We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Immunotherapy against Gliomas

Mathew Sebastian, Bayli DiVita Dean and Catherine T. Flores

Abstract

Immunotherapy has been demonstrably effective against various cancers, particularly those in the hematopoietic system and those with a high tumor-specific antigenic burden. Unfortunately, the development of immunotherapeutic strategies has proven more challenging against central nervous system (CNS) malignancies due to several unique characteristics of brain tumors that pose extraordinary barriers. To date, there is a lack of phase III trials demonstrating improved progression-free survival (PFS) and/or overall survival (OS) using immunotherapies in brain cancers. However, a better mechanistic understanding of current resistance to immunotherapies along with data from novel innovative techniques to overcome these barriers has been encouraging. This chapter gives an overview of current immunotherapies in the development of brain cancers. We will evaluate the present studies available in the clinical setting and any of their potential findings. The chapter will also discuss pertinent preclinical strategies whose translation for human use would potentially prove efficacious or provide invaluable scientific discovery.

Keywords: immunotherapy, brain cancer, immune system, malignancy

1. Introduction

Primary malignant brain tumors remain one of the most lethal and clinically challenging of all cancers. Despite comprising only an estimated 1.3% of all new cancer cases, brain tumors represent one of the highest causes of cancer mortality with 18,600 (3.1%) deaths predicted in 2021 [1]. Glioblastoma (GBM) is one of the most common and aggressive of the primary malignant adult brain tumors with a median survival of less than 21 months despite standard of care which includes surgical resection, targeted radiation therapy, high-dose chemotherapy, and tumor-treating fields [2–8].

Cancer immunotherapies have emerged as new therapeutic mainstays in a variety of cancers [9–14]. However, the unique characteristics of brain tumors pose extraordinary barriers that, thus far, have foiled efforts and the success of immunotherapeutic approaches. These characteristics include high tumor heterogeneity and relatively few coding mutations [15, 16], an immunosuppressive microenvironment [17–23], a relative lack of immune effector cell types [19, 24], and relative isolation from systemic circulation because of the blood-brain barrier [25–29]. This chapter will discuss some of the current immunotherapy types with emphasis on the prominent clinical trials for each and the limitations observed.

However, despite a lack of phase III trials demonstrating improved progression-free survival (PFS) and/or overall survival (OS) in many of these immunotherapies, incremental progress continues to be made in brain malignancies in both the clinical and preclinical settings. Novel immunotherapeutic strategies and combinations are

currently being tested in the preclinical setting. This chapter will also discuss novel preclinical strategies to enhance immunotherapies, including modified chimeric antigen receptor (CAR) T cells, small molecular inhibitors that target immunologic pathways, and combinatorial checkpoint approaches.

2. Current immunotherapies

2.1 Cancer vaccines

Cancer vaccines involve exogenous administration of tumor antigens that can stimulate an adaptive immune system against tumor cells. The basic requirements for cancer vaccines include the delivery of tumor-specific antigens to antigen-presenting cells (APCs) such as dendritic cells (DCs), DC activation, activation of both T cell subsets and infiltration into the tumor microenvironment to exert durable responses [30]. Vaccine strategies have been employed against primary brain tumor targets using a variety of antigen substrates, including peptides, full-length proteins, RNA, and DNA in various formulations including antigens alone, antigens in combination with various local or systemic adjuvants, or dendritic cell vaccines. Though vaccination strategies have demonstrated a survival benefit in early phase clinical trials, there have yet to be any phase III clinical trials in patients with GBM demonstrating survival benefit. However, vaccination strategies continue to hold great promise with the rationale and hope that they would stimulate effective tumor-specific immunity, target tumor cells but not normal brain, and provide immunological memory against tumor recurrence [31].

2.1.1 Single peptide vaccines

Multiple single peptide vaccines have been generated to target a variety of tumor antigens including mutated isocitrate dehydrogenase 1 (IDH-R132H), survivin, Wilms Tumor 1 (WT1), and epidermal growth factor receptor variant III (EGFRvIII). Peptide vaccinations are highly specific and provide the benefit of reduced off-target effects, preventing autoimmune toxicities.

Mutated IDH1 defines a molecular subtype of diffuse glioma. A phase I trial of an IDH1(R132H)-specific peptide vaccine was conducted in 33 patients with newly diagnosed WHO grade 3 and 4 astrocytomas [32]. This study met its primary safety endpoint and demonstrated a three-year progression-free rate of 63% and a three-year death-free rate of 84% [33]. This study assessed intratumoral inflammatory reactions associated with the use of vaccines by the presence of pseudoprogression. Intriguingly, this study found high frequencies of pseudoprogression, 37.5% in the treatment group compared to 16.7% in a molecularly matched control cohort, indicating intratumoral inflammatory reactions. In one patient with pseudoprogression, the analysis found that a cluster of T cells was dominated by a single IDH1(R132H)-reactive T cell receptor.

Survivin is an anti-apoptotic protein expressed in malignant gliomas. One early phase study assessed the survivin peptide vaccine in nine patients with survivin-positive malignant gliomas and found it to be safe and tolerable [34]. The treatment group had a median PFS of 17.6 weeks and a median OS of 86.6 weeks compared to an analysis of phase II chemotherapy trials of patients with recurrent glioma with a PFS of 10 weeks and OS of 30 weeks [35]. A phase II trial was initiated with the survivin peptide vaccine in 63 participants with newly diagnosed glioblastoma [36]. In 2020, a trial update found 96.8% of patients did not experience disease

progression within 6 months with a 93.5% survival rate a year after diagnosis [37]. This is an ongoing study.

Wilms Tumor 1 (WT1) is a pleiotropic transcription factor with functional roles in GBM that range from driving cellular proliferation [38] to inhibiting apoptosis [38, 39]. An uncontrolled nonrandomized phase II trial of WT1 peptide vaccination for patients with recurrent WT1-positive GBM was conducted with 21 patients. This study demonstrated that the vaccination was safe and produced a clinical response with a median PFS period of 20.0 weeks, median overall survival after initial vaccination of 36.7 weeks, and a 6 month PFS of 33.3% [40]. The median PFS and median OS found in this study were said to be comparable to various combination regimens of chemotherapy and/or radiotherapy.

Epidermal growth factor receptor (EGFR) amplification is enriched in the classical subset of GBM and is seen in 57.4% of primary GBM patients [41, 42]. Epidermal growth factor receptor variant III (EGFRvIII) regulates EGFR activity by inducing the expression of EGFR ligands [43]. A phase II trial assessed the immunogenicity of an EGFRvIII-targeted peptide vaccine [44]. The 6-month PFS after vaccination was 67% (versus 59% in the historical cohort) with a median overall survival of 26.0 months (versus 15.0 months in the matched control group) [45]. However, no benefit was observed in a randomized phase III trial [46]. Further analysis found significant loss of EGFRvIII expression in a subset of patients with tumor tissue available at recurrence in both those that received the vaccine and in those receiving standard-of-care chemoradiation [47].

To date, single peptide vaccines have yet to lead to clinical benefit in phase III trials in brain cancers. The EGFRvIII work hints that the selection of a single molecular target as a peptide vaccine might be inadequate to overcome the considerable challenges of tumor antigen down-regulation and tumor heterogeneity. Thus, targeting multiple targets could lead to robust durable responses. Thus, studies investigating multi-peptide vaccines, with several tumor antigen targets, have now been initiated.

