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REVIEW

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Prospects of biological and synthetic pharmacotherapies for glioblastoma

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ABSTRACT

Introduction: The field of neuro-oncology has experienced significant advances in recent years. More is known now about the molecular and genetic characteristics of glioma than ever before. This knowledge leads to the understanding of glioma biology and pathogenesis, guiding the development of targeted therapeutics and clinical trials. The goal of this review is to describe the state of basic, translational, and clinical research as it pertains to biological and synthetic pharmacotherapy for gliomas.

Areas covered: Challenges remain in designing accurate preclinical models and identifying patients that are likely to respond to a particular targeted therapy. Preclinical models for therapeutic assessment are critical to identify the most promising treatment approaches.

Expert opinion: Despite promising new therapeutics, there have been no significant breakthroughs in glioma treatment and patient outcomes. Thus, there is an urgent need to better understand the mechanisms of treatment resistance and to design effective clinical trials.

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1. Introduction

Gliomas are a group of primary brain neoplasms, which include genotypically and phenotypically heterogeneous brain tumor subtypes. They represent 27% of the tumors of the central nervous system (CNS) and 80% of the malignant brain tumors [1]. They are classified according to the World Health Organization (WHO) classification, which assigns a grade (WHO grades I-IV) based on their degree of anaplasia and clinical characteristics [2]. WHO grade I is assigned to tumors with slower progression and better prognosis; and WHO grade IV is assigned to aggressive brain tumor lesions, which are designated as high-grade gliomas (HGG) or glioblastomas (GBM) [2,3]. The histopathological features are also considered by the WHO for glioma classification, defining astrocytoma, oligodendroglioma, and GBM as principal histologic groups[4]. Recently, analysis of molecular profiles in glioma patients has improved this classification, introducing the genomic alterations as criteria to differentiate glioma subtypes [3,5]. The distribution of molecular markers, including alterations in TP53, IDH1, PI3K, ATRX, EGFR, H3F3A TERT, PDGFR, PTEN [4,6], distinguishes these tumor types based on their association with recurrent genetic lesions and histology [4,7,8].

One of the most distinctive criteria for the molecular classification in gliomas is the mutational status of isocitrate dehydrogenase 1 (IDH1). Almost 50% of the adult glioma patients harbor mutations in IDH1, usually at arginine 132 (R132H) [8–10]. This proportion reaches 80% in patients with low-grade gliomas (LGGs; WHO grade II) and anaplastic astrocytomas (WHO grade III) [10–12]. In addition, 70% of the secondary HGG (WHO grade IV) also have IDH1 mutations [10,11]. IDH1-R132H produces 2-hydroxyglutarate which induces an epigenetic reprogramming of the tumor transcriptome [8,9,12,13] and is associated with better prognosis [7,9,14]. In LGG, two mutant IDH1 glioma subtypes have been identified according to mutually exclusive genomic alterations: i) ATRX mutation or ii) loss of 1p/19q chromosomal segments (1p/19q-codel) [3,7,8,12] (Table 1). Mutant IDH1 LGGs with inactivating mutations in ATRX co-expresses TP53 mutation, and are associated with astrocytoma [7,8,12]. Mutant IDH1 LGGs with 1p/19q-codel subtype present TERT promoter (TERTp) and CIC mutations are associated with oligodendroglioma [8,13] (Table 1).

The IDH1 wild-type molecular subgroup represents the other 50% and includes primarily WHO grade IV gliomas. In adults, IDH1 wild-type glioma patients retain ATRX function and typically express TERTp mutations and alterations in regulators of the RTK-RAS-PI3K signaling cascade [3,4,6] (Table 1). Pediatric gliomas are mostly IDH1 wild type, harboring TP53, and ATRX inactivating mutations, as well as H3F3A mutations which are associated with malignancy and poor prognosis [13,15].

The molecular markers incorporated in the classification of gliomas are important for diagnosis, prognosis, and treatment strategy. Molecular alterations present in the tumor may allow us to predict therapeutic responses [13,16]. Additionally, an accurate understanding of tumor biology is also valuable for developing new targeted therapeutic strategies. A number of

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Article Highlights

- In this review we cover the breadth of new therapeutic strategies that have emerged as potential treatment options for glioma.
- We provide an insight into the basic mechanisms involved in the development of resistance against targeted therapies.
- We present the importance of multi-modal therapies to address the heterogeneity of known mutations present in the different glioma subtypes.

Table 1. Molecular alterations in glioma.

Histopathology / Genetic Lesions	WHO Grade II and III		WHO Grade IV
	Oligodendroglioma	Astrocytoma	Glioblastoma
IDH1	Mutated (82%)	Mutated (68%)	Mutated (7%)
1p/19q	Co-deleted (70%)	Retained	Retained
ATRX	Mutated (19%)	Mutated (48%)	Mutated (7%)
P53	Mutated (24%)	Mutated (65%)	Mutated (30%)
CDKN2A	Deletion (2%)	Deletion (22%)	Deletion (54%)
RTK Pathway	EGFR Amp/Mut (5%)	EGFR Amp/Mut (5%)	EGFR Amp/Mut (64%)
	PTEN Del/Mut (2%)	PTEN Del/Mut (2%)	PTEN Del/Mut (38%)
	PDGFRA Amp/Mut (4%)	PDGFRA Amp/Mut (4%)	PDGFRA Amp/Mut (16%)

targeted therapies are currently being investigated in ongoing clinical trials (Table 2). In this review, we will cover the latest progress in the biological and synthetic pharmacotherapy in the glioma field.

2. Immune checkpoint inhibitors

Preclinical studies showed promising results when using immune checkpoint inhibitors individually or in combination with other immunotherapeutic strategies [17–20]. The effectiveness of immune checkpoint inhibitors has been linked to the enhanced levels of the neo-antigens (reflected by the mutation burden) within the tumor [21,22]. Compared to other tumors, GBM does not have a higher incidence of mutations [23,24]. A recent report showed a positive correlation between mutational load and the effectiveness of immune checkpoint inhibition in several cancers, but not in glioma [24]. This suggests that the mutational load is not a valid predictor for the response to immune checkpoint inhibitors in glioma patients. This could contribute to the failure seen in multiple clinical trials currently testing the benefits and safety of immune checkpoint blockade in GBM [18,25].

