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Development of molecularly targeted agents and immunotherapies in glioblastoma:

A personalized approach.

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Abstract

Over the past decade, precision cancer medicine has driven major advances in the management of advanced solids tumours with the identification and targeting of putative driver aberrations transforming the clinical outcomes across multiple cancer types. Despite pivotal advances in the characterization of genomic landscape of glioblastoma, targeted agents have shown minimal efficacy in clinical trials to date, and patient survival remains poor. Immunotherapy strategies similarly have had limited success. Multiple deficiencies still exist in our knowledge of this complex disease, and further research is urgently required to overcome these critical issues. This review traces the path undertaken by the different therapeutics assessed in glioblastoma and the impact of precision medicine in this disease. We highlight challenges for precision medicine in glioblastoma, focusing on the issues of tumour heterogeneity, pharmacokinetic-pharmacodynamic optimization and outline the modern hypothesis-testing strategies being undertaken to address these key challenges.

Background

Glioblastoma (GBM, WHO Grade IV glioma) is the most common primary malignant tumour of the CNS, accounting for 12-15% of all intracranial tumours and 50-60% of gliomas [1]. It is an aggressive and incurable disease with an annual age-adjusted incidence rate of 3.2 per 100,000 individuals in the United States [2,3] and a median survival of only 12 to 15 months, even with optimal treatment [4,5]. Current standard of care involves maximal safe surgical resection, followed by adjuvant chemotherapy with temozolomide combined with radiotherapy [6,7]. Due to its infiltrative and invasive nature, the disease invariably recurs, and progression typically occurs after six to nine months [5]. On relapse, treatments options are limited, with minimal clinical efficacy [7], and only approximately 3-5% of patients survive longer than 3 years [8].

Despite recent significant progress in our understanding of the molecular pathology of gliomagenesis and the epigenetics of GBM[9], as yet this has not translated successfully to improved clinical outcomes. There is extensive inter-patient cellular and genetic heterogeneity in GBM, but also significant intra-tumoral heterogeneity, which may contribute to therapeutic failure[10–13]. Analysis of data from The Cancer Genome Atlas (TCGA), offering insights into genetic regulation of GBM, has led to the stratification of GBM into major molecular subgroups with recognised signaling pathways and differing prognostic significance [14,15]. These subgroups - proneural, classical, and mesenchymal - were identified using transcriptional tumour profiling,

and are based on dominant genes expressed in each group. The classical subgroup is marked by amplifications or mutations in the epidermal growth factor receptor (EGFR) in over 95% of cases, with high rates of concordant amplification in chromosome 7 and deletions of chromosome 10 (93%), and a complete absence of *TP53* mutations [14,15]. The proneural subset by contrast is commonly associated with *TP53* mutations (54%) and isocitrate dehydrogenase-1 (*IDH*-1) mutations, whilst the mesenchymal subtypes have a high rate of aberrations in *NF1* signalling. Overall, the TCGA data demonstrated that most GBMs tumours were found to harbour alterations in common oncogenic pathways receptor tyrosine kinase (RTK) signalling through mutations/amplifications in receptors such as EGFR and PDGFRA, mutations in downstream partners of AKT pathway such as PI3K and PTEN and apoptosis signalling through mutations in p53, and cell cycle control signalling through alterations in CDKs [14,15]. Indeed, 57% of GBM showed evidence of mutation, rearrangement, altered splicing and/or focal amplification of EGFR [14,15].

However, despite evidence of biologically distinct transcriptional profiles, the clinical relevance of these subgroups is questionable. Apart from the observation that most secondary GBMs represent the proneural subtype, the clinical outcomes of each subgroup are similar, with a slight observed survival advantage with chemo-radiotherapy in the proneural subgroup. The reality is that the impact on treatment and prognoses of these GBM subgroups, is limited by genetic landscape of these tumours continually evolving at a remarkably rapid pace [16–18], and generating an incredible degree of cellular complexity and heterogeneity within a single tumour [19–21]. GBM tumours are complex; they are not usually defined by a single genetic or

molecular alteration. Consequently, isolating signalling pathways responsible for GBM oncogenesis has been difficult, and therapeutic outcomes from single-agent targeted therapies have been modest.

Of course, further glioma classification systems exist, and as of the 2016 edition of the WHO classification, gliomas are classified based not only on histopathologic appearance, but also on well-established molecular parameters [22]. The incorporation of molecular features has most notably impacted the classification of astrocytic and oligodendroglial tumors, which are now grouped together as diffuse gliomas, on the basis of growth pattern, behavior, and shared *IDH-1* status. Mutations in *IDH1* and, less commonly, *IDH2*, are a defining feature of the majority of WHO grade II and III diffuse astrocytic and oligodendroglial tumors and confer significantly improved prognosis compared with IDH-wildtype tumors[23–25]. Meanwhile, IDH-wildtype GBM, WHO grade IV, are densely cellular, pleomorphic tumors with either microvascular proliferation or necrosis, or both, and include a number of histologic variants, including giant cell GBM, gliosarcoma, and epithelioid GBM [22]. IDH-mutant GBMs conversely comprise approximately 10 percent of all GBMs, and while they are histologically similar to IDH-wildtype GBM, they are more likely to contain cells with oligodendroglial morphology [22], occur in younger adults (mean age 45 years) and have a more favorable prognosis[26,27]. This recent progress in the classification of the different types of glioma is indeed encouraging, and while these advances are crucial to ensure that gliomas are diagnosed and treated accurately, the hope is that these advances in classification will eventually translate into improved outcomes for patients.

