaptive anti-tumour immune response. Initially designed as a mechanism of gene delivery to provide tumour cells with susceptibility to chemotherapy, is now recognised as a form of immunotherapy. Viruses are recognised by the immune system through pathogen-associated molecular patterns and pattern recognition receptors. Furthermore, viruses often activate macrophages through receptors, such as TLRs [121]. As a secondary effect, activated myeloid cells can improve the infiltration of T cells into tumours to promote an inflamed microenvironment. As a result, viral therapies are a very interesting approach to overcoming the immunosuppression of GBM. The excitement about this therapy is largely driven by the population of long-term survivors which was recently reviewed [122]. To-date, two therapeutic viruses have entered testing in phase III clinical trial, ASPECT and Toca5. Initial results on ASPECT, a replication-deficient adenovirus, showed prolonged time to death or to reintervention. However, no difference in median overall survival was observed [123]. Toca5 a non-lytic retrovirus expressing cytosine deaminase was compared to standard therapies in recurrent high-grade gliomas(NCT01470794). 5 patients out of 23 who received Toca 511 showed durable responses and as of August 2017,

all of those patients were still alive, one over 4 years [124]. Several other viral therapies that include replication competent HSV1 (G207), parvovirus (ParvOryx01), adenovirus (DNX-2401), and poliovirus (PVS-RIPO) [12], have reported durable responses in patients with GBM [122]. The future direction of oncolytic viral therapies seems to be focused on combinations with immunotherapy strategies, to potentially induce a durable anti-cancer immune response initiated by the viral infection and to elicit prolonged clinical responses.

5.5 CAR T-Cell therapy

Chimeric antigen receptor (CAR) T-cell therapy has achieved tremendous successes in treatment of haematological malignancies [125]. CAR T-cell therapy has the advantage of bypassing the need for MHC antigen presentation, the development of an adaptive immune response and hence, the need for co-stimulatory signals. Despite the success in haematological malignancies the response to CAR T-cell therapy in solid tumours including GBM have been disappointing. This has been attributed to the complexity of solid tumours. This therapy faces multiple obstacles in solid tumours such as the hostile tumour microenvironment, on-tumour/off-tumour toxicities, and undesired antigen specificity, reviewed in [126]. The tumour antigens that have been most investigated for CAR T therapy in GBM to-date are EGFRvIII, HER2 and IL-13Rα2. Encouragingly, none of the clinical trials in GBM using CAR T-cell therapy reported unmanageable CNS side effects, a concern that arose in CAR T-cell therapy in B cell lymphoma which led to elevated intracranial pressure and associated encephalopathy [127]. Various CAR T-cell constructs and routes of administration are currently under investigation. We summarise here the results of three recent reports. As mentioned/noted above, EGFRvIII has also been targeted

using CAR T-cell therapy. Infiltration of CAR T-cells showed elevated levels of intratumoural EGFRvIII CAR T-cell DNA and a decrease in EGFRvIII expression, suggesting effective infiltration of the tumour [128]. Although CAR T-cells efficiently infiltrated and eliminated EGFRvIII+ tumour cells, no partial or complete responses were observed [128]. CAR T-cells targeting HER2, a receptor tyrosine kinase with high expression in a proportion of GBM, has been explored [129]. A phase I clinical trial, enrolling 10 adult and 7 paediatric patients, with heavily pre-treated recurrent HER2+ GBM were treated with HER2-specific CAR T-cells [130]. The study demonstrated relative safety and persistence of HER2 virus-specific CAR T-cells in peripheral blood for up to one year. One patient had a partial response for more than 9 months and seven patients had stable disease for between 8 weeks to 29 months. The median overall survival was 11.1 months from the first T-cell infusion and 24.5 months after diagnosis [130]. A safety and efficacy trial of CAR T-cells targeting IL-13Rα2 in GBM was performed on a group of three patients. IL-13Rα2 modulates activation of the rapamycin pathway and is typically associated with a worse overall prognosis in GBM [131, 132]. Patients received an intracranial infusion post-resection, followed by an intertumoural infusion and an intraventricular infusion. Two out of three GBM patients showed a radiological response and a significant decrease in IL13Rα2 expression after therapy [133]. One patient with recurrent multifocal *IDH1* wild-type, *MGMT* non-methylated GBM showed a dramatic response that lasted for 7.5 months. However, disease ultimately recurred in this patient [134]. An important observation in this study was that the route of delivery appeared decisive with complete regression of multi-focal tumours following intraventricular administration. This CAR T target is continuing to be studied in combination with check point inhibitors (NCT04003649).