2.1.2 Multi-peptide vaccines

To identify multiple tumor-associated peptides for immunotherapy, a study set out to assess the potential of using HLA-associated tumor peptidomes as a source of tumor-associated antigens to be used in immunotherapy [48]. The components found gave rise to the multi-peptide vaccine IMA950. A phase I/II set out to assess IMA950 and its 11 tumor-associated peptides which include brevican (BCAN); chondroitin sulfate proteoglycan 4 (CSPG4); fatty acid-binding protein 7, brain (FABP7); insulin-like growth factor 2 messenger mRNA-binding protein 3 (IGF2BP3), neuroligin 4, X-linked (NLGN4X); neuronal cell adhesion molecule (NRCAM), protein tyrosine phosphatase, receptor-type, z polypeptide 1 (PTPRZ1); tenascin C (TNC); Met proto-oncogene (MET); baculoviral IAP repeat-containing 5 (BIRC5); and hepatitis B virus core antigen [48]. In this study, IMA950 was adjuvanted with poly-ICLC (polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose) [49]. The multi-peptide vaccine was used in 19 patients, 16 with GBM and 3 with grade III astrocytoma. Results showed a median overall survival of 19 and 17 months for the whole cohort and GBM patients-only, respectively, with a PFS of 68% at 6 months for the whole cohort and 69% for GBM patients only when calculated from the study entry [50]. There was no mention of a historical control group used as a comparator in this study. Due to the findings in this study, a follow-up trial is actively recruiting patients with recurrent GBM to test IMA950/poly-ICLC alone or in combination with pembrolizumab, a checkpoint inhibitor that will be discussed later [51].

Another multi-peptide vaccine was generated based on observations of three tumor-associated antigens that were observed to be highly expressed in pediatric gliomas. This vaccine targets the peptide epitopes of EPH receptor A2 (EphA2; a tyrosine kinase), interleukin-13 receptor alpha 2 (IL-13R α 2), and survivin. This study was conducted in 26 pediatric patients with diffuse brainstem gliomas (BSG) or high-grade gliomas (HGG) [52]. Results showed a median survival of 13.3 months from diagnosis in the overall cohort with a median survival of 12.7 months in the BSG group and a median survival of 25.1 months in the HGG group. Though no historical control group was discussed in this phase I study, the authors mentioned that for children with BSGs, current therapies at the time failed to increase median overall survival beyond 9–12 months [53].

Though these studies are showing promising results, the lack of clear indication of efficacy and eventual tumor progression in these phase I-III trials may be attributed to the multiple obstacles in place by brain cancers including the high degree of heterogeneity of antigenic expression, an outgrowth of subclones not expressing the antigens, lack of major histocompatibility complex molecules and/or an immunosuppressive tumor microenvironment.

2.1.3 Dendritic cell (DC) vaccines

The aforementioned peptide cancer vaccines require uptake and activation of endogenous antigen-presenting cells (APCs) such as DCs. These DCs then present antigens to tumor-specific T cells leading to T cell activation. To circumvent the reliance of endogenous DC antigen loading and activation, some studies utilize DC vaccines and load DCs *ex vivo* with a variety of tumor antigens including autologous tumor lysates, tumor-associated peptides, and tumor-associated viral antigens. DC vaccines have a variety of advantageous characteristics making them an ideal choice for antitumor vaccines. They are considered to be the professional APC and most effective in sensitizing naïve T cells to specific antigens. They also are able to cross-prime, allowing them to present exogenous antigens for presentation on major histocompatibility complex (MHC) class I molecules, activating cytotoxic T lymphocytes.

A phase I trial of the DC vaccine DCVax-L was completed which loads autologous DCs with tumor lysate from newly diagnosed or recurrent GBM participants [54]. In this trial that enrolled 23 patients, the 1-year survival rate was 91% with a median OS of 31.4 months from the time of initial surgical diagnosis. The authors compared this median OS to the median OS of 18.6 months found in a large study of GBM patients who underwent tumor resection and chemoradiotherapy [55]. However, the study noted that it was unclear whether the extended survival of participants is a direct result of the vaccine effects or good responses to follow-up therapies after failing the vaccine [56]. DCVax-L has since gone on to a large phase III clinical trial with 331 participants with the primary endpoint of PFS and the secondary endpoint of OS [57]. Preliminary results of the study reveal a median OS of 23.1 months from surgery in the overall intention-to-treat population (ITT) and 34.7 months from surgery in patients with a methylated O⁶-methylguanine-DNA-methyltransferase (MGMT) gene promoter. The authors compared the median OS in the ITT population to a median OS of 15–17 months from surgical intervention typically achieved with a standard of care in past studies. The PFS was not evaluated in this interim analysis. In this blinded interim survival analysis, the authors found that patients were living longer than expected and that this warrants further follow-up and analyses [58].

ICT-107 is another DC vaccine loaded with synthetic tumor-associated peptides of antigens commonly overexpressed in CD133-positive cancer stem cells that

includes Erbb2 (HER2), second tyrosinase-related protein (TRP-2), glycoprotein 100 (gp100), melanoma-associated antigen 1 (MAGE-1), IL-13R α 2, and absent in melanoma 2 (AIM-2). In a phase I trial of 21 participants who were HLA-A1 or HLA-A2-positive and with newly diagnosed GBM (n = 17), recurrent GBM (n = 3), or with a brain stem glioma (n = 1), the median PFS was 16.9 months with a median OS of 38.4 months. These results suggest a correlation with prolonged OS and PFS though no comparator group or historic controls were mentioned [59]. The same group then conducted a phase II randomized, double-blind, placebo-controlled study using ICT-107 in 124 participants with newly diagnosed GBM following resection and radiotherapy with concomitant temozolomide [60]. The primary endpoint of median OS was not increased but a significant increase in the PFS by 2.2 months was observed in the intent-to-treat population treated with ICT-07 (11.2 months versus 9 months) [61]. A phase III trial was halted due to insufficient financial resources [62].

Another pair of studies made use of the immunodominant cytomegalovirus (CMV) antigen phosphoprotein 65 (pp65) in their DC vaccines. This antigen is expressed in GBM but not in normal brains [63]. The first was a randomized blinded phase I clinical trial in 12 patients with newly diagnosed GBM who received pre-conditioning in the form of tetanus/diphtheria toxoid (a potent recall antigen) or unpulsed mature DCs before bilateral vaccinations with DCs pulsed with CMV pp65 RNA [64]. Td pre-conditioning led to a significant increase in both median PFS and median OS compared to the DC alone cohort which had a median PFS and OS of 10.8 and 18.5 months (consistent with patients treated with standard of care) [65]. A later study from the same group evaluated DCs pulsed with CMV pp65 RNA along with dose-intensified temozolomide (TMZ) and adjuvant GM-CSF [64]. Here they observed a median PFS of 25.3 months and a median OS of 41.1 months in the treatment group compared to 8.0 months and 19.2 months in historical controls, respectively [66]. A phase II randomized, blinded, placebo-controlled trial of DCs pulsed with CMV pp65 and Td is underway with a target of 120 patients [67]. Another phase II trial utilizing DCs pulsed with CMV pp65 was recently completed with results pending which is assessing whether basiliximab, a monoclonal anti-CD25 antibody, may inhibit the functional and quantitative recovery of T-regulatory cells after TMZ-induced lymphopenia in newly diagnosed GBM [68].