The recent remarkable success of immunotherapy agents in other cancer subtypes, together with the considerable medical need in the absence of approved targeted therapies in GBM, has led to the questioning of the previously-held belief that the central nervous system (CNS) is immune-privileged, and thus inaccessible to antitumor immunity. Encouraging pre-clinical data in experimental models has led to therapies targeting immune checkpoints reaching the clinic, and an invigorated interest in the immunotherapy of GBM. Here, we describe the current state of play in the development of molecularly targeted agents and immunotherapies in GBM. We summarise the data on current clinical trials for these systemic treatments in GBM and and address the successes, pitfalls, and opportunities of precision medicine in this disease.

Angiogenesis inhibition

The path to the era of personalized medicine in GBM was first paved by the recognition of MGMT hypermethylation as a valid prognostic and predictive marker in patients undergoing treatment with temozolomide [9]. Subsequent progress in this era of molecularly targeted strategies has been characterised by promising discoveries, with a failure to translate to clinically meaningful improved outcomes for patients.

One of the initial molecularly targeted strategies for GBM was with angiogenesis inhibitors, in light of the fact high-grade gliomas (HGGs) are highly vascularized

tumour [28,29]. In particular, the vascular endothelial growth factor receptor (VEGF) family of receptors have been identified as the main molecular driver of angiogenesis, although other targets including adhesion molecules such as integrins, have also been identified [30]. Preclinical studies had shown that GBMs express high levels of vascular endothelial growth factor (VEGF)[31], with the degree of overexpression correlating with tumour aggressiveness[32]. Several mechanisms for the potential activity of antiangiogenic therapies in GBM have been posited including normalisation of tumour vasculature [33] and improving tumour oxygenation[34], thereby increasing the efficacy of chemotherapy and radiotherapy.

The initial suggestion that VEGF inhibitors may be of benefit in GBM, came in 2005, when a response rate of 43% was observed in a single-arm study with bevacizumab combined with irinotecan[35]. Subsequent studies suggested that most, if not, all of the benefit of this combination could be attributed to bevacizumab [36]. Multiple single-arm studies subsequently confirmed unprecedented response rates in the recurrent GBM setting[28]. These unprecedented response rates, prompted accelerated FDA approval for the use of bevacizumab in the recurrent setting, the commencement of two large clinical trials in the first-line setting as well as the development of a host of other anti-angiogenic agents[37–40]. Unfortunately, the initial promise of high response-rates did not lead to a clear survival benefit, with a large meta-analysis demonstrating consistently improved progression-free survival (PFS) without a correlating overall survival (OS) benefit[29]. These results have not only called into question the validity of PFS as an appropriate endpoint in GBM trials, but have also illuminated the difficulties in neuro-imaging assessment, in particular with

the use of anti-angiogenic agents which may reduce contrast-enhancement resulting in a pseudo-response[41]. More recently, randomised data has even called into question the utility of bevacizumab in the recurrent setting, with no evidence of a survival benefit compare to chemotherapy[42]. Additionally, although bevacizumab is widely noted to have a steroid sparing effect[28], two large randomised controlled trials demonstrated discrepancies with regards to the quality-of-life benefit of bevacizumab in the adjuvant setting[37,38]. The lack of efficacy of bevacizumab has been mirrored in the results of other antiangiogenic therapies in GBM, with negative trials with cilengitide, an integrin inhibitor[43,44] and cediranib, a small molecule pan-VEGF inhibitor[39].

Nevertheless, despite the purported lack of survival benefit, recent efforts have focused upon identifying a population of likely to derive a benefit from anti-angiogenic therapy. Sandmann et al, demonstrated a survival benefit of bevacizumab in patients with proneural, IDH-1 wild-type, GBM[45]. Other markers potentially correlating with bevacizumab response include a microRNA profile[46], as well as imaging biomarkers such as cerebral blood volume[47]. Although these biomarkers are promising, they are in need of clinical validation prior to more widespread adoption.

The Epidermal Growth Factor Receptor

More recent efforts have focused on targeting genetic alterations in GBM. The underlying genetic landscape of GBM is complex, however, there are a number of

recurring alterations in the PI3K/MAPK, p53 and Rb pathways[48]. More recently, TERT promoter alterations have also been identified as comprising a significant subset of genomic alterations in GBM[24]. Of these pathways, alterations (mutations, and/or amplifications) in EGFR are found in more than 50% of GBM[48], and therefore represent a particularly attractive therapeutic target, particularly in light of the clinically validated benefit of inhibition of the epidermal growth factor receptor (EGFR) mediated pathways in other tumour types[49]. In particular, 50-60% of tumours found to have EGFR amplification in GBM also contain the mutant *EGFR gene*, EGFRvIII, which is a truncating mutation characterised by the deletion of exons 2 to 7[50]. This causes an in-frame deletion of 267 amino acids in the extracellular domain, which results in functional changes leading to ligand independent constitutive tyrosine kinase activity[51].

Preclinical data supporting EGFR kinase inhibition as a viable therapeutic option, particularly in tumours co-expressing EGFRvIII and PTEN [52], rapidly led to the commencement of multiple clinical trials of erlotinib in GBM. Despite promising results in non-randomised studies[53], a large negative randomised phase II trial in the recurrent setting found a lack of discernible clinical activity[54]. A study evaluating gefitinib, a first generation EGFR tyrosine kinase inhibitor (TKI) after at least five days of continuous oral daily dosing prior to planned surgery, shed more light on the difficulties targeting this pathway in GBM[55]. This study demonstrated that gefitinib penetrated the blood-brain barrier and reached concentration in tumour tissue similar to that achieved in non-small cell lung cancer, caused decreased phosphorylation of the EGFR, but did not significantly reduce downstream signal

transductors, a finding which was replicated in a xenograft model but not in a cell line model[55].