Category	Therapy	Target	Phase	Clinical trials
Immune checkpoint inhibition	Nivolumab	PD-1	III	NCT02017717
			III	NCT02617589
			III	NCT02667587‡
	Pembrolizumab	PD-1	II	NCT02337491
	Durvalumab	PD-L1	I	NTC02866747
	Ipilimumab	CTLA-4	I	NCT03233152
	Anti-LAG3	LAG3	I	NCT02658981
Vaccines	Rindopepimut	EGFRvIII	II	NCT01498328
			III	NCT03068650
	DC-Vax	tumour lysate antigen vaccine	II	NCT03014804
	DSP-7888 in combination with bevacizumab	Wilms tumour gene 1 (WT1) protein + anti-VEGFA	II	NCT03149003
	IDH1 peptide vaccine	IDH1	I	NCT02193347
	CMV pp65 DC	pp65	I	NCT03299309
	CMV pp65 DC in combination with bevacizumab nivolumab	pp65 + PD-1	I	NCT02529072
	TVI-Brain-1	cancer cell vaccine	II	NCT01290692
	HSPPC-96	tumor-derived heat shock protein peptide-complex	II	NCT00905060‡
	ICT-107	tumor associated antigens	III	NCT02546102‡
	SL701/poly-ICLC	IL-13Ra2, ephrin A2, survivin	I	NCT02078648
	PVS-RIPO	Poliovirus	I	NCT01491893‡
	ASPECT [123]	Adenovirus-mediated	III	2004-000464-28
	Toca511	Retroviral replicating vector	I	NCT01470794
CAR T-Cells	EGFR-806	EGFRvIII	I	NCT03638167
	HER2-specific CAR T-cells	HER2	I	NCT03500991
	IL-13Ra2-specific CAR T-cells	IL-13Ra2	I	NCT04003649 NCT02208362
	CMV-specific CAR T-cells	CMV specific antigens	I	ACTRN12609000338268

Table 1: Representative immune therapy clinical trials in recurrent glioblastoma (‡ studies on newly-diagnosed glioblastoma). Interventions are categorised based on their mode of action.

Multiple studies indicate the human cytomegalovirus (CMV) is a contributing factor to glioma progression [135]. This finding is further emphasised by the presence of CMV sequences in malignant T-cells [136, 137] and that vaccination with autologous GBM lysate elicited a CMV-specific immune response. A clinical phase I trial for adoptive immunotherapy using CMV-specific T-cells in patients with recurrent GBM proved the safety of adoptive immunotherapy and was coincident with disease stabilisation [138]. Studies of recurrent GBM have revealed resistance mechanisms at all phases of the immune response. Intrinsic resistance prevents the initiation of a response, adaptive resistance deactivates tumour-infiltrating immune cells and acquired resistance protects a tumour from specific targeting. Future trials utilising immune therapy will target multiple antigens in each patient in an attempt to address tumour antigen heterogeneity in the recurrent setting.

6. Future Directions

Multiple small-cohort clinical trials using specialist techniques have shown promising results in recurrent disease, often with responses only observed in a subset of enrolled patients. The challenge moving forward will be to select the best candidate approaches, define tests to determine which patients will respond and broaden patient access. Technical approaches that improve BBB therapy penetrance such as CED and MgFUS hold significant promise and revive previously failed systemic therapy-based clinical trials. In theory, all previously targeted agents and antibodies that have failed in the vein could be reassessed using CED or MgFUS. These approaches are not without limitation, requiring specialist CED surgical techniques or equipment and expertise in the case of MgFUS. A significant cost is also attached to these procedures and scalability is an

issue. Interestingly, the BTB appears to be less stringent in the recurrent setting compared to upfront disease. This is hopeful for therapeutic interventions at recurrence. Depatux-M (ABT-414), developed by Abbvie, showed significant promise during initial testing in a recurrent cohort but was subsequently ceased during phase III clinical trial in the upfront setting. Pharmaceutical companies could be encouraged through federal incentive schemes to continue therapies that show positive signals in recurrent GBM, as the need is so great and almost all patients invariably experience relapse.

The success of immunotherapy in GBM faces several obstacles including the highly immunosuppressive nature of GBM and the limitations of the immune response in the central nervous system. Learning from phase III clinical trial failures, the future of immunotherapy for GBM appears most hopeful for combination therapies driven by biomarkers for appropriate patient selection. Given the extreme need for improved survival in GBM, current clinical trials are evaluating checkpoint inhibition in combination with novel therapies including vaccines, CAR-T cell therapy, and viral therapy. The biggest breakthrough in recent times in recurrent GBM has been the use of neoadjuvant IO. This relies on the existing recurrent tumour acting as sink of both immune cells and tumour cells to raise an appropriate response prior to surgical resection. The benefits of neoadjuvant IO include accessibility, scalability, well-established toxicity profiles with minimal changes to standard practices. A number of clinical trials are either underway or in preparation and the effectiveness of these approaches will be further refined in the coming years. Deciphering the biology driving recurrent GBM might harbour the promise of targeted therapy at the stage of the primary disease. Hence, preventing recurrence by managing

the initial disease. Here we summarise exciting biological discoveries and recent progress in the treatment of patients with recurrent GBM. GBM remains a particularly challenging disease as little progress has been made towards improving patient outcomes and survival. A better understanding of the origins of this cancer and the molecular biology driving glioma genesis at recurrence is still needed to develop therapies addressing the main obstacles discussed in this review.

7. Conclusions

Now is time for hope for brain cancer sufferers. A significant build up in our knowledge of the disease has occurred in the past decade. This, in essence, has "primed the pump" and will lead future discoveries and clinical trial design into the future. Substantial financial support through federal programs such as the Cancer Moon-Shot in the United States and Australian Brain Cancer Mission (ABCM) have been leveraged to bring discoveries from bench to bedside. These programs have also strengthened awareness and community philanthropic support. Based on the advances discussed in this review the next decade should see significant breakthroughs for patients suffering from this aggressive disease.

Conflicts of Interest

Authors declare no conflict of interest.

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