The potential for DC vaccines is vast in their ability to generate antitumor immunity however, to date, they have provided suboptimal and overall unsatisfactory clinical benefits in large trials. Work now includes methods to improve *in vitro* APC generation [69, 70], improve DC vaccine activity with additional treatments [65], and increase inflammation at the vaccine site [56, 66, 71]. It is now thought that the next major advances in DC vaccines will come from their combination with other immunotherapies such as checkpoint inhibitors [20].

2.2 Immune checkpoint inhibitors

The principal breakthrough in cancer treatment over the last 15 years is the introduction of immune checkpoint inhibitors (ICIs) blocking the immune checkpoints programmed death 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic lymphocyte antigen 4 (CTLA-4). Immune checkpoints are negative regulators of T-cell immune function and are central for the modulation of physiological immune responses and the maintenance of self-tolerance. T cells are created in the thymus where they undergo positive and negative selection and undergo apoptosis if they fail to recognize self-MHC or bind too strongly to MHC with self-peptides. This process is called central tolerance [72]. T cells that appropriately respond to MHC molecules are then sent into the circulation where they eventually interact

with APCs displaying mutated self-proteins (in cancers) or foreign antigens (in infection) [73]. However, central tolerance is sometimes incomplete and some T cells escape and become autoreactive. To prevent autoreactivity, there are multiple inhibitory checkpoint pathways that regulate the activation of T cells at multiple levels during an immune process called peripheral tolerance [74].

Central to cancer immunotherapy is that tumor cells can take advantage of peripheral tolerance and hijack these checkpoint mechanisms, inhibiting T cells from attacking. The arrival of checkpoint inhibitors in 2011 introduced a new mechanism to treat cancer and revolutionized cancer management in a variety of solid tumors [75–78]. There are now several FDA-approved monoclonal antibodies against solid tumors including ipilimumab targeting CTLA-4, pembrolizumab, and nivolumab targeting PD-1, and atezolizumab and durvalumab targeting PD-L1. However, despite numerous articles describing preclinical efficacy of checkpoints in central nervous system (CNS) tumors, activity against brain metastases from melanoma and non-small-cell lung cancer [79, 80], and multiple studies describing increased PD-L1 expression in GBM [81, 82], no FDA approval has occurred for immune checkpoints in GBM. Here, we will discuss some of the phase III trials that have occurred with immune checkpoint inhibitors, what has been learned, and where the research is going.

2.2.1 Phase III trials

One randomized phase III study assessed the effect of nivolumab versus bevacizumab (anti-vascular endothelial growth factor A) in 439 patients with recurrent glioblastoma [83]. The study found no statistical difference between the median OS of nivolumab monotherapy (9.8 months) and bevacizumab (10.0 months) [84]. Interestingly, this study observed that corticosteroid use at baseline seemed to be associated with worse outcomes in the nivolumab group. This may be due to the direct effects of corticosteroids on T cell function which may abrogate activation or priming of the immune system.

Additionally, a phase III study compared nivolumab versus temozolomide in newly diagnosed patients with unmethylated MGMT GBM [85]. In 2019, Bristol-Myers Squibb announced that the study did not meet its primary endpoint, which assessed overall survival [86].

Another randomized phase III single-blind study set out to compare TMZ plus radiation therapy combined with nivolumab or placebo in newly diagnosed patients with MGMT-methylated glioblastoma [87]. In 2019, Bristol-Myers Squibb provided an update that the nivolumab group did not meet one of its primary endpoints, progression-free survival, but that the data monitoring committee recommended continuing the trial to allow the other primary endpoint, overall survival, to mature [88]. The final results are pending.

It remains to be seen whether the lack of demonstrated efficacy of checkpoint therapeutic efficacy is due to difficulty getting to the tumor site or the tumor itself. Though it has been shown that T cells can traffick to the CNS, the relatively immune-privileged CNS may prove to be a limitation if checkpoint inhibition must enter into these tumors to be effective [20]. However, at least one study demonstrated clinically meaningful intracranial efficacy with ipilimumab combined with nivolumab in patients with melanoma with untreated brain metastasis, suggesting that immune checkpoint strategies can target tumors located intracranially [80]. Lack of effective checkpoint strategies in primary CNS tumors could be due to a variety of challenges that interplay with one another. First, glioblastomas generally are considered cold tumors, lacking intratumoral inflammatory cells though this is also considered to be heterogenous. Lack of efficacy could also be due to

the relatively low mutational burden since it has been consistently shown that malignancies with a high burden of clonal neoantigens have a higher response rate to checkpoint inhibition [89]. Also, the high degree of heterogeneity found within gliomas, makes specific immunological targeting difficult. Lastly, the observed systemic T cell dysfunction and sequestration imposed by an intracranial tumor remain another domineering challenge as this singly does away with the requirement of a viable T cell compartment for immune checkpoints to act on [90].

Though multiple challenges must be overcome for immune checkpoint inhibitors to overcome glioblastoma specifically, a better understanding of treatment resistance in addition to many promising synergistic combinatorial approaches will provide important incremental advances to efficacy. Finally, as seen in other solid tumors, resistance to immune checkpoint blockade leads to upregulation of a host of alternative inhibitory immune checkpoint molecules that are currently also being targeted in ongoing clinical trials. These new inhibitory immune checkpoint targets potentially offer increased therapeutic targets to be used as single agents or in combination with other immunotherapies [91].

2.3 Adoptive cellular therapy (ACT) immunotherapy

Immunotherapy can be considered active or passive. The difference between each centers on how they modulate the immune system. Active immunotherapy, such as the aforementioned vaccines, relies on the process of endogenous immune cells activation, producing a durable response and generation of immunological memory. Passive immunotherapy, however, produces an immediate response due to the administration of cytokines, antibodies, or immune cells. A form of passive immunotherapy is adoptive cellular therapy (ACT) which specifically allows for the *ex vivo* generation and expansion of autologous immune cells that can then be given back to patients. This section will first discuss the non-specific adoptive cellular therapies such as lymphokine-activated killer (LAK) cells and natural killer (NK) cells followed by adoptive T cell therapies.

2.3.1 Lymphokine-activated killer (LAK) cells

LAK cells were thought to be a promising candidate for adoptive cellular therapies due to their ease of generation (culturing peripheral blood lymphocytes in the presence of IL-2), rapid expansion, the long shelf life *in vitro*, and tumor lysing capabilities [92]. These characteristics and favorable results in other cancers led to a phase I/II clinical trial in adult patients with recurrent or progressive supratentorial malignant glioma who were candidates for reoperative surgery. In this study, 19 eligible patients underwent craniotomy with debulking and placement of LAK cells and IL-2 in a reservoir inserted in the tumor resection cavity. Compared to an institutional historical control group of GBM after reoperation with a median OS of 28 weeks, LAK-treated patients had a median OS of 53 weeks. After treatment, the 1-year survival was 53% compared to less than 6% in a control contemporary chemotherapy group after reoperation suggesting improved long-term survival [93].

Another phase I/II trial was initiated in 40 patients with GBM who had autologous LAK cells placed in the tumor cavity. Findings from this study showed a median survival from the original diagnosis of 17.5 months compared to 13.6 months in a contemporary age-matched group [94]. The same group conducted a phase II trial with LAK cell treatment in 33 GBM patients who had not experienced clinical or radiographic evidence of progressive disease during or shortly after completion of initial therapy which showed a median survival from diagnosis of 20.5 months with a 1-year survival of 75%. The authors stated that 20.5 months

median survival is 88% longer than the 12-month survival associated with GBM and 33% longer than the 15-month median survival observed in the clinical trials that established the benefit of temozolomide therapy [95].