In part, lack of sensitivity to kinase inhibition, may be due to the fact that the most common mutant found in GBM, EGFRvIII mutation, is found in the extracellular domain of the EGFR[51,56]. Indeed, one key difference between EGFR in GBM and lung cancer is the distribution of mutations within the EGFR coding sequence; EGFR mutations in lung cancer are located in the intracellular kinase domain, whereas EGFR mutations in GBM cluster in the extracellular domain, and include in-frame deletions (such as the common EGFRvIII mutation (and missense mutations) [57]. It has been proposed instead, that these GBM mutants are preferentially inhibited by EGFR inhibitors that can only be accommodated by the inactive conformation of the EGFR catalytic pocket due to their bulky aniline substituents (lapatinib)[58,59]. Given the lack of single agent activity observed with EGFR TKIs, multiple early phase combination trials were performed with chemotherapy, mTOR inhibitors and anti-angiogenic were also performed which failed to show any significant clinical activity[60].

Nevertheless, given the frequent amplification of EGFR in GBM, novel therapeutic strategies targeting this pathway have recently been developed. The two most clinically advanced strategies have been the development of a therapeutic conjugate peptide vaccine, rindopepimut[61], targeting EGFRviii and the antibody-drug conjugate ABT-414[47]. Rindopepimut, is a peptide vaccine targeting the neo-epitope created by a 13 amino acid sequence unique to EGFRvIII, chemically conjugated to the carrier protein KLH to induce an immune response[62]. Promising initial results[63], culminated

in the ACT III clinical trial, a single-arm study in newly diagnosed GBM, which resulted in an unprecedented median overall survival of 21.8 months, suggesting clinical activity[64]. These results prompted the FDA to grant breakthrough status to rindopepimut. Unfortunately, the randomized phase III study, ACT IV, failed to confirm the survival benefit of this compound; median OS with rindopepimut was 20.4 months compared with 21.1 months in the control arm[65] (HR=1.01; p=0.93), with no substantial differences in progression-free survival (PFS).

Cetuximab and Nimotuzumab, both unconjugated antibodies that bind the extracellular domain of EGFR and suggested to cause internalization of EGFRvIII, have little benefits in patients regardless of their EGFR gene amplification status [66,67]. The antibody-drug conjugate ABT-414 consists of a unique antibody targeting active EGFR or mutant EGFRvIII linked to a potent anti-microtubule agent and has shown promising results in initial phase I studies[68]. Multiple phase II and III trials are currently ongoing evaluating this therapy, but it remains to be seen as to whether the elusive goal of a clinically effective therapy targeting EGFR in GBM can be achieved.

Novel approaches

In addition to EGFR amplification, other genetic events are commonly found in GBMs. Of note, TCGA data has shown a high prevalence of mutations affecting *PTEN* in GBM[14]. Preclinical data have shown a strong association between mutations in *PTEN* and reduced homologous recombination function[69], giving a strong preclinical rationale for synthetic lethality with poly-ADP ribose polymerase (PARP)

inhibitors[70,71] . This combined with possible synergy between PARP inhibition and two of the core components of standard GBM management, temozolomide and radiation[72,73], has led to the commencement of clinical trials of PARP inhibitors in GBM which are currently recruiting.

Isocitrate dehydrogenases 1 and 2 (*IDH1, IDH2)* are frequently mutated in low-grade glioma (LGG) and are found in 12% of GBM; they comprise a large proportion of secondary GBM and are rarely found concomitantly with *EGFR* mutations[27]. In glioma pathogenesis, the IDH genes are strongly correlated with the CpG island methylator phenotype, which is markedly associated with improved survival clinically[74]. Moreover, although *IDH1* is strongly implicated in glioma pathogenesis, it has been unclear what role it plays in progression. A recent study demonstrated that IDH1/2 mutations induce a homologous recombination (HR) defect rendering tumor cells exquisitely sensitive to PARP inhibitors [75]; this IDH1-dependent PARP inhibitor sensitivity was demonstrated in a range of clinically relevant models, including primary patient-derived glioma cells in culture and genetically matched tumor xenografts in vivo, providing the basis for a possible therapeutic strategy exploiting the biological consequences of mutant IDH, rather than attempting to block 2HG production, by targeting the 2HG-dependent HR-deficiency with PARP inhibition[75]. Another recent study demonstrated in paired initial LGG tumour samples and post-progression samples that *IDH1* mutation is preserved, suggesting that it plays a role not only in tumour initiation, but also in tumour maintenance[76]. These preclinical data have led to the clinical development of *IDH1* inhibitors which

are currently in the process of undergoing phase I clinical trials and have already shown promising activity[77].

Viral strategies

Oncolytic viruses (OVs) are an emerging class of experimental treatments for malignant glioma, currently under investigation in the clinic, following the recent successes of talimogene laherparepvec (T-vec) in malignant melanoma [78]. Progress, in GBM has, however, been more muted. OVs are live viruses that are selectively toxic to cancer cells; as well as their direct oncolytic properties, OVs are also considered a form of immunotherapy, as they can induce effective anti-viral and anti-tumour immune responses, although many of these immune-mediated mechanisms are being recognised[79]. Several OVs have been investigated for glioma in the preclinical setting, including poliovirus, HSV, adenovirus, reovirus, parvovirus, Newcastle disease virus, measles virus (MV), and retrovirus[80]. While clinical trials involving OVs in GBM as single agents have largely been safe, demonstrated acceptable toxicity, and in certain studies, shown signs of efficacy by radiological evaluation and the presence of live virus in tumor biopsies a week or more after treatment [81–83], the overall efficacy of single-agent OV therapy has at best been modest at best.