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152, is constitutively expressed in Tregs and activated T-cells upon antigen stimulation and has been shown to be upregulated in cancer [26,27]. The anti-CTLA-4 blocking antibody, Ipilimumab, was the first immune checkpoint inhibitor to be tested and approved treatment in cancer patients [28,29]. In GBM, preclinical testing suggests that blocking CTLA-4 alone results in enhanced long-term survival [29,30]. Another critical immunosuppressive pathway in GBM is the PD-L1/PD-1 interaction. PD-L1 is a major immunosuppressive molecule expressed by antigen-presenting cells (APC) and glioma cells [31]. Evidence shows that levels of PD-L1 expression correlate with unfavorable outcome in glioma patients [31–33]. Blockade of PD-L1 is necessary for dendritic cells (DCs) to prime CD8 T-cells and prevent T-cell exhaustion [34].

Several phase I and II clinical trials are currently examining the role of checkpoint inhibitors in combination with other therapies. In a phase I clinical trial Ipilimumab (anti-CTLA4) was tested in combination with Nivolumab (anti-PD-1) or Temozolomide (TMZ) to treat newly diagnosed GBM [35,36] (Table 2). In the same trial, Nivolumab was also tested in combination with TMZ. The study demonstrated that Ipilimumab and Nivolumab were safe and tolerable with similar toxicity profiles. In a phase III trial, Nivolumab was tested in combination with Bevacizumab (anti-VEGF), an antibody that is currently being used clinically to treat recurrent GBM, patients in this study did not show an increase in the overall median survival [35,36] (Table 2). These trials have stratified patients based on PD-L1 expression, although the majority of them are expected to express high levels of PD-L1 due to the dominance of the wild-type IDH1 phenotype in GBM [31,32]. The anti-PD-1 (Nivolumab and Pembrolizumab) antibodies are the most frequently used immune checkpoint inhibitors in clinical trials for GBM (Table 2). A recent report showed that neoadjuvant administration of Pembrolizumab prior to surgical resection of the tumor mass in a phase I clinical trial resulted in local and systemic anti-glioma immune response [37].

The mechanisms leading to both primary and acquired resistance to immune checkpoint inhibition are varied and can be both multifactorial and overlapping in an individual patient. Resistance to checkpoint inhibition therapy could be due to the lack of penetration of the blocking antibodies throughout the tumor, the ineffective effector T-cell infiltration, and/or T-exhaustion in the TME [27,38]. Currently, there are is no checkpoint inhibition monotherapy to treat patients with GBM; however, the combination of checkpoint inhibition with other immune stimulating therapies could be a prospective treatment strategy.

3. EGFRvIII-mediated vaccine

Mutation in the epidermal growth factor receptor (EGFR variant III (EGFRvIII)) is the most common gain of function mutation in high-grade glioma [39,40]. This tumor-specific gain of function causes constitutive activation of the receptor, which promotes growth and proliferation signals in tumor cells. The mutation occurs in the extracellular domain of the receptor resulting in the formation of an immunogenic peptide sequence that can be detected by monoclonal antibodies [41]. This can be used as a diagnostic biomarker for glioma [42]. Rindopepimut (CDX-110) was the first EGFRvIII-targeted vaccine developed. In order to promote immunogenicity, CDX-110 peptide has been conjugated to the potent immunogenic keyhole, limpet hemocyanin (KLH) (CDX-110-KLH). Preclinical data showed that rindopepimut was able to effectively target EGFRvIII tumor and promote an immune response [43–45]. In a phase II clinical trial, CDX-110-HLH was tested in combination with TMZ and radiation to treat newly diagnosed GBM. In

Table 2. Current clinical trial outcomes for glioma therapeutics.

Targeted therapy	Mechanism	GBM setting	Phase	Clinical trial identifier	Outcomes
Ad-hCMV-TK+ Ad-hCMV-Flt3L	Combined direct tumor cell killing and immune stimulation	Newly diagnosed GBM	Phase I	NCT01811992	• A dose-escalation safety study has recently concluded enrolling patients harboring primary GBM, that were treated with both vectors delivered simultaneously into the peritumoral region after tumor resection and results are expected by the end of 2020.
HSV-TK	Direct tumor cell killing	Newly diagnosed GBM	Phase III	NCT00002824	• Did not show an increase in the median overall survival.
Nivolumab + Bevacizumab	Anti-PD-1 + anti-VEGF	Recurrent GBM	Phase III	NCT02017717	• Did not show an increase in the median overall survival. • Nivolumab alone did not demonstrate an improved overall survival compared with bevacizumab. • Overall radiographic response was lower in nivolumab group compared to bevacizumab.
Ipilimumab and/or Nivolumab in Combination with Temozolomide	Anti-PD-1 + anti-CTLA4 + TMZ	Newly diagnosed GBM	Phase I	NCT02311920	• Ipilimumab and/or nivolumab are safe and tolerable with similar toxicity profiles when given with adjuvant TMZ for newly diagnosed GBM.
Toca 511	Combination retroviral encoding cytosine deaminase with targeted delivery of prodrug, 5-fluorocytosine	Recurrent GBM	Phase III	NCT02414165	• The trial did not meet the primary endpoint, demonstrating 11.1 months of overall median survival compared to 12.2 months with standard of care.
DNX-2401 (Delta-24-RGD-4C)	Tumor selective oncolytic adenovirus	Recurrent GBM	Phase I	NCT00805376	• Results demonstrated that Delta-23-RGD replicates and spreads within the tumor, leading to immunogenic tumor cell death and enhancement of T lymphocyte tumor infiltration.
ABT-806	EGFRvIII antibody	Advanced Solid tumors	Phase I	NCT01472003 NCT01255657 NCT01406119	• mAb806 has significant antitumor activity without nonspecific binding to normal tissues with safe and tolerable toxicity profile.
IDH305	Mutant IDH1 small molecule inhibitor	Recurrent glioma with R132H mutation	Phase I	NCT02381886	• Lowered 2HG production by 70% after 1 week of treatment. • Signs of tumor progression were identified based on FLAIR volume.
IDH305	Mutant IDH1 inhibitor	IDH1 Mutant Grade II or III Glioma	Phase II	NCT02977689	• Withdrawn after 1 year.
SonicCould	Ultrasound Devise to open BBB	Recurrent GBM	Phase II	NCT02253212	• BBB was disrupted in 11 patients and they showed a median progression-free survival of 4.11 months and median overall survival of 12.94 months.
ABT-414	EGFRvIII antibody	Newly diagnosed and recurrent GBM	Phase II/III	NCT02573324 NCT02343406	• Randomized studies are ongoing to determine efficacy in newly diagnosed and recurrent glioblastoma.
CDX-110-KLH + TMZ+ IR	EGFRvIII Vaccine	Newly diagnosed GBM	Phase II	NCT00458601	• The trial for this vaccine was terminated early as it was deemed likely that the study would fail to meet primary end point.
Pembrolizumab	Anti-PD1	Recurrent GBM	Phase II	NCT03899857	• Median progression-free survival was 7 months and progression-free survival at 6 months. • Median overall survival was not reached. • Tumor microenvironment was enriched with CD68 macrophages but exhibited paucity of effector T-cells.
CART-EGFRvIII + pembrolizumab	Autologous CAR-T targeted to the EGFR variant III plus PD-1 inhibition	Newly diagnosed GBM with EGFRvIII and unmethylated MGMT	Phase I	NCT03726515	• There was a variable response to CART-EGFRvIII therapy due to the inherent heterogeneity in GBM.