Overall, the use of LAK has since fallen out of favor [20, 96]. In phase III randomized trial of IL-2 with or without LAK in the treatment of patients with advanced renal cell carcinoma (RCC), the addition of LAK did not improve the response rate against RCC [97]. It is thought the efficacy of LAK cell ACT is due to the amplification of a subset of therapeutic cells found in the peripheral blood that are reactive against tumors [96]. Thus, the use of tumor-infiltrating lymphocytes (TILs; discussed later), which are more specific to the target tumor, might have better potential.

2.3.2 Natural killer (NK) cells

The NK cell ACT field is rapidly expanding in both biological understanding of NK cells, including their distinct immune checkpoints [98, 99] in addition to clinical development of NK cell ACT. These cytotoxic cells are part of the innate immune system and have many advantageous characteristics which include rapid *ex vivo* activation and expansion without the use of autologous tumor cells and are not MHC restricted [100]. It is recognized that NK cells target other cells types based on a lack of MHC-I expression [101]. Glioblastoma is known to employ immune evasion tactics including downregulation of MHC-I [102–104] which may make it amenable to ACTs using NK cells.

An early preliminary trial was conducted in nine patients with recurrent malignant gliomas using autologous NK cells injected into the tumor cavity, using a reservoir system, and intravenously. This study found that NK cell therapy was safe with some clinical benefit demonstrating three patients with partial response (50% decrease in tumor volume), two with a minor response (25% decrease in tumor volume), seven with progressive disease (increase of 25% in tumor volume), and four with no change [100].

Currently, there is at least three phase I trials in the process utilizing NK cells in high-grade gliomas [105–107].

2.3.3 Tumor-infiltrating lymphocytes (TILs)

As mentioned before, ACT allows for *ex vivo* generation and expansion. During expansion, several modifications and enhancements can occur to confer advantageous characteristics in antitumor activity. T cells can be positively selected based on specificity to tumor antigens and increased effector function. Or, they can also be transduced to express specific tumor-associated T cell receptors (TCRs) that, though MHC-restricted and MHC-dependent, can target intracellular antigens. Alternatively, T cells can be modified to express chimeric antigen receptors (CARs) for specific tumor cell surface proteins.

As the name implies, tumor-infiltrating lymphocytes are thought to have undergone *in vivo* recognition of their cognate antigen and migration into the tumor. Thus, the administration of autologous TILs have produced durable objective responses in patients with advanced melanoma [108]. However, TILs are less feasible in GBM owing to the difficulty in isolating and expanding them [109] and T cell exhaustion while within the tumor microenvironment [110]. A more feasible approach is the aforementioned targeting of the ubiquitously expressed human CMV antigen pp65 in GBM tissue [111]. This approach was conducted in an early phase clinical trial and was able to successfully expand CMV-specific T cells from 13 out of 19 patients of which 11 received all four T-cell infusions and found that

the median overall survival of these patients since the first recurrence was 403 days. The overall median OS in this study was >57 weeks (a range of 19–345 weeks) and a median PFS of >35 weeks (a range between 15.4–254 weeks). No comparator group or historic controls were mentioned in this early phase trial. Interestingly, molecular profiling of CMV-specific T cells from the patients revealed distinct gene expression signatures which correlated with their clinical response [111]. Another phase I randomized study was initiated in 22 CMV-seropositive, newly diagnosed GBM patients. This study assessed CMV pp65-specific T cells that were generated *ex vivo* with autologous CMV pp65 RNA-transfected DCs with or without a CMV-DC vaccination [112]. Though this study was not powered to detect differences between cohorts with regard to PFS and OS, the study found an association between higher IFN γ ⁺, TNF α ⁺, and CCL3⁺ polyfunctional, CMV-specific CD8⁺ T cells and OS [113].

2.3.4 Chimeric antigen receptors T cells (CAR T cells)

A major recent advancement in adoptive cellular therapies has been the development of chimeric antigen receptors as a means for T cells to bypass MHC restriction, and dependence and have specificity for a cell surface antigen. CAR T cell therapy recently received approval targeting CD19 in B cell leukemia and lymphoma [114]. CAR T cells are genetically modified to express an extracellular single-chain variable fragment that specifically recognizes a tumor cell's surface antigen. The extracellular binding fragment is bound to intracellular signaling domains and/or co-stimulatory domains that allow for T cell activation when the fragment is bound to its cognate antigen. CAR T cells have the advantage of recognizing target antigens independent of HLA and also disregarding tumor cell immunoevasion by MHC expression reduction.

A phase I safety study was conducted using autologous CAR T cells targeting EGFRvIII in 10 recurrent EGFRvIII⁺ GBM patients [115]. The median OS was 251 days (~8 months; PFS could not be calculated due to the confounding factor of neurosurgical intervention in most of the patients). No specific historical controls were mentioned though the authors stated that GBM patients with significant residual disease after surgery have an average survival that is around ~6 months. The group demonstrated that EGFRvIII specific CAR T cells were found in the brain tumor and exerted antigen-directed activity. They also found that most of the patients had decreased expression of EGFRvIII in tumors resected after CAR T therapy [116].

Another member of the family of EGFR-related receptor tyrosine kinase is HER2. HER2 is commonly overexpressed in high-grade gliomas [117–120]. A phase I dose-escalation study was initiated to assess the safety and antitumor efficacy of autologous HER2-specific CAR T cells in 17 patients with progressive recurrent GBM [121]. This study found that though HER2-specific CAR T cells did not expand, they were detected in peripheral blood for up to 12 months. They found that eight patients had clinical benefit from either partial response or stable disease. The median OS was 11.1 months from the first CAR T cell infusion and 24.5 months from diagnosis with an 18-month OS of 29.4% [122]. As a comparator, this study mentions achieving similar outcomes as another study that used bevacizumab and lomustine where the median OS was 12 months with an 18-month OS of 20% [123].

Similar to the aforementioned peptide and DC vaccines, there are CAR T approaches targeting IL-13R α 2 due to its expression in a majority of adult and pediatric GBM tumors but not in normal brains [124, 125]. One group demonstrated that administration of IL-13R α 2-specific CAR T cells was feasible and showed evidence for transient anti-glioma responses in two out of three patients with recurrent GBM [126, 127]. The same group has initiated an ongoing phase I

study utilizing IL-13R α 2-specific CAR T cell administration into the resected tumor cavity and the ventricular system in patients with recurrent or refractory malignant glioma [128]. A case report derived from this phase I study observed regression of all CNS tumors along with concomitant increases in cytokines and immune cells in the cerebrospinal fluid. Subsequent relapse was later found to be due to IL-13R α 2-negative tumors [129].