Combination strategies involving checkpoint inhibitors are currently being explored; CAPTIVE (NCT02798406), which explores the Combination of Adenovirus and Pembrolizumab to Trigger Immune Virus Effects is one such study. Other oncolytic viruses currently in the process of undergoing clinical trials include the oncolytic

polio virus, which utilizes the aberrant expression of the poliovirus receptor, CD155, in solid tumours to mediate viral cell entry[84].

Immunotherapy

Immunotherapy is a new paradigm in cancer care, and recent advances in the field of immune checkpoint blockade have led to dramatic results, most notably with the inhibition of the programmed cell death—1 (PD-1) and programmed cell death ligand -1 (PD-L1) interaction. Immunotherapy of HGGs has been hindered by poor definition of relevant antigens and selective measures to target the central nervous system (CNS), but this has evolved in recent years. Driven by the high medical need in the absence of approved targeted therapies, we now have novel neuro-oncology-specific concepts, providing new approaches, with individualized immunotherapy trials.

CNS Immunology

A major determinant of cancer pathogenesis is the interaction of tumour cells with the immune system. The central nervous system (CNS), in large part due to the protective nature of the blood-brain-barrier (BBB), was traditionally believed to be an immune-privileged site. However, the discovery that lymphatic vessels exist in the CNS[85–87] and that immune cells can cross the BBB [88] radically changed this assumption. Recent data indicate that leukocytes can traffic to the CNS, even in the presence of an intact BBB[89,90], and the flow of CSF connects the CNS to lymphatics by draining into cervical and nasal lymph nodes, providing another route for antigen

and immune cell circulation[91,92]. Taken together, these findings suggest that the immune system can combat gliomas, in addition to other tumour types (Figure 1).

An immune response to cancer occurs through a series of precise and stepwise actions beginning with tumor antigen presentation by antigen-presenting cells (APCs) and progressing through to priming and activation of T cells, trafficking of cytotoxic T cells (CD8+ cells) to tumors, and ultimately the killing of tumor cells[93]. This interaction is regulated by immune checkpoints, which can be inhibitory or stimulatory. PD-1 and its ligand PD-L1, represent an inhibitory immune checkpoint at the tissue level, wherein PD-L1 expressed on tumour tissue binds PD-1 on cytotoxic T cells and leads to T-cell anergy[94,95]. Targeting this checkpoint has proven successful in other tumour types[96–102] and its activity in GBM is currently being explored.

In HGGs however, it is not known whether glioma antigen cross-presentation occurs peripherally or within the CNS; is also debateable which cell types are most responsible for glioma-antigen presentation. Preclinical models have shown that microglia are capable of cross-presenting tumor antigens to CD8-positive T cells; microglia however, even when activated express less MHC and co-stimulatory markers than similarly activated dendritic cells (DCs)[103]. Tumour-infiltrating DCs, macrophages, and pericytes are also candidates for antigen presentation within the tumour bed[104,105]. Tumour antigens could also potentially drain outside the CNS to the peripheral lymphatics for antigen presentation.

Higher grade gliomas, typically associated with BBB disruption and tumour necrosis, result in antigen expulsion, and have increased numbers of immune cells throughout the tumour bed[106]. Although higher numbers of tumour-infiltrating leukocytes may

theoretically suggest a more robust immune reaction within the microenvironment of high-grade versus low-grade gliomas, this does not necessarily correlate with better clinical outcomes[107]. It is possible that, despite increased leukocyte access to HGGs, other tumour-related factors may diminish the immune response.

Generalized immunosuppression has long been an established feature in patients with GBM, and it has been well documented that gliomas have various mechanisms to suppress the immune system. Numerous mechanisms lead to a suppressed immune response in patients with GBM[108]. Individuals with GBM have reduced response to pro-inflammatory signals and impaired T cells with reduced proliferative potential[108,109]. Glioma cells can also down-regulate their own MHC I complexes making them invisible to immune cells[110], and in the presence of glioma, proinflammatory cytokines, such as interleukin 12 (IL-12), IL-18, and interferon c (IFN-c), are notably reduced while soluble inhibitory molecules are abundant (including IL-10, VEGF, and transforming growth factor)[103]. A subclass of dendritic cells, plasmacytoid DCs, secrete large amounts of IFN-α in the periphery which provokes effector T-cell maturation; a recent murine study, however, demonstrated that plasmacytoid DCs within the glioma, lacked IFN-a secretion and were associated with immune tolerance[111]. Regulatory T cells (Tregs), which are thought to downregulate the immune response, have also been identified throughout gliomas, and there are data which indicate that a higher tumor-infiltrating CD8-positive T-cell/Treg ratio is clinically favorable[112]. Furthermore, glioma cells express surface proteins that bind to leukocyte receptors – this leads to secondary signaling pathways, further dampening lymphocyte activation, such as PD-L1, which, as

reported previously, leads to an increase in the Treg/ effector T-cell ratio[113].

Immunotherapeutic strategies can be broadly divided into four major classes; checkpoint inhibitors, adoptive strategies such as using chimeric antigen receptor (CAR) T cells, active immunotherapy such as with cancer vaccines and immune stimulatory gene therapy and passive immunotherapies utilising antibodies.