(*Continued*)

Table 2. (Continued).

Targeted therapy	Mechanism	GBM setting	Phase	Clinical trial identifier	Outcomes
Autologous T-cells	Autologous CMV specific cytotoxic T-cells	Newly diagnosed and recurrent GBM	Phase I/II	NCT02661282	• Repeated infusions of CMV-TC were associated with a significant increase in circulating CMV+ CD8 + T-cells • Adoptive infusion of CMV-TC after lymphodepleting therapy with MZ was well tolerated. • The final dose level is currently being enrolled. Thereafter, efficacy will be evaluated in cohorts of newly diagnosed and recurrent GBM patients.
PVSRIPO	Oncolytic polio:rhinovirus recombiant virus	Recurrent GBM	Phase I	NCT01491893	While the phase I dose escalation trial for convection-enhanced delivery of PVS-RIPO in recurrent GBM is currently enrolling (NCT01491893), preliminary re-sults are promising, with 3 of 7 patients demonstrating a com-plete response While the phase I dose escalation trial for convection-enhanced delivery of PVS-RIPO in recurrent GBM is currently enrolling (NCT01491893), preliminary re-sults are promising, with 3 of 7 patients demonstrating a com-plete response • Intratumoral infusion of PVSRIPO in patients with recurrent GBM confirmed the absence of neurovirulent potential.
CART- IL-13Rα2	Autologous CAR-T targeted to IL-13Rα2	Recurrent GBM	Phase I	NCT02208362	• Ongoing Recruiting
CART-HER2	Autologous CAR-T targeted to HER 2	Recurrent GBM	Phase I	NCT01109095	• Ongoing Recruiting

this study, median progression-free survival and overall survival from histopathological diagnosis were 12.3 and 24.6 months, respectively. The study also demonstrated that EGFRvIII was eliminated in 4/6 (67%) of tumor samples obtained after >3 months of treatment [43–45] (Table 2). As an alternative treatment approach, patients were vaccinated with dendritic cells (DCs) pulsed with CDX-110-KLH [43–45]. A phase III clinical trial for this vaccine was terminated early as it was deemed likely the study would fail to meet its primary end point [46].

Agents targeting EGFR have been shown to be associated with on-target toxicities as a consequence of disrupting normal EGFR function [47]. MAb 806 is a novel EGFR antibody that selectively targets a tumor-selective epitope suggesting that a mAb 806-based therapeutic would retain antitumor activity without the on-target toxicities associated with EGFR inhibition [47]. MAb806 (now known as ABT-806) inhibited the growth of EGFRvIII-positive human glioma xenografts [48,49]. This therapy did not show signs of adverse effects and could cross the blood–brain barrier (BBB). Currently, it is the only monoclonal antibody that has been tested clinically against GBM in a phase I clinical trial for advanced solid tumors [50] (Table 2). Early results from the trial demonstrate that mAb806 has significant antitumor activity without nonspecific binding to normal brain tissue and tolerable toxicity profile [50]. ABT-414 is another antibody derived from ABT-806. It is conjugated to anti-microtubule agent, monomethyl auristatin F [51], which has demonstrated promising results in cancer patients [52,53]. Randomized studies are ongoing to determine ABT-414's efficacy in newly diagnosed and recurrent glioblastoma in phase 2 clinical trials (Table 2).

From the above results, it can be concluded that ablation of EGFRvIII-positive glioma cells does not yield effective glioma regression in the clinical setting. The mechanisms of resistance to EGFRvIII vaccines can be multifactorial [39,54]. First, intratumoral heterogeneity allows for the expansion of non-targetted EGFRvIII-negative glioma cells. Additionally, GBM cells can activate other cell proliferation pathways that render the tumor independent of EGFRvIII signaling [39,47]. Finally, the presence of an immune-suppressive intratumoral milieu can yield T-cells at exhausted states and therefore dampen vaccine-induced antitumor immune responses [39,47].

4. Chimeric antigen receptor-T adoptive cell therapies

Adoptive cellular therapy of chimeric antigen receptors (CAR) T-cell therapy specific for tumor antigens has been shown to induce antitumor immunological memory in preclinical glioma models [55]. CAR T-cell therapy is based on gene transfer technology capable of reprograming the patient's cytotoxic T-cells to express recombinant surface molecules that combine the antigen-recognizing variable region of an antibody in tandem with intracellular T-cell signaling moieties [25,56]. Specifically, CARs are composed of: single-chain variable fragment derived from a B-cell receptor, a CD3ζ domain derived from a T-cell receptor (TCR), and intracellular co-stimulatory domains [57,58]. Unlike the classic TCR, this structure allows CAR T-cells to target specific antigens in an HLA-independent

fashion. This is important because downregulation of HLA is a common strategy of immune evasion by tumors [58].

CAR T-cell therapies that target interleukin-13 receptor alpha 2 (IL-13Rα2) [59], human epidermal growth factor receptor 2 (HER2) [60], and epidermal growth factor receptor vIII (EGFRvIII) [61], are currently in phase I clinical trials (Table 2). IL-13Rα2-CAR T-cells were administered intracavitary following resection, intratumorally, or intraventricularly. HER2 and EGFRvIII-CAR T-cells were administered peripherally [62]. Unfortunately, 7.5 months after the administration of CAR T-cells, tumor recurrence was detected at four new locations at non-adjacent areas. These tumors displayed a lower expression of IL13Rα2, which could explain tumor evasion driven by targeted killing by IL13Rα2-CAR T-cells [63].