These studies demonstrate the barriers found in targeting single antigens in a highly heterogeneous tumor. Newer approaches for enhanced CAR T therapy efficacy will require targeting multiple antigens, a combinatorial approach with other immunotherapies, or the development of CAR T cell designs that induce significant epitope spreading [20]. Aside from antigen target constructs, current work in CAR T therapy looks toward maximizing and maintaining the activity of the administered CAR T cells to overcome barriers in the solid tumor microenvironment [130]. As mentioned with cancer vaccines, the benefit will likely occur with the combination of CAR T therapy and immune checkpoint blockade. Another strategy is to express chemokine receptors in CAR T cells to improve their tumor-directed trafficking (discussed below) or, conversely, express blocking chemokines and receptors expressed by tumor cells to inhibit recruitment of inhibitory immune cells. Another strategy is disrupting the tumor vasculature with anti-VEGFR CAR T therapy. Strategies are also looking into the combination of depleting immune-inhibitory cells to then allow for CAR T therapies to maintain durable responses. Though CAR T therapy remains a promising therapy for GBM, further work is needed to lead to clinical benefit.

3. Novel preclinical strategies

3.1 Targeting glioma stem cells (GSCs)

Glioma stem cells (GSCs) are a subpopulation of glioma cells with stem-like properties. These cells are thought to promote tumor initiation, chemo- and radio-resistance, and tumor invasiveness. GSCs were first defined by their expression of prominin 1 or CD133, however, it was later discovered that CD133-negative cells were also capable of causing tumor initiation. In addition, several different models of GSC initiation have been proposed.

Vora *et al.* generated three different therapeutic modalities to target CD133⁺ GSCs and tested their efficacy using human GBM models. The first modality, a CD133-binding IgG, was found to be ineffective at causing a significant reduction in proliferation *in vitro* and *in vivo* tumor burden. The second modality, a dual-antigen T cell engager or DATE, specific for CD133 and CD3, caused significant tumor-killing both *in vitro* and *in vivo*. Finally, the CD133-specific CAR T cells provided profound T cell proliferation and secretion of the anti-tumor cytokines IFN γ and TNF α upon co-culture with various human GBM cells. In addition, when mice were intracranially injected with human GBM cells followed by subsequent intracranial injection of CD133-specific CAR T cells, a significant reduction in tumor burden and prolonged survival was observed relative to control-treated mice. Importantly, they found administration of CD133-specific CAR T cells did not significantly impair hematopoiesis [131].

An additional novel method of targeting GSCs is through the use of NK cells. These cells are cytotoxic lymphocytes capable of killing target tumor cells. GSCs have been shown to express activating ligands of NK cells, such as CD155 and B7-H6. In addition, NK cells were shown to be able to lyse GSCs *in vitro* upon co-culture with target GSCs. Contrarily, GSCs were found to promote NK cell

dysfunction that was determined to be contact-dependent. Mechanistically, the NK cell dysfunction was found to be mediated via TGF β -1 released by GSCs and upon treatment with a TGF β inhibitor, the dysfunction could be significantly diminished. When evaluated in an *in vivo* model of human GSC, the combination of allogeneic NK cells and a TGF β inhibitor provided superior survival relative to any control groups. These results suggest combinatorial NK cell therapy and TGF β inhibitor may provide promising anti-tumor responses [132].

3.2 Modified CAR T cells

CAR T cells combine the single-chain variable fragment (scFV) of monoclonal antibodies with the internal component of the T cell receptor. There are three main generations of CAR T cells—first-generation CAR T cells include an scFV as well as CD3 ζ endodomain. The second generation built upon this by adding a costimulatory molecule such as CD28 or 4-1-BB to promote expansion. Finally, third-generation CAR T cells consist of an scFV, CD3 ζ , as well as two or more costimulatory molecules. CAR T cells, especially third-generation CAR T cells, have had great success in patients with B cell malignancies [133].

However, single-agent CAR T cells have had limited success in patients with CNS malignancies. This is likely due to several factors, including a high degree of heterogeneity in the tumor microenvironment (TME), loss of antigen during tumor progression, exhaustion of the CARs within the TME, and finally upregulation of immunosuppressive molecules that inhibit CAR T cell killing [134].

Bielamowicz *et al.* utilized human GBM cells to identify three antigens expressed on human glioma cells: HER2, IL-13R α 2, and EphA2. Single-agent CAR T cells, bispecific CAR T cells targeting IL-13R α 2 and EphA2, as well as trivalent CAR T cells specific for all three antigens were developed and tumor-killing was first assessed *in vitro*. Upon coculture with target human glioma cells, secretion of IL-2 and IFN γ were significantly higher upon treatment with trivalent CAR T cells relative to nontransduced T cell controls. In addition, the specific lysis of target cells was significantly greater when co-cultured with trivalent CAR T cells relative to controls. Efficacy was also evaluated using intracranially injected human glioma cells followed by intracranial injection of single CAR T (targeting IL-13R α 2), bivalent CAR T (targeting EphA2 and IL-13R α 2), trivalent CAR T cells (targeting HER2, IL-13R α 2, and EphA2), or nontransduced T cell controls. The authors found the trivalent CAR T cells provided superior anti-tumor efficacy relative to controls [135].

Several other modified CARs have shown increased efficacy relative to their first-generation counterparts. Krenciute *et al.* modified IL-13R α 2-specific CAR T cells to secrete IL-15, a cytokine that promotes activation, proliferation, and cancer cell lysis. Relative to IL-13R α 2 CAR T cells that did not secrete IL-15 (first-generation), these second-generation CAR T cells showed increased lysis of target tumor cells and increased proliferation *in vitro*. In addition, when evaluated *in vivo*, mice treated with the second-generation CAR T cells had significantly increased PFS and OS relative to those treated with the first-generation CAR T cells. The authors found mice that succumbed to the tumor after treatment CAR T cell therapy downregulated the expression of IL-13R α 2 [136].

In the context of neuroblastoma, disialoganglioside (GD2) represents a promising tumor-associated target for CAR T cell therapy. GD2 has been shown to promote malignant phenotypes such as proliferation, migration, and invasion [137]. In a phase I clinical trial, GD2-specific CAR T cells were evaluated in neuroblastoma patients in combination with cyclophosphamide and fludarabine as well as the checkpoint inhibitor, anti-PD-1 [138]. Although the therapy was found to be safe,

only modest anti-tumor responses were observed [139]. To improve upon the efficacy of these CAR T cells, Moghimi *et al.* modified GD2-specific CAR T cells to express B7H3 and found enhanced anti-tumor responses both *in vitro* and *in vivo* relative to untreated controls. They go on to determine the enhanced efficacy is likely due to improved metabolic function [140].

Another promising CAR T cell target for brain tumors is CD70. In terms of normal immunology, CD70 is a co-stimulatory molecule expressed in activated immune cells. However, Jin *et al.* found CD70 to be overexpressed in tumor samples isolated from IDH wild-type low-grade glioma and GBM patients [141]. Using a model of high-grade glioma, modified CD70 CAR T cells that express CXCR1 or CXCR2 improved T cell migration to the tumor site. In addition, survival of tumor-bearing mice was improved when treated with CXCR1 or CXCR2 modified CD70 CAR T cells relative to unmodified CD70 CAR T cells [142]. Collectively, these results suggest modified CAR T cells may hold promising anti-tumor responses relative to their first-generation counterparts.