Checkpoint Inhibitors

Tumours can manipulate the central function of the immune system to maintain self-tolerance, to prevent autoimmunity and thus escape immune-driven destruction. The two most intensely investigated co-inhibitory checkpoints in this new era of cancer immunotherapy are Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)/B7 and PD-1/PD-L1. CTLA-4, expressed on APCs, interacts with B7, on T cells, resulting in inhibition of clonal expansion of naïve T cells[113]. Conversely, PD-1 on activated T-cells interacts with PD-L1 expressed in target tissue to result in T-cell anergy[112]. PD-1 has an additional ligand, PD-L2 which has limited expression. This receptor-ligand interaction, via down-stream signalling advances apoptosis of antigen-specific T-cells, and decreases apoptosis of Tregs[113]. As such, the ligands for these immunosuppressive checkpoints, often overexpressed in the GBM microenvironment to inhibit T-cell response against tumor cells, have become the targets for therapies, and pre-clinical efforts aimed at inhibiting the PD-1/PD-L1 pathway have shown promising results[113]. A pre-clinical glioma study using the GL261 mouse model, for example, demonstrated the combination of anti-PD-1

antibodies and radiotherapy doubled median overall survival and resulted in long-term survival in 15–40% of mice compared with either treatment alone[114].

Whether this success can be replicated in the clinic, is currently being addressed by a large number of ongoing clinical trials – indeed there has been a veritable explosion in the number of clinical trials for both newly diagnosed and recurrent HGG (Table 1). Reardon et al previously presented safety and efficacy data from the Checkmate-143, a study of nivolumab alone versus nivolumab plus ipilimumab for recurrent GBM[115]. This demonstrated that nivolumab was well tolerated with tolerability profiles consistent with observations in other tumor types, and OS was reported as an encouraging 40% at 12-months. However, 90% of patients who received combination therapy had grade 3 or 4 treatment-related adverse events (TRAE), and 50% of patients in that arm had to discontinue treatment early due intolerability[115]. Disappointingly however, CheckMate-143 did not meet its primary endpoint of improved overall survival (OS), as presented by Reardon et al at World Federation of Neurooncology Societies (WFNOS) 2017 [116]. The reported median OS was 9.8 months with nivolumab [95% CI 8.2, 11.8] and 10.0 months with bevacizumab[116]; 12-month OS rate was 42% in both arms and PFS medians were 1.5 months with nivolumab and 3.5 months bevacizumab[116]. Furthermore, documented response rates were lower with nivolumab than bevacizumab, in spite of the more durable responses noted with nivolumab[116].

Reardon et al previously presented encouraging data on the single agent activity of checkpoint-inhibitor pembrolizumab at the Annual Society of Neuro-oncology (SNO)

Meeting 2016 [117]. KEYNOTE-028 (NCT02054806) evaluated the safety and efficacy of the anti–PD-1 monoclonal antibody pembrolizumab in 20 advanced solid tumor types. In the GBM cohort, pembrolizumab demonstrated a manageable safety profile with grade 3–4 TRAEs observed in 15.4% of patients (lymphopenia, type 2 diabetes mellitus, arthritis, and syncope). Promising anti-tumour activity was noted; while only 1 partial response (PR) was observed, 12 patients (46%) experienced stable disease (SD) at a median duration of 39.4 weeks (95% CI: 7.1–85.9), median PFS 2.8 months (95% CI: 1.9–9.1), and median OS 14.4 months (95% CI: 10.3–not reached). Furthermore, durable response was suggested in 4 patients who continued therapy >54 weeks following enrolment.

Further encouraging preliminary safety and efficacy data from the ongoing phase II study of the anti–PD-L1 antibody MEDI4736 (durvalumab) (NCT02336165) were presented for the recurrent bevacizumab-naïve GBM patients[118]. In these 31 patients treated with durvalumab monotherapy, no grade 4/5 serious TRAEs were observed; grade 3 TRAEs were reported in 9.7%[118]. Response rate was 13%, median PFS was 13.9 weeks (95% CI: 8.1–24.0), and 6-month PFS was 20% (90% CI: 9.7–33.0) with 5 of these 6 patients remaining progression free at 1 year[118]. It is the durability of response in this cohort which is most exciting; all six patients who were progression-free at 6 months remain progression-free for over a year, suggesting that perhaps with this PD-L1–targeting immunotherapeutic for recurrent GBM, there is a tail of the curve which has been witnessed in other cancers – a subset of patients who are having a remarkably durable benefit. The study is also investigating immuno-correlative biomarkers with the aim of better identifying those responders.

The majority of glioma checkpoint-inhibitor trials are in early phases, but two further phase III studies are assessing nivolumab in GBM: CheckMate-498 and CheckMate-548, evaluating the combination of nivolumab with radiation therapy with or without temozolomide in O6-methylguanine-DNA methyltransferase (MGMT)-unmethylated and methylated patients. Active checkpoint-inhibitor trial information obtained from clinicaltrials.gov are summarized in Table 1.

The lack of survival benefit demonstrated in the CheckMate-143 trial is, of course, discouraging[116]. A proposed hypothesis as to why gliomas display a reduced sensitivity to checkpoint inhibition alone is thought to be due to a relatively low mutational load. Checkpoint inhibition releases mutation-specific T cell responses[119], and gliomas typically contain 40–80 non-synonymous single-nucleotide variations (nsSNVs), which is comparatively lower than in melanoma or small-cell lung cancer, both of which tend to respond well to single-agent checkpoint inhibition[120]. Supporting this hypothesis are the exceptional case reports of significant clinical responses to nivolumab seen in two siblings with biallelic MisMatch Repair Deficient (bMMRD) recurrent multifocal GBM, both of which exhibited very high mutational loads[121].