Expression of antigens targeted by CAR T-cells varies across tumors, enabling the outgrowth of non-targeted cells following treatment. In turn, the efficacy of CAR T-cell therapy can vary drastically but can have significantly positive results in particular cases. For example, in a clinical trial using CAR T-cells designed to target IL-13Rα2, one of the patients showed a powerful clinical response, demonstrating a complete regression of all metastatic tumors in the spine [64]. Currently, much effort is being put into the development of the next generation of CAR T-cells. These developing approaches involve stimulatory cytokine overexpression, gene editing, and multi-antigen targeting [63].

In the case of glioma, CAR T-cells targeting both HER2 and IL13Rα2 have been designed to prevent antigen escape and they have been tested in preclinical models [65]. These engineered T-cells have a bispecific CAR molecule that incorporates two antigen recognition domains for HER2 and IL13Rα2, joined in tandem (TanCAR). In *in vivo* orthotropic glioma mouse models, the mice treated with the TanCAR T-cells exhibited an improved survival and a more effective antitumor immunity, compared to the controls treated with both monospecific CAR T-cells or with CAR T-cells co-expressing separate CARs against HER2 and IL13Rα2. Moreover, other modifications have been tested to increase the proliferation and persistence of CAR T-cells in the tumor microenvironment [66]. This study in a preclinical model setting demonstrated that the overexpression of stimulatory cytokines is a feasible strategy to improve CAR T-cell therapy's outcomes.

There are still many challenges that should be addressed to improve the efficacy and persistence of CAR T-cells for GBM therapy. Tumor cell heterogeneity is a characteristic of brain tumors, and specifically of GBM, representing a critical limitation to targeted therapies [67]. In recent years, and in line with the development of single-cell RNA-sequencing techniques, different transcriptomic clusters within a single tumor mass could be identified, illustrating glioma heterogeneity. In this regard, the expression of the antigens selected for the development of CAR T-cells is not homogeneous across the tumors, which enables the outgrowth of antigen-negative tumor cells after therapy administration. For example, in one of the clinical trials for CAR T-cells designed to target IL-13Rα2, one of the patients showed a powerful clinical response, demonstrating a complete regression of all metastatic tumors in the spine, while the patient experienced an improved life quality [64]. The antigen escape as a pathway of therapeutic resistance could be overcome by employing CAR T-cells with multiple specificities or by combining CAR T-cells specific for different antigens. This approach has been tested and trivalent CAR T-cells targeting HER2, IL13Rα2, and EphA2 have been designed and assessed in preclinical studies, showing promising results to overcome tumor heterogeneity [60]. In addition, GBMs display an immunosuppressive tumor microenvironment (TME) that could hinder the efficacy of CAR T-cells. The anatomical location of these tumors, the presence of immune inhibitory cytokines and immunosuppressive cells, and the lack of nutrients are some of the factors that contribute to a suppressed TME [68]. A strategy developed to by-pass this situation was to administer CAR T-cells in combination with checkpoint blocking antibodies, such as PD-1/PD-L1 inhibitors [69]. Also, these immunosuppressive molecules could serve as the antigens for the CAR T-cells' design [62].

5. Gene therapy

Non-replicating recombinant viral vectors have been extensively evaluated in GBM patients in clinical trials [60]. Many of these studies have evaluated the efficacy of local delivery of retroviral or adenoviral vectors encoding the conditionally cytotoxic HSV1-thymidine kinase (TK) gene in combination with systemic ganciclovir or similar prodrugs. The rationale for this approach is that TK-expressing cells are able to phosphorylate ganciclovir, inhibiting DNA synthesis in proliferating cells and leading to cell death. This strategy was shown to be safe and was evaluated in a large phase III trial in patients with newly diagnosed GBM. Although it increased time to progression or re-intervention, it failed to improve OS (Figure 1) [70].

Local overexpression of pro-inflammatory cytokines could overcome the immunosuppressive tumor microenvironment and the CNS immune-privilege; therefore, administration of gene therapy vectors encoding cytokines has also been evaluated in preclinical and clinical trials for GBM patients. Local delivery of IFN-β gene using adenoviral vectors showed promising results in GBM preclinical models [71]. Additionally, a pilot clinical trial showed the safety of interferon-β gene transfer when used on patients with malignant glioma. In this study, two patients demonstrated a partial response (<50% tumor reduction) and two other patients had stable disease 10 weeks after beginning therapy [72]. Nevertheless, definitive evidence of its potential will require a randomized and controlled phase III study.

By combining suicide gene therapy with immune-stimulatory gene therapy strategies (e.g., encoding pro-inflammatory cytokines) the efficacy of gene therapy for GBM could be improved (Figure 1). Co-delivery of IL-2 and TK in GBM patients using retroviral vector-producing cells led to an increase in circulating pro-inflammatory cytokines without adverse events; however, it failed to display therapeutic efficacy[73]. A strategy that combines TK and Flt3L gene delivery using adenoviral vectors has shown to trigger antitumor immunity and long-term immunological memory, impairing GBM recurrence in multiple preclinical models of GBM (with no significant toxicity) [74–76]. The efficacy of this strategy relies on the cytotoxic effect of TK, promoting the release of antigens and DAMPs from dying tumor cells and on the immune stimulatory effect of Flt3L, which induces the expansion and recruitment of dendritic cells into the tumor