A huge limitation of CAR T cells is the eventual expression of exhaustion molecules, leading to a lack of anti-tumor efficacy. Weber *et al.* recently utilized transient periods of rest using a small molecule as well as dasatinib, a tyrosine kinase inhibitor that inhibits T cell signaling. The authors utilized GD2.CD28 ζ CAR T cells in a model of human osteosarcoma. The use of rest in pre-exhausted CAR T cells redirected their fate from a state of exhaustion toward a memory-like state. Furthermore, in T cells that already acquired markers of exhaustion, the use of rest reversed the exhaustion phenotype and caused epigenetic remodeling similar to non-exhausted controls. CAR T cells subjected to intermittent rest through alternating CAR expression or dasatinib-treatment demonstrated superior anti-tumor effects. These findings have profound translational implications to improve therapeutic response using CAR T cells [143].

3.3 Small molecule inhibitor

Myeloid-derived suppressor cells (MDSCs) have been shown to be expanded in the periphery of GBM patients [144]. MDSCs within the TME have been shown to contribute to tumor immunosuppression via the secretion of immunosuppressive molecules such as arginase 1 and inducible nitric oxide synthase (iNOS). Alban *et al.* expanded upon these findings and found the monocytic subset of MDSCs (mMDSCs) express high levels of CD74 and its ligand, macrophage migration inhibitor factor (MIF). They used MN-166, a small molecule inhibitor of phosphodiesterase capable of penetrating the blood-brain barrier to inhibit the CD74/MIF interaction on myeloid cells and therefore prevent mMDSC formation. They found MN-166-treated MDSCs prevented MDSC-mediated T cell suppression. In addition, increased intratumoral CD8⁺ T cells were found when tumor-bearing mice were treated with MN-166. Despite no difference in survival being observed, the authors suggest this therapy could combine well with activating immunotherapies [145].

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor shown to be upregulated in GBM and is correlated with decreased survival. Wightman *et al.* have shown treatment of GBM cells with IL-6 increased phosphorylation and overall STAT3 expression. The authors used bazedoxifene, a selective estrogen receptor modulator, to inhibit IL-6-mediated STAT3 activation. Importantly, they show treatment of GBM cells with bazedoxifene decreases markers of GSCs, such as SRY-box transcription factor 2 (SOX2) and octamer-binding transcription factor 4 (OCT4). In addition, they demonstrate treatment of tumor-bearing mice with bazedoxifene significantly prolongs survival relative to vehicle-control treated mice [146].

3.4 Gene therapy

Alghamri *et al.* recently published a thorough investigation of mutant versus wild-type IDH (wtIDH) gliomas in both murine and human models. Focusing on the murine data, the authors found wild-type IDH gliomas possessed more suppressive CD11b⁺Ly6G⁺ granulocytic MDSCs (gMDSCs) as well as increased PD-L1, iNOS, and Arg1 relative to gMDSCs derived from mutant IDH (mIDH) glioma bearing mice. Furthermore, murine mIDH glioma neurospheres were found to secrete significantly more G-CSF relative to their wtIDH counterparts. This increased secretion was determined to be caused by enrichment of H3K4me3 in the *Csf3* gene, which encodes G-CSF. Finally, when the immune-stimulatory gene therapy herpes simplex virus 1—thymidine kinase/Feline McDonough sarcoma (Fms)—like tyrosine kinase 3 ligand (TK/Flt3L) was used in combination with recombinant G-CSF (rG-CSF) in a wtIDH mouse model, a significant survival benefit was observed relative to TK/Flt3L or rG-CSF alone [147].

3.5 Combination checkpoint inhibition

Tumor-treating fields (TTFs) work as a non-invasive anti-cancer therapy via alternating electric fields. As stated earlier, TTFs are already FDA-approved for GBM in combination with temozolomide. Voloshin *et al.* expanded upon these findings and found TTFs elicited tumor cell death in murine models of lung and colon cancer. In addition, the authors found TTFs could induce maturation of bone marrow-derived DCs. Furthermore, using an orthotopic model of murine lung cancer, the combination of TTFs and the ICI, anti-PD-1, was found to reduce growth relative to control-treated mice. This anti-tumor effect was found to be mediated by the expansion of macrophages, DCs, and CD8⁺ T cells within the TME. In addition, when subcutaneous colon cancer-bearing mice were treated with anti-PD-1, TTFs, or the combination, a reduction in tumor growth was observed in combination-treated mice relative to controls. Combination-treated mice were found to have a decrease in intratumoral DCs and macrophages but increased CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells. These results suggest the combination of an ICI such as anti-PD-1 and TTFs could enhance anti-tumor responses in the context of brain tumors [148].

As stated earlier, ICI as monotherapies has had limited success in patients with CNS-derived malignancies. Therefore, several groups are evaluating combinatorial ICI approaches to enhance anti-tumor effects. Flores *et al.* found the combination of lineage-negative hematopoietic stem and progenitor cells (HSPCs) and the ICI, anti-PD-1 provided significantly prolonged survival relative to HSPC or anti-PD-1 monotherapy. The authors found the enhanced survival is likely due to increased secretion of IFN γ by T cells in the TME. In addition, they found the CCR2⁺ HSPCs were the population responsible for providing the enhanced anti-tumor efficacy. Interestingly, they observed that utilizing CCR2⁺ HSPCs in the context of an adoptive cellular therapy (ACT) platform, which combines tumor RNA-pulsed DCs, tumor-reactive T cells, and radiotherapy, significantly enhanced survival relative to ACT using bulk lineage-negative HSPCs. These results suggest these CCR2⁺ HSPCs cells may be combined with various types of immunotherapies to enhance anti-tumor efficacy [149].

Alternatively, Flores-Toro *et al.* identified an expansion of CCR2⁺ myeloid cells within the TME using two models of intracranial glioma. The authors used a small molecule inhibitor of CCR2, CCX872, in combination with the ICI, anti-PD-1 to enhance survival using a murine model of high-grade glioma as well as a GSC model. They went on to determine this mechanism of anti-tumor efficacy was likely due to a combination of reduced recruitment of Ly6C⁺ myeloid cells to the TME,

an increase in intratumoral CD4⁺, CD8⁺, CD3⁺ IFN γ ⁺ cells, and a reduction in CD8⁺ TIM3⁺ PD-1⁺ T cells relative to vehicle control-treated mice [150].

Finally, Sabbagh *et al.* used novel combinatorial immunotherapy approach to enhance anti-tumor efficacy. They utilized low-intensity pulsed ultrasound (LIPU) to open the BBB for better penetration of various therapeutics. Although LIPU as monotherapy did not provide a robust anti-tumor response, when combined with anti-PD-1, enhanced median survival was observed relative to IgG control-treated mice. In addition, the authors used EGFRvIII-specific CAR T cells in combination with LIPU and found increased trafficking of administered CAR T cells to the TME as well as enhanced survival relative to CAR T cells alone. These results suggest utilizing combinatorial immunotherapeutic approaches with LIPU may lead to enhanced anti-tumor efficacy [151].