PD-L1 is expressed not only in the tumor microenvironment of gliomas[112,122,123], but also elevated in circulating antigen presenting cells (APCs) in glioma patients[124]. This of course may indicate biological activity, even if the therapeutic antibody does not reach sufficient intra-tumoral levels. As such, anti-PD-L1 anti-bodies such as atezolizumab represent an appealing strategy, where intratumoral or even peripheral PD-L1 expression may serve as a biomarker[125,126].

Chimeric Antigen Receptors

Chimeric antigen receptors (CARs) are a novel type of adoptive T-cell transfer currently garnering interest in immuno-oncology. CARs involve the extraction of T-cells from a patient and subsequently transducing the cells, using a lentiviral vector, to express a modified T-cell receptor with specific affinity to a tumour surface antigen[127]. A weakness of adoptive T-cell transfer is that effective tumour antigen-induced T-cell activation can be hindered by weak affinity of the T-cell receptor to the peptide/MHC complex; subsequent tumour cells have a tendency to down-regulate their MHC expression[127]. CAR-T cells are activated independent of MHC, and as such, avoid the difficulty of MHC restriction. One concern is the damage that can occur to normal tissues if the antigen expression is not tumor specific; thus it is essential to select targets that show tumour-restricted expression.

Clinically, adoptive T-cell therapy has demonstrated its effectiveness with CAR-based treatment for B-cell malignancies[128], and dramatic results have been achieved in early clinical studies in relapsed acute lymphoblastic leukaemia (ALL), with one phase I dose-escalation trial examining CD19 CAR T cells for refractory ALL demonstrating a remarkable 70% complete response (CR)[129]. The effects of CAR-T cells have been further investigated in renal cell carcinoma and neuroblastoma[130–133]. In brain tumours, using CARs as a therapeutic strategy was first tested by the Jensen group, who showed that intratumoral delivery of IL-13 zetakine CAR eliminated orthotopic human glioma tumors in immune compromised mice[134]. The clinical trial assessing the safety and feasibility of this therapy in patients with recurrent GBM involved autologous cytotoxic T cells with CARs that bind to IL13Ra2 (a protein overexpressed

in more than one-half of GBMs) being directly inserted into the resected tumor cavity. This therapy resulted in minimal side effects, and two of the three patients who received repeated intracranial infusions experienced transient anti-glioma immune responses[135]. Indeed, Brown et al recently updated the results of one of these patients and reported their remarkable findings in the New England Journal of Medicine[136]. In one patient who received weekly intra-cavitary infusions of cytotoxic T cells with CARs that bind to IL13Ra2, regression of all intracranial and spinal tumors was observed, along with corresponding increases in levels of cytokines and immune cells in the CSF[136]. This response was sustained for 7.5 months; however, recurrence did eventually occur and preliminary results suggest that tumours downregulated IL-13α2 expression at progression[136].

HER2-specific CAR T cells have also been investigated, and in xenograft mouse GBM model this led to tumour regression and a HER2-dependent anti-tumour response with increased production of IFN- γ and IL-2[137]. A phase I trial is currently ongoing and will test the safety and efficacy of using HER2-specific CARs in patients with recurrent GBM (NCT02442297). The Rosenberg group at NCI (NCT01454596) and the University of Pennsylvania/Novartis (NCT02209376) are also testing the safety and feasibility of administering T cells expressing anti-EGFRvIII CAR to patients with gliomas expressing EGFRvIII.

The most common and severe side effect of CAR T-cell therapy is cytokine release syndrome (CRS), a life-threatening complication involving the release of cytokines from leukocytes; this manifests clinically as fever, headache, nausea, dyspnea, tachycardia, hypotension, and rash[138]. The acute inflammatory reaction can cause

vascular permeability and multi-organ failure; it has been observed in almost two-thirds of patients receiving CAR T-cells, typically days after the infusion. As such, while there is excitement in this developing field, the risk involved in CAR T-cell therapy is not insignificant, and as always, recognition of adverse events is paramount, given that CRS can be rapidly reversed with corticosteroids and/ or anticytokine agents.

Cancer Vaccines – Active immunotherapy

With the aim of stimulating adaptive immune responses that target tumor-specific antigens, cancer vaccine strategies currently used include delivery of tumor-associated antigens, administration of tumor antigen loaded DCs and tumor cell vaccines.

DC Vaccination

DC-based vaccine therapy involves the extraction of DCs from the patient, harvested in culture while being exposed to tumour lysate or particular tumor antigens, and then returned to the patient to promote a T-cell–mediated reaction. Currently, there are two anticipated ongoing phase III DC vaccine trials for newly diagnosed GBM, the most advanced using an autologous DC vaccine- DCVax-L (NCT00045968). This vaccine was investigated in two phase I/II studies[139]; 20 patients with newly diagnosed GBM and 19 with recurrent tumors received biweekly vaccines followed by monthly booster injections. The long-term survival analysis was encouraging: 33% of patients achieved a median survival of at least 48 months, and 27% achieved a median survival of at least 72 months[139].

ICT-107, targets 6 GBM markers, and is the current DC vaccine ongoing phase III investigation(NCT02546102). Targeting absent in melanoma 2 (AIM-2), melanoma-associated antigen 1 (MAGE-1), tyrosine-related protein 2 (TRP-2), glycoprotein 100 (gp100), HER-2, and interleukin 13 receptor a2 (IL- 13Ra2), and previous phase II data[140] of ICT-107 for newly diagnosed GBM also was promising. ICT-107 was well tolerated, and it was associated with a two-month increase in PFS and a trend toward improved OS[140].