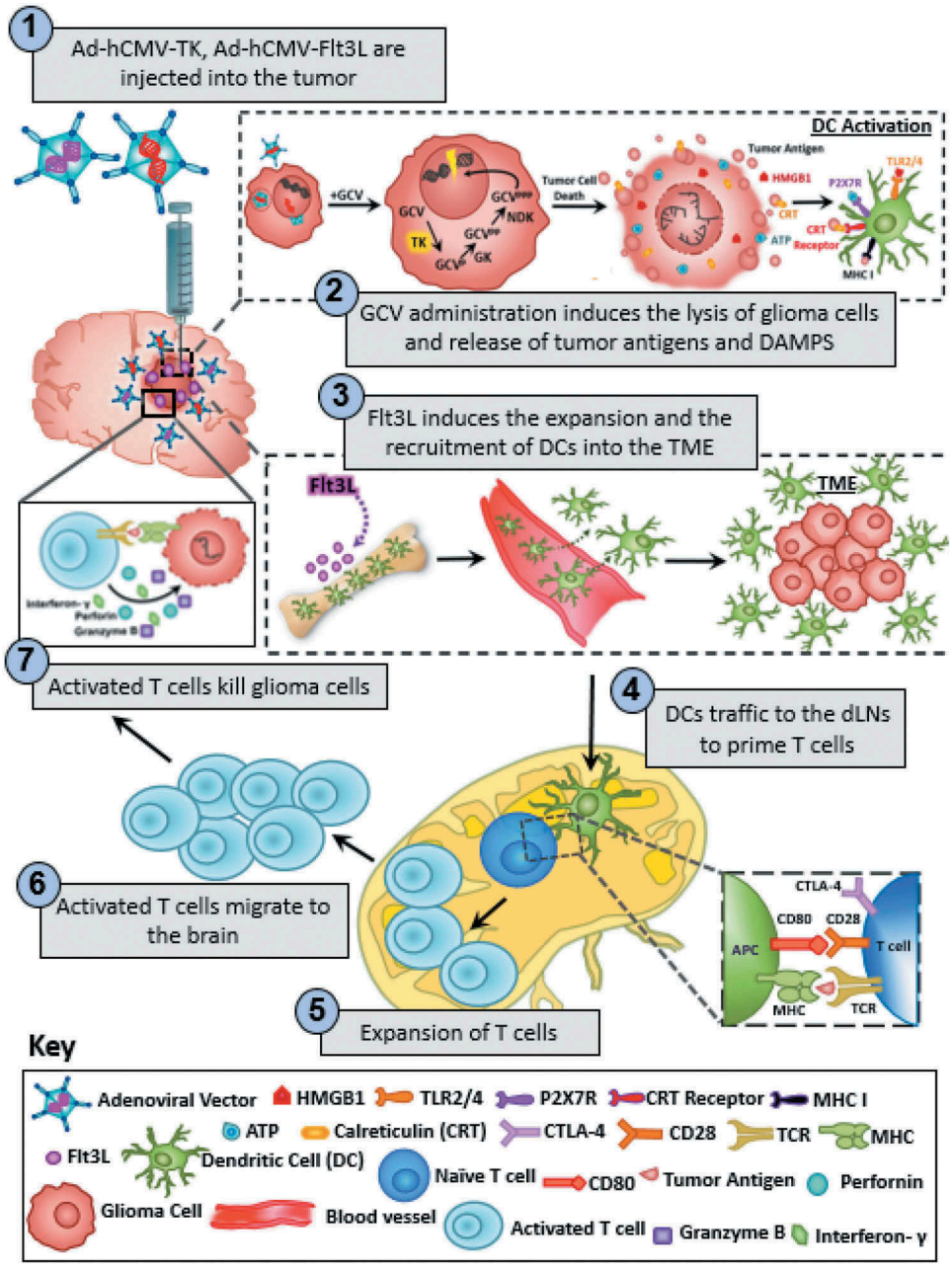

Figure 1. Mechanism underlying the anti-glioma immune response following TK/Flt3L gene therapy. First-generation adenoviral vectors encoding HSV1-Thymidine Kinase (TK) and HSV1-Flt3L are intratumorally injected. This is followed by systemic administration of prodrug ganciclovir (GCV). TK is capable of converting GCV to GCV-triphosphate, a purine analog that selectively inhibits DNA replication in proliferating tumor cells. The expression of TK in the presence of GCV mediates the release of damage-associated molecular patterns (DAMPs), i.e. HMBG1, calreticulin, and ATP from dying tumor cells. Expression of Flt3L recruits dendritic cells (DCs) into the tumor milieu where they take up brain tumor antigens released from the dying glioma cells and present them on their MHC complexes. HMGB1 binds to TLR2/4, which promotes the production of cytokines and tumor antigen cross-presentation. The binding of extracellular ATP to purinergic receptor P2X7R promotes the recruitment of DCs to the tumor milieu. The DCs loaded with tumor antigens migrate to the cervical draining lymph nodes where they present tumor antigens to naïve T-cells, priming tumor-specific anti-glioma effector T-cells. The tumor-specific effector T-cells then migrate back into the brain and kill residual glioma cells via the production of granzyme B, perforin, and effector cytokine IFN-γ.

microenvironment. [74–76]. A dose-escalation safety study has recently concluded enrolling patients harboring primary GBM that were treated with both vectors delivered simultaneously into the peritumoral region after tumor resection. Dose-limiting toxicity for the vectors was not encountered in the study, and an overall survival of ~5 months was observed in patients treated with the gene therapy vs. contemporary controls.

Local delivery of the viral vectors at the time of the surgery offers its advantages for treating residual disease and potentially extending the period to recurrence. Factors that might hinder the efficacy of gene therapy strategies include (i) presence of circulating antibodies against viral vectors [77], (ii) insufficient diffusion of the viral vectors or transgenes from the site of intratumoral injection [77], or (iii) variance in the persistence of therapeutic transgene expression at the tumor site [77].

6. Oncolytic virus therapy

Oncolytic viruses (OVs) selectively replicate in tumor cells, promoting the lysis of cancer cells and the dissemination of OVs to neighboring tumor cells without affecting normal cells. There are two main types of OVs: 1) viruses that are nonpathogenic in humans, but naturally replicate in cancer cells (e.g. parvoviruses, poxvirus, Newcastle disease virus, reovirus, picornavirus) and 2) viruses that are genetically manipulated to selectively inhibit their replication in normal cells, but not in cancer cells (e.g. Delta-24-RGD, Toca 511, ONYX-015, PVSRIPO) [78]. OVs trigger an antitumor response that not only depends on the lysis of tumor cells but also on the subsequent enhancement of antitumor immunity. OVs can also be genetically engineered to express therapeutic transgenes. Armed OVs encoding cytokines, chemokines, and tumor-associated antigens have been developed to further boost antitumor immunity [79].

Genetically modified adenoviral vector ONYX-015 has been designed to selectively replicate in p53-deficient tumor cells. Interestingly, additional mechanisms seem to allow the replication of ONYX-015 in p53-competent gliomas. An early Phase I dose-escalation clinical trial was performed in patients with recurrent GBM that received injections of ONYX-015 within the tumor bed after surgical resection [80]. ONYX-015 was well tolerated but did not yield therapeutic benefit (Table 2).

A recent Phase I clinical trial using the conditionally replicating adenoviral vector, Delta-24-RGD, showed long-term survival (over 3 years post-treatment) in 5/25 of patients with recurrent high-grade gliomas [81]. This trial also demonstrated that Delta-24-RGD replicates and spreads within the tumor, leading to immunogenic tumor cell death and enhancement of T lymphocyte tumor infiltration (Table 2).