4. Conclusions

Malignant brain tumors pose a unique and difficult set of challenges including high tumor heterogeneity and tumor antigen loss, low mutation burden, an immunosuppressive microenvironment, systemic T cell dysfunction, and relative isolation from systemic circulation due to the blood-brain barrier. These overwhelming obstacles have, thus far, limited immunotherapy efficacy. Despite these hurdles, immunotherapies are making incremental advances to overcome these challenges simultaneously [152, 153]. New developments are occurring in the peptide vaccine platforms by the conjugation with toll-like receptor agonists which can enhance activation of DCs to elicit tuned immune responses [154–156]. Studies are also moving forward to focus on targeting multiple antigens simultaneously to combat tumor antigen loss in CAR T therapy [157]. Other groups are working on addressing the immunosuppressive tumor microenvironment and T cell exhaustion with several studies underway in a variety of cancers that combine vaccines and immune checkpoint inhibitors [158]. In the CAR T therapy arena, groups are overcoming T cell exhaustion by knocking out the checkpoint molecules [159, 160], endowing CAR T cells with the capabilities of secreting anti-PD-L1 antibodies [161], and linking the PD-1 extracellular domain to the CD28 intracellular domain to lead to an activation signal instead of inhibition [162, 163]. Other groups are working on overcoming the blood-brain barrier challenge by using laser interstitial thermal therapy or the aforementioned low-intensity pulsed ultrasound to cause local disruption and permeability which may increase trafficking of therapies to the tumor site [151, 164, 165]. These approaches utilizing various combinations and novel technologies may provide solutions to the aforementioned obstacles.

In summary, the next advances in immunotherapies for CNS malignancies will come from enhanced foundational understanding of immune cells and the tumor microenvironment, better mechanistic understandings of current immunotherapy resistance, increased rational combinations of current immunotherapies with complementary mechanisms of action, and novel immunotherapeutic approaches. Together, the above-mentioned clinical studies and novel preclinical work provide an optimistic future in cancer with much-needed improvement in patient survival.

Acknowledgements

This work was supported by the University of Florida Clinical and Translational Science Institute, which is supported in part by the NIH National Center for Advancing Translational Sciences under the award TL1TR001428. NIH funding

was also received through the National Cancer Institute (F30CA232641 to MS) (T32CA257923 to BDD) and the National Institute of Neurological Disorders and Stroke (R01NS112315 and R01NS111033 to CF). Additionally, this work was supported by Alex's Lemonade Stand Foundation and the University of Florida MD-PhD Training Program.

Conflict of interest

CF holds interest in iOncologi, Inc., a biotechnology company focused on immuno-oncology. Other authors declare no conflicts of interest.

Notes/thanks/other declarations

We thank all of the patients and their families who participated in the referenced studies that made this work possible and allows us to move the needle forward on patient outcomes.

Author details

Mathew Sebastian, Bayli DiVita Dean and Catherine T. Flores*
Preston A. Wells Jr. Center for Brain Tumor Therapy, Lillian S. Wells Department of Neurosurgery, University of Florida College of Medicine, Gainesville, FL, USA

*Address all correspondence to: cfdrake@ufl.edu

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

References

- [1] Cancer Stat Facts: Brain and Other Nervous System Cancer. 2021. Available from: <https://seer.cancer.gov/statfacts/html/brain.html>
- [2] Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *The New England Journal of Medicine*. 2014;**370**(8):699-708
- [3] Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *The New England Journal of Medicine*. 2005;**352**(10):997-1003
- [4] Imperato JP, Paleologos NA, Vick NA. Effects of treatment on long-term survivors with malignant astrocytomas. *Annals of Neurology*. 1990;**28**(6): 818-822
- [5] Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: A randomised controlled multicentre phase III trial. *The Lancet Oncology*. 2006;**7**(5): 392-401
- [6] Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England Journal of Medicine*. 2005;**352**(10):987-996
- [7] Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: A randomized clinical trial. *Journal of the American Medical Association*. 2017;**318**(23):2306-2316
- [8] Walker MD, Green SB, Byar DP, Alexander E Jr, Batzdorf U, Brooks WH, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *The New England Journal of Medicine*. 1980;**303**(23):1323-1329
- [9] Esfahani K, Roudaia L, Buhlaiga N, Del Rincon SV, Papneja N, Miller WH Jr. A review of cancer immunotherapy: From the past, to the present, to the future. *Current Oncology*. 2020; **27**(Suppl 2):S87-S97
- [10] Kelly PN. The cancer immunotherapy revolution. *Science*. 2018;**359**(6382):1344-1345
- [11] Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nature Reviews. Cancer*. 2012;**12**(4): 265-277
- [12] Sharpe AH. Introduction to checkpoint inhibitors and cancer immunotherapy. *Immunological Reviews*. 2017;**276**(1):5-8
- [13] Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: From T cell basic science to clinical practice. *Nature Reviews. Immunology*. 2020;**20**(11):651-668
- [14] Yang Y. Cancer immunotherapy: Harnessing the immune system to battle cancer. *The Journal of Clinical Investigation*. 2015;**125**(9):3335-3337
- [15] Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*. 2014; **344**(6190):1396-1401
- [16] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;**339**(6127):1546-1558

- [17] Gong D, Shi W, Yi SJ, Chen H, Groffen J, Heisterkamp N. TGFbeta signaling plays a critical role in promoting alternative macrophage activation. *BMC Immunology*. 2012;**13**:31
- [18] Perng P, Lim M. Immunosuppressive mechanisms of malignant gliomas: Parallels at non-CNS sites. *Frontiers in Oncology*. 2015;**5**:1
- [19] Quail DF, Joyce JA. The microenvironmental landscape of brain tumors. *Cancer Cell*. 2017;**31**(3):326-341
- [20] Sampson JH, Gunn MD, Fecci PE, Ashley DM. Brain immunology and immunotherapy in brain tumours. *Nature Reviews. Cancer*. 2020;**20**(1):12-25
- [21] Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nature Medicine*. 2003;**9**(10):1269-1274
- [22] Wainwright DA, Balyasnikova IV, Chang AL, Ahmed AU, Moon KS, Auffinger B, et al. IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. *Clinical Cancer Research*. 2012;**18**(22):6110-6121
- [23] Zhang I, Alizadeh D, Liang J, Zhang L, Gao H, Song Y, et al. Characterization of arginase expression in glioma-associated microglia and macrophages. *PLoS One*. 2016;**11**(12):e0165118
- [24] Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nature Immunology*. 2013;**14**(10):1014-1022
- [25] Bechmann I, Galea I, Perry VH. What is the blood-brain barrier (not)? *Trends in Immunology*. 2007;**28**(1):5-11
- [26] Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: A new view. *Immunology Today*. 1992;**13**(12):507-512
- [27] Huber JD, Egleton RD, Davis TP. Molecular physiology and pathophysiology of tight junctions in the blood-brain barrier. *Trends in Neurosciences*. 2001;**24**(12):719-725
- [28] Kipnis J. Multifaceted interactions between adaptive immunity and the central nervous system. *Science*. 2016;**353**(6301):766-771
- [29] Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. *The Journal of Cell Biology*. 2015;**209**(4):493-506
- [30] Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. *Nature Reviews Cancer*. 2021;**21**(6):360-378
- [31] Hoang-Minh LB, Mitchell DA. Immunotherapy for brain tumors. *Current Treatment Options in Oncology*. 2018;**19**(11):60
- [32] Phase I Trial of IDH1 Peptide Vaccine in IDH1R132H-mutated Grade III-IV Gliomas (NOA-16). US National Library of Medicine; 2015 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02454634>
- [33] Platten M, Bunse L, Wick A, Bunse T, Le Cornet L, Harting I, et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature*. 2021; **592**(7854):463-468
- [34] Vaccine Therapy and Sargramostim in Treating Patients With Malignant Glioma. US National Library of Medicine; 2010. Available from: <https://clinicaltrials.gov/ct2/show/NCT01250470>
- [35] Fenstermaker RA, Ciesielski MJ, Qiu J, Yang N, Frank CL, Lee KP, et al. Clinical study of a survivin long peptide

vaccine (SurVaxM) in patients with recurrent malignant glioma. *Cancer Immunology, Immunotherapy*. 2016;**65**(11):1339-1352