Challenges

The power of molecular targeted therapy, and how to practically implement precision medicine in GBM, has been limited by diverse factors, ranging from the complex molecular biology underlying gliomagenesis to challenges such as CNS penetration of agents, target selection and evaluation of treatment response.

Firstly, although many agents have therapeutic potential for GBM, few of these agents have been clinically used because of concerns of its ability to penetrate the blood-brain barrier and patients with brain tumours have also been historically excluded from the majority of early experimental trials of novel agents. This thinking is now largely archaic, on a number of fronts. We, and others, have shown that patients with primary malignant brain tumours who meet standard strict phase I eligibility criteria and are enrolled onto trials of appropriately chosen compounds, successfully meet phase I end points, such as safety and toxicity[141]. Furthermore, surgical and radiological studies have shown that the blood-brain barrier is disrupted

in all GBM patients[142]. This has important implications clinically, as drugs that do not show pre-clinical brain penetration, may in fact have utility in patients with GBM. For example, the PARP inhibitor, olaparib, penetrates both core and margins of recurrent GBM despite failing to penetrate the intact blood brain barrier[143], and is now in Phase II combination studies with temozolomide and radiation[144]. Additionally as we understand the CNS cancer immunity cycle, antigen presentation and the generation of an active immune response is likely to take place peripherally within lymphatic system and as such drugs targeting various facets of the anti-cancer immune response may not need to penetrate the brain at all.

Secondly, as discussed in considerable detail earlier, genomic heterogeneity represents a major challenge for precision medicine in GBM. Molecular studies to date use small samples, typically one slide from initial surgical resection samples or diagnostic biopsies and are insufficient to comprehensively integrate temporal or spatial tumour evolution data. The key question arising is whether critical molecular drivers are being missed given a randomly selected single slide is used for molecular stratification at diagnosis. Treatment-mediated selective pressure is likely to subsequently facilitate the selection of the resistant clone or clones, but given the inherent risks of repeat neurosurgical procedures, patients with GBM almost never have further tissue sampling.

Circulating biomarkers such as circulating-free DNA (cfDNA) and circulating tumour cells (CTC) are promising sources for obtaining tumour genomic material through a minimally invasive form of a liquid biopsy that can be repeated over time to account

for tumour evolution, and are now in use in translational clinical studies for multiple solid tumours, for example in breast and prostate cancer[145,146]. Circulating tumour cells from GBM tumours do cross the blood-brain-barrier, and can be detected peripherally; work is currently ongoing to refine various platforms for their detection[147]. ctDNA has been reported to be more abundant than circulating tumour cells, and can certainly be detected in patients with GBM where targeted next-generation sequencing for IDH1 for example has been performed[148]. This poses the exciting possibility of remote monitoring of the evolution of brain tumours in response and resistance to treatment for patient care. These molecular profiles can be further complemented with the molecular analysis of nucleic acids, lipids and proteins contained within extracellular vesicles, such as exosomes which may contain a higher amount of clinically relevant key signalling components[149] (Figure 1), and thus be used as a tumour biomarker for tracking cancer progression, and as a potential therapeutic target/delivery system. Given that, intriguingly, exosomes may play a role in a range of biological processes within the progression of GBM [150,151]it is no surprise that targeting exosome-mediated cellular interactions is becoming an area of interest for therapeutics. Indeed, dendritic cell-derived exosomes appear to express both MHC class I and II, and given the role of exosomes in modulating immune response, the appliance of immunotherapy utilizing exosomes for the treatment of gliomas, while still in its infancy [152], is a thought-provoking concept.

Prioritizing the numerous available therapies, and biomarkers that may be detected, requires creative efficient clinical testing platforms. INSIGhT [INdividualized

Screening Trial of Innovative GBM Therapy] (NCT02977780) is the first GBM umbrella trial where patients are assessed for multiple pre-specified genetic aberrations using NGS or other platforms, and then randomised to either standard therapies or matched to biomarker-based targeted treatment arms agents that is currently ongoing[153].

The greater challenge moving forward is how to integrate the potentially complementary fields of both targeted therapies and immunotherapies, to improve precision cancer treatments for patients with GBM. Emerging biology is unraveling the myriad of ways in which tumour oncogenic drivers can modulate the tumour microenvironment, and how targeted therapies can therefore impact the host immune response[147]. For example, PTEN loss has been shown to increase PD-L1 expression in gliomas[148] and has also been associated with resistance to immune checkpoint inhibitors in other tumours settings[154], supporting the evaluation of combinatorial strategies targeting the PI3K-AKT pathway to increase the efficacy of immunotherapy. The interaction between EGFR-driven cancers and the immune system is much less clear, with patients with NSCLC harbouring EGFR mutations having poor outcomes with immunotherapy[155].

Conclusions

In this era of precision medicine, the sluggish progress in the advancement of therapy in GBM is insupportable. Results from single agent targeted therapy trials have been modest, and the success of single agent immunotherapeutic agents to date has been mixed, although encouragingly there are a multitude of ongoing trials.

Future successes in molecularly targeted agents and immunotherapies in neuro-oncology will likely depend on the development of rationally designed combination trials – trials incorporating both surgical arms, allowing for further tumour molecular characterization and creative biomarker selection and development. However, given the innumerable permutations of possible combination regimens with targeted agents, chemotherapy, radiation and immunotherapy, a deep understanding of the cancer biology of GBM, and its interaction with the immune system must underpin robust biology-driven approaches.