Toca 511, a retroviral OV based on the murine leukemia virus has also been used to treat recurrent high-grade gliomas. Toca 511 encodes cytosine deaminase, a conditionally cytotoxic enzyme which converts the prodrug,5-fluorocytosine, into the antimetabolite, 5-fluorouracil. This conversion induces tumor cell death and depletion of myeloid-derived suppressive cells and tumor-associated macrophages [82]. This strategy was granted Breakthrough Therapy designation in recurrent high-grade glioma by the FDA and a recent early phase I clinical trial showed that treatment of these patients with Toca 511 followed by oral 5-fluorocytosine led to complete responses and long-term survival (over 34 months post-treatment) in 5/23 patients [82]. However, a phase III clinical trial for Toca 511 did not meet the primary endpoint. This study demonstrated 11.1 months of overall median survival for Toca 511-treated patients compared to 12.2 months with the standard of care [83] (Table 2). This failure could be due to the fact that this therapeutic modality was tested in the recurrent setting; testing Toca 511 in primary GBMs at the time of surgical resection would be warranted.

Oncolytic polio:rhinovirus recombinant virus, PVSRIPO, is a live-attenuated poliovirus type 1 virus, in which the internal ribosome entry site has been replaced with that of the human rhinovirus type 2 virus, blocking neurovirulence [84]. PVSRIPO tropism toward CD155, present in tumor cells and APCs, enables tumor cell cytotoxicity and activation of an inflammatory response. The survival of recurrent GBM patients treated with convection-enhanced delivery of this vector in a Phase I clinical trial reached a plateau of 21% overall survival at 24 months, with a subset of patients surviving over 57 months [84] (Table 2). PVSRIPO was also granted Breakthrough Therapy designation by FDA. Nevertheless, success in a double-blind controlled, randomized Phase 3 clinical trial is necessary in order to draw conclusive results.

The results of the first dose-escalating clinical trial of the rat parvovirus H-1PV were recently reported [85]. Patients with recurrent GBM received systemic or local injections of H-1PV, which was safe and well tolerated. Both cohorts showed markers of viral replication in the tumor and signs of an immunogenic tumor microenvironment, suggesting that systemic therapy could be an alternative strategy to treat inoperable tumors [85].

Resistance to oncolytic virus therapy may arise from the following: (i) anti-bodies present in the host's system could recognize viral epitopes resulting in an immune response against the oncolytic viral vectors [79], or (ii) insufficient diffusion of the viral vectors from the site of intra-tumoral injection throughout the tumor bed [79]. Although early phase trials suggest that subsets of GBM patients may benefit from oncolytic virotherapy, larger trials are required to confirm the efficacy of these strategies and identify which patients will benefit from these treatments.

7. Targeting metabolism: IDH1 mutation

IDH1 is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) [86]. α-KG is a key metabolite involved in the Krebs cycle. It is also important for the activity of α-KG dependent enzymes including the DNA hydroxylase ten-eleven translocation (TET) enzymes and histone demethylases (KDM) enzymes [87]. As described, mutation in IDH1 (IDH1-R132H) is a hallmark genetic marker in a subset of gliomas [12]. This mutation generates a gain of function in IDH1 enzymatic activity, producing 2-hydroxyglutarate (2-HG) from α-KG [12]. 2-HG is an 'oncometabolite' which acts as a competitive inhibitor to α-KG. This alters the glioma cell metabolism and impairs the activity of α-KG dependent demethylases, resulting in hypermethylation of DNA and histones [88]. As a consequence, mutant IDH1 glioma cells exhibit metabolic and epigenetic reprogramming that impacts tumor development and cellular signaling [89]. Glioma patients harboring IDH1-R132H are younger at the time of diagnosis and have a better prognosis compared with wild-type DH1 glioma patients [7,14]. Despite this relative survival benefit, gliomas with IDH1-R132H are invasive and can progress to grade IV [90]. The molecular mechanisms contributing to the increased median survival in IDH1-R132H tumors are not completely understood. The mechanisms are likely closely related to the epigenetic changes in gene expression induced by mutant IDH1 activity. It has been reported that mutant IDH1 blocks cell differentiation [91,92] and inhibition of 2-HG production decreases cell proliferation, delaying growth of mutant IDH1 expressing xenografts [93].

Recently, the use of a brain penetrant inhibitor resulted in improved median survival in an intracranial mutant IDH1 glioma model [94]. Based on these results, several IDH1-

R132H inhibitors have been developed. Disruption of mutant IDH1 is a potential therapeutic target for glioma patients that express this molecular alteration [95]. A phase I clinical trial demonstrated a 70% reduction of 2-HG in mutant IDH1 gliomas with an impact on metabolic reprograming and cell density [96]. In addition, IDH1-R132H expression has been associated with changes in DNA-repair and DNA-damage response (DDR) efficiency [13] with variance among mutant IDH1 glioma subtypes. PARP inhibitors have been suggested as a potential therapeutic approach for glioma subtypes that show decreased homologous (HR) DNA-repair capacity [97]. Our team recently reported that IDH1-R132H in combination with loss of TP53 and ATRX increases HR DNA repair and induces radioresistance in glioma, a phenomenon that is reversed by using DDR response inhibitors [13]. Disruption of DDR via ATM or CHK1/2 inhibition, combined with radiation increased the median survival of mice harboring brain tumor expressing IDH1-R132H with loss of TP53 and ATRX suggesting a novel potential therapeutic strategy for this specific glioma molecular subtype [13].

Preclinical data showed that treatment of human glioma xenografts with small molecule inhibitors against mIDH1 impaired tumor growth and did not affect outcomes in wildtype-IDH1 glioma xenografts [98]. IDH305 a small molecule inhibitor developed by Novartis has advanced to Phase I clinical trial and the safety study demonstrated lower 2-HG levels within a week of treatment. [98] Although mutations in IDH1 are found in 50% to 80% of low-grade glioma, only 12% of GBMs express this mutation [98]. Thus, the mIDH1 small molecule inhibitors are not suitable for treating primary GBM patients.

The loss of mIDH1 expression from primary tumors could lead to early tumor recurrence due to clonal expansion. For instance, in a longitudinal analysis of 50 mutant IDH1 patients, six cases had copy number alterations (CNA) at the IDH1 endogenous locus in recurrent tumor samples when compared to the primary mIDH1 glioma [99]. Deletion or amplification of mutant IDH1 locus led to reduced 2HG and transformation to more aggressive grade IV glioblastoma [99]. These findings indicate that heterogeneity within the primary tumor could lead to resistance to mIDH1 inhibitor treatment, making mutant IDH1 a passenger upon tumor recurrence.