[36] Study of Pembrolizumab Plus SurVaxM for Glioblastoma at First Recurrence. US National Library of Medicine; 2019. Available from: <https://clinicaltrials.gov/ct2/show/NCT04013672>

[37] Ahluwalia MS, Peereboom DM, Ciolfi M, Schilero C, Hobbs B, Ciesielski MJ, et al. Phase II study of pembrolizumab plus SurVaxM for glioblastoma at first recurrence. *Journal of Clinical Oncology*. 2020; **38**(Suppl 15):TPS2581-TPS

[38] Kijima N, Hosen N, Kagawa N, Hashimoto N, Kinoshita M, Oji Y, et al. Wilms' tumor 1 is involved in tumorigenicity of glioblastoma by regulating cell proliferation and apoptosis. *Anticancer Research*. 2014;**34**(1a):61-67

[39] Tatsumi N, Oji Y, Tsuji N, Tsuda A, Higashio M, Aoyagi S, et al. Wilms' tumor gene WT1-shRNA as a potent apoptosis-inducing agent for solid tumors. *International Journal of Oncology*. 2008;**32**(3):701-711

[40] Izumoto S, Tsuboi A, Oka Y, Suzuki T, Hashiba T, Kagawa N, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastoma multiforme. *Journal of Neurosurgery*. 2008;**108**(5):963-971

[41] Jawhari S, Ratinaud MH, Verdier M. Glioblastoma, hypoxia and autophagy: A survival-prone 'menage-a-trois'. *Cell Death & Disease*. 2016;**7**:1

[42] Brennan CW, Verhaak RGW, McKenna A, Campos B, Nounshmehr H, Salama SR, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013;**155**:462. Erratum in:2014;**157**(3):753

[43] Ramnarain DB, Park S, Lee DY, Hatanpaa KJ, Scoggin SO, Otu H, et al. Differential gene expression analysis reveals generation of an autocrine loop by a mutant epidermal growth factor receptor in glioma cells. *Cancer Research*. 2006;**66**(2):867-874

[44] Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme (ACTIVATE). US National Library of Medicine; 2008 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00643097>

[45] Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *Journal of Clinical Oncology*. 2010;**28**(31):4722-4729

[46] Phase III Study of Rindopepimut/GM-CSF in Patients With Newly Diagnosed Glioblastoma (ACT IV). US National Library of Medicine; 2011 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01480479>

[47] Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): A randomised, double-blind, international phase 3 trial. *The Lancet Oncology*. 2017;**18**(10):1373-1385

[48] Dutoit V, Herold-Mende C, Hilf N, Schoor O, Beckhove P, Bucher J, et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. *Brain*. 2012;**135**:1042-1054

[49] Phase I/II Trial of IMA950 Multi-peptide Vaccine Plus Poly-ICLC in Glioblastoma. US National Library of

Medicine; 2013 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01920191>

[50] Migliorini D, Dutoit V, Allard M, Grandjean Hallez N, Marinari E, Widmer V, et al. Phase I/II trial testing safety and immunogenicity of the multi-peptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients. *Neuro-Oncology*. 2019;**21**(7):923-933

[51] Pembrolizumab in Association With the IMA950/Poly-ICLC for Relapsing Glioblastoma (IMA950-106). US National Library of Medicine; 2018 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT03665545>

[52] A Pilot Study of Glioma Associated Antigen Vaccines in Conjunction With Poly-ICLC in Pediatric Gliomas. US National Library of Medicine; 2010 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01130077>

[53] Pollack IF, Jakacki RI, Butterfield LH, Hamilton RL, Panigrahy A, Potter DM, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. *Journal of Clinical Oncology*. 2014;**32**(19):2050-2058

[54] Vaccine Therapy in Treating Patients With Malignant Glioma. US National Library of Medicine; 2003 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00068510>

[55] Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year

analysis of the EORTC-NCIC trial. *The Lancet Oncology*. 2009;**10**(5):459-466

[56] Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. *Clinical Cancer Research*. 2011;**17**(6):1603-1615

[57] Study of a Drug [DCVax®-L] to Treat Newly Diagnosed GBM Brain Cancer (GBM). US National Library of Medicine; 2002 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00045968>

[58] Liau LM, Ashkan K, Tran DD, Campian JL, Trusheim JE, Cobbs CS, et al. First results on survival from a large phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *Journal of Translational Medicine*. 2018;**16**:142

[59] Yu JS, Phuphanich S, Wheeler C, Rudnick J, Mazer M, Wang HQ, et al. Phase I trial of a multi-epitope pulsed dendritic cell vaccine targeting cancer stem cells in patients with newly diagnosed glioblastoma. *Journal of Immunotherapy*. 2012;**35**(9):747-748

[60] A Study of ICT-107 Immunotherapy in Glioblastoma Multiforme (GBM). US National Library of Medicine; 2011 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01280552>

[61] Wen PY, Reardon DA, Armstrong TS, Phuphanich S, Aiken RD, Landolfi JC, et al. A randomized double-blind placebo-controlled phase II trial of dendritic cell vaccine ICT-107 in newly diagnosed patients with glioblastoma. *Clinical Cancer Research*. 2019;**25**(19):5799-5807

[62] Phase 3 Randomized, Double-blind, Controlled Study of ICT-107 in Glioblastoma. US National Library of

Medicine; 2015 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02546102>

[63] Rahman M, Dastmalchi F, Karachi A, Mitchell D. The role of CMV in glioblastoma and implications for immunotherapeutic strategies. *Oncoimmunology*. 2019;**8**(1):e1514921

[64] Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme (ATTAC). US National Library of Medicine; 2008 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00639639>

[65] Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature*. 2015;**519**(7543):366-369

[66] Batich KA, Reap EA, Archer GE, Sanchez-Perez L, Nair SK, Schmittling RJ, et al. Long-term survival in glioblastoma with cytomegalovirus pp65-targeted vaccination. *Clinical Cancer Research*. 2017;**23**(8):1898-1909

[67] Vaccine Therapy for the Treatment of Newly Diagnosed Glioblastoma Multiforme (ATTAC-II). US National Library of Medicine; 2015 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02465268>

[68] DC Migration Study for Newly-Diagnosed GBM (ELEVATE). US National Library of Medicine; 2015 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02366728>

[69] Van Brussel I, Berneman ZN, Cools N. Optimizing dendritic cell-based immunotherapy: Tackling the complexity of different arms of the immune system. *Mediators of Inflammation*. 2012;**2012**:1-14

[70] Mailliard RB, Wankowicz-Kalinska A, Cai Q, Wesa A,

Hilkens CM, Kapsenberg ML, et al. alpha-type-1 polarized dendritic cells: A novel immunization tool with optimized CTL-inducing activity. *Cancer Research*. 2004;**64**(17):5934-5937

[71] Okada H, Kalinski P, Ueda R, Hoji A, Kohanbash G, Donegan TE, et al. Induction of CD8(+) T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with alpha-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. *Journal of Clinical Oncology*. 2011;**29**(3):330-336

[72] Sprent J, Kishimoto H. The thymus and central tolerance. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*. 2001;**356**(1409):609-616

[73] Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways similarities, differences, and implications of their inhibition. *American Journal of Clinical Oncology*