Glioblastoma tumours are profoundly complex. While there is unlikely to be a single "magic bullet" for GBM, there is much to be hopeful about as we focus on innovative biomarker-driven trial designs with greater collaborations between academic and industry partners to truly achieve precision medicine for glioblastoma.

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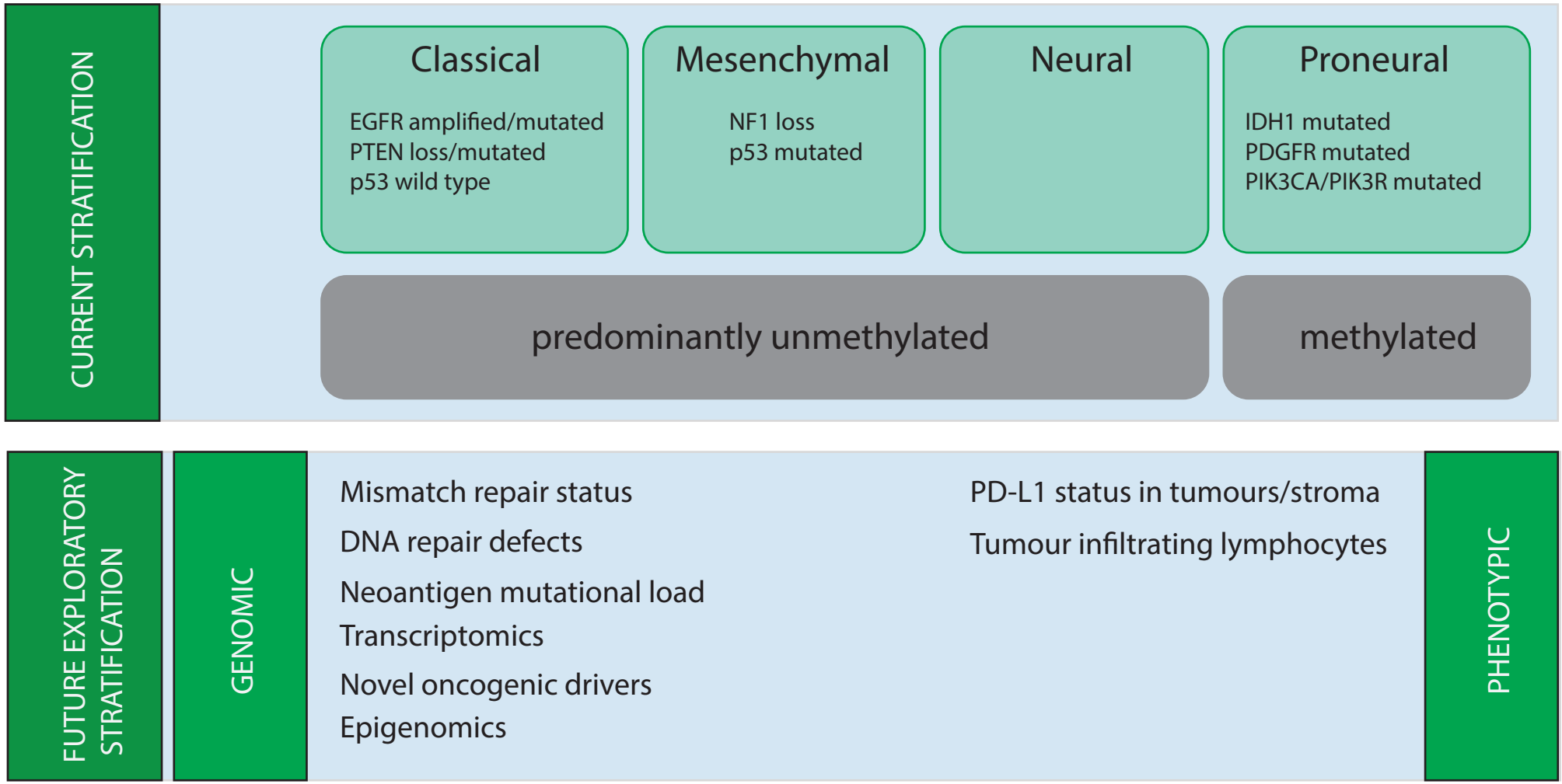
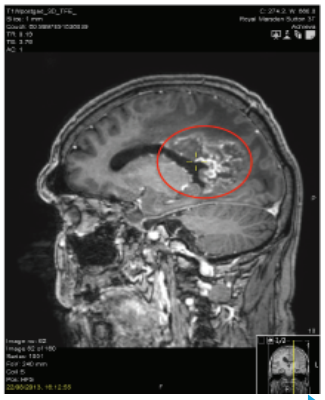
FIGURE 1 Molecular Characterisation of Gliblastoma



FRAMEWORK FOR PRECISION CANCER MEDICINE FOR GLIOBLASTOMAS



Patient with glioblastoma

surgical resection/ biopsy

GENOMICS

EGFR
IDH1/2
ATRX
PDGFR
BRAF
p53
NF1
Mismatch repair
DNA damage repair
Mutational load
Novel oncogenic drivers

IHC

PTEN
PD-L1
TILs

EPIGENOMICS

Methylation of MGMT promoter

Global methylation profiling

Molecular profiling

CLINICAL TRIAL

CTC/ctDNA monitoring exosomes

RESPONSE

DRUG RESISTANCE

repeat resection/ re-biopsy