In conclusion, IDH1 mutant tumors are unique entities and understanding this biology may lead to novel treatment strategies. The effects of IDH1-R132H are highly dependent on the genetic context in which this mutation is found. Therefore, subtypes of mutant IDH1 glioma should be studied independently in order to best define potential novel targeted therapies. Inhibition of 2-HG production and modulation of the signal cascade involved in IDH1-R132H activity, including DDR, may serve as effective adjuvant treatment approaches for patients with mutant IDH1 gliomas.

8. Nanoparticle formulations for glioma therapeutics

Alternative drug delivery approaches have been developed to overcome several limitations of glioma treatments. Nanoparticles (NPs) are emerging as an effective and noninvasive delivery system for treating brain tumors [100]. They are engineered using natural (e.g. albumin) [101], synthetic (e.g. polylactids) [102], lipid (e.g. liposomes) [103], or lipoprotein (e.g. sHDL nanodiscs) [104] biodegradable materials. Due to their small size (average diameter less than 200 nm), NPs are able to overcome the BBB, allowing for systemic delivery [105]. The encapsulation of hydrophilic and hydrophobic therapeutic agents into NPs protects them from enzymatic or chemical degradation when administered via different delivery routes (e.g. oral, transdermal, nasal, and intravascular) [106]. They also allow for targeted delivery of multi-modal treatments and can also be used as imaging agents (theranostics) [107–110]. The size of the particles, the high stability (i.e. long half-life) [104], and the capacity of conjugating multiple active compounds into their matrix are some of the characteristics that make NPs an attractive therapy [104]. Therefore, NPs offer a potential means to optimize drug delivery at the disease site, enabling increased drug bioavailability and reduction of the dosing frequency [111].

Efforts have been made to improve drug delivery to the tumor site in order to offset any putative systemic toxicity and maximize the therapeutic benefits [112–114]. A genotype-targeted molecular-based treatment study demonstrated that the delivery of NPs loaded with a therapeutic agent to the glioma site reduced the incidence of tumor relapse in mice [115]. In this study, PLGA microparticles encapsulated with nicotinamide phosphoribosyltransferase inhibitor (GMX-1778), which exerts anti-tumor activity by selectively antagonizing NAD^+ biosynthesis, were stereotactically injected at the tumor site. Glioma cells significantly depend on NAD^+ to support the high levels of ATP production necessary for rapid cell proliferation [115]. Thus, a single stereotactic injection of GMX-1778 resulted in the suppression of the intracerebral mutant IDH1 tumor growth when compared to control mice that were injected with blank PLGA microparticles [115]. In another preclinical study, intratumoral delivery of lipopolymeric NP (LPNPs) loaded with siRNAs targeting transcription factors SOX2, OLIG2, SALL2, and POU3F2 (which drive proneural brain tumor-initiating cells), resulted in GB43 tumor growth suppression in a xenograft model compared to the non-targeting siRNA loaded control LPNPs [116].

Work from our team showed that local treatment of glioma with NPs loaded with a chemotherapeutic agent coupled with an adjuvant capable of stimulating an immune response could elicit tumor cell death, tumor regression, and immunological memory prevents relapse [117]. We utilized sHDL nanodiscs that had previously been administered to humans in Phase I/II studies for treating acute coronary syndrome and were proven to be well tolerated [100,118–120]. In this study, docetaxel (DTX), a widely used chemotherapeutic agent that suppresses microtubule depolymerization was incorporated into synthetic apolipoprotein-I (ApoA-I) peptide-based sHDL nanodiscs coupled with CpG (a TLR9 agonist). Local delivery of these sHDL nanodiscs resulted in sustained release of the drug formulation at the tumor site while avoiding adverse off-target toxicity [119]. The findings from this study suggest a potentially new approach for glioma chemo-immunotherapy. Local drug delivery at the time of surgery offers its advantages for treating residual disease and combatting recurrence due to the immunological memory response elicited by this NP-mediated therapy.

Given that the brain is a delicate organ susceptible to toxic substances, NPs pose specific safety issues that need to be addressed carefully. Factors such as the structure of the NPs, abnormal tumor microenvironment, and the heterogeneity across tumors can compromise the efficiency of the NPs [121]. In addition, off-target distribution of the NPs to non-tumor stromal cells due to the heterogeneity of the tumor microenvironment could result in their accumulation in the brain, inducing drug resistance and compromising clinical outcomes [122]. NPs with varying composition, size, and functionality offer attractive therapeutic options for glioma treatment. However, these will need to be extensively validated in preclinical models before proceeding to their implementation and testing in Phase I clinical trials in human GBM patients.

9. BBB disruptive therapies

The blood-brain barrier is a complex passive and active structure that protects the brain from exposure to potentially dangerous substances [123]. While critical for protection against otherwise dangerous circulating compounds, the BBB also prevents the delivery of systemically administered drugs to the brain under pathological conditions. The BBB limits the efficacy of systemically administered therapeutics due to the fact that the body acts as a sink for the therapeutic agent with very limited concentrations of the compound actually reaching the target brain tissue or tumor [109]. Numerous invasive approaches have been developed; however, they can be problematic in the clinical setting, causing damage in the surrounding brain tissues. Alternatively, BBB disruptive therapies have been studied as a method for improving the delivery of compounds to the brain in neurologic conditions as well as for patients with brain tumors [124].

One popular method for disrupting the BBB is pulsed ultrasound. This method has been shown to effectively increase drug concentration and slow tumor growth in preclinical studies [123]. There have also been phase 1/2a clinical trials using implantable ultrasound device systems in combination with carboplatin chemotherapy for patients with recurrent GBM (Table 2) [125]. It has been demonstrated that repeated opening of the BBB using pulsed ultrasound in combination with systemic microbubbles is safe and well tolerated, displaying the potential to allow effective delivery of chemotherapy to the brain. Resistance to this therapeutic strategy was observed in some patients due to the architecture of the microvessels in the tumor, which may be more resistant to damage through microbubble/vessel interaction [125]. Biochemical methods to circumvent the BBB are also well established in preclinical models [110]. The traditional method involves osmotic BBB disruption which is based on the principle that injection of a hyperosmotic agent will cause temporary shrinkage of endothelial cells and subsequent opening of the tight junctions, allowing entry of systemically administered therapeutic compounds into the brain [102]. Other methods for bypassing the BBB include bradykinin receptor-mediated BBB opening [126], inhibition of drug efflux transporters [127], or exploitation of receptor-mediated transport systems [128]. While each of these methods holds potential, evidence of safety and efficacy to date is largely limited to preclinical models and small early clinical studies. Further translational and clinical research is required to determine whether these therapies will improve patient outcomes.

10. Conclusion

Although innovative therapeutic modalities have been designed to treat glioma, they have failed in improving patient outcomes. Currently available standard of care (SOC) treatment modalities include surgical resection, radiotherapy (IR) and/or chemotherapy [129]. These treatment strategies have been based on the 2016 WHO brain tumor classification guidelines [3]. Radiotherapy and adjuvant temozolomide (TMZ) have been SOC for treating GBM for 15 years [130]. Patients receiving TMZ and IR after surgery showed a 2.5-month survival advantage compared with those receiving adjuvant radiotherapy alone. Modest advances in SOC treatment have been made recently, where maximal safe surgical resection is being followed by radiotherapy, procarbazine, lomustine, or vincristine chemotherapy [130]. The median survival time is doubled in patients receiving a combination of radiotherapy and chemotherapy versus surgery alone in randomized clinical trials [131]. There is only a meek increase of 1–2 years' survival following the combined radiotherapy and chemotherapy treatment. There is also evidence that indicates that whole brain radiotherapy and chemotherapy impair patient cognitive functions [130].

Many innovative therapies have been designed to treat glioma; however, they have ultimately failed in Phase III clinical trials despite showing promise in the research setting. The failure of these treatments can be attributed to tumor heterogeneity, tumor evasion, the blood-brain barrier, its anatomical location, invasiveness, and the immune suppressive tumor microenvironment [132]. While new therapies are attempting to address these challenges, none to date has been effective in the clinical setting. This prompts the necessity to understand the poor translational potential of these therapies in order to increase the clinical efficiency.

Currently, mouse models and in vitro experiments are used for glioma translational research [131]. These models are useful for understanding the biological influence of particular mutations, but they have their limitations. The cells in these models are designed to express a particular set of genetic lesions; however, clinical gliomas are heterogeneous [133]. Therefore, the efficacy of these therapies may only apply to a portion of the tumor, accounting for the poor clinical translation.

It has been proposed that intratumoral heterogeneity significantly influences the efficacy of immune therapies. Increased heterogeneity makes it more difficult to detect specific neoantigens and allows for subclonal evasion of immune detection. Currently, tumor xenograft models are often used to study immune therapies. While these models are capable of generating solid tumors, they may not accurately simulate the tumor-immune microenvironment seen in patients. In these models, tumor cells are introduced to a competent-immune environment. This ignores the crosstalk with the immune system that plays a critical role during tumor development in the clinical setting.

These issues are starting to be addressed with advanced molecular analysis such as single-cell RNA-sequencing [133]. An increased understanding of intratumoral and intertumoral heterogeneity would allow for more combination treatments and could provide a more accurate basis for heterogeneous tumor models.

Another constraint is the size of the tumor in the rodent models. Due to the small size of the mouse brain, tumors can only grow to a limited size. This limited tumor volume may contribute to the higher success rate seen in the preclinical research setting. The smaller size means there are fewer cells to kill and also may allow therapies to diffuse to a higher percentage of the tumor mass. Animal models which allow for greater tumor growth may address this issue and create a more accurate therapeutic model. Pet dogs with GBM constitute an ideal model to address the regression of a large tumor mass [134].

In conclusion, gliomas are heterogeneous central nervous system neoplasms that are associated with poor prognosis in the case of higher-grade tumors. There are no effective treatment strategies available for high-grade glioma. The mainstays of therapy for high-grade glioma include maximal safe surgical resection, radiation, and treatment with toxic and nonspecific chemotherapeutic agents that have been in use for decades. As scientific discoveries uncover mechanisms for tumorigenesis, attractive targets for the development of highly specific and novel therapeutics continue to emerge. Popular areas for drug development today are focused on the interaction between the tumor and the immune system. These therapies along with targeting known mutations, such as in mutant IDH1, represent exciting avenues for future drug development. There is an urgent need for translational research and novel clinical trials to determine the potential efficacy of these exciting therapies in patients with glioma.

11. Expert opinion

The landscape for basic science in glioma research is rapidly evolving. Recent advances in the understanding of tumor heterogeneity and the detailed characterization of chromosomal and molecular alterations provide an accurate approach for classifying gliomas. This is reflected in the recent update to the WHO Classification of CNS tumors [3]. For the first time, genetic and molecular alterations are significant factors in how brain tumors are classified, supplanting historical systems based on histopathologic appearance. These novel methods for characterizing gliomas provide a more accurate foundation on which to develop novel therapeutics and design effective clinical trials.

The next step for glioma research is to use the strides made in the expansion of the basic science knowledge to develop novel-targeted therapeutics and test them in patients. Unfortunately, drug design and clinical trial conduct come at a significant economic cost that often limits the development of potentially promising treatments. In order to address this issue, preclinical in vivo models that recapitulate the disease processes are essential to enable the scientific and medical communities to differentiate effective from ineffective therapies before implementing treatment in a clinical patient population.

This review highlights that there are more exciting therapeutics for glioma under development at present than at any other time. Immune-based strategies hold great promise for the treatment of patients with glioma. CAR-T therapy and immune checkpoint blockade have drastically improved outcomes for patients with other cancers such as hematologic malignancies and melanoma. These successes are rooted in a strong understanding of the underlying molecular mechanisms involved in the interaction between a tumor and the immune system. A critical question looming over the field of neuro-oncology has been the lack of an explanation of why similar targeted therapies have not realized the same successes for patients with glioma as has been observed for other cancer patients. This is another understudied area in neuro-oncology. A better understanding of the basic mechanisms for resistance to targeted therapies will avoid the costs incurred in the development of therapeutics that are unlikely to succeed and will promote proper allocation of resources to the highest yield clinical trials.

In this review, we cover the breadth of new therapeutic strategies that are emerging as potential treatments for glioma. Accurate preclinical models for drug design and assessment of their efficacy and safety are important to identify the most promising treatment approaches. Rigorous, well-designed clinical trials are also essential to identify patients that will benefit most from novel therapeutics. Despite the exciting challenges, the present day is a more promising time than ever for glioma research and clinical implementation and there is a sense that effective novel treatments are on the horizon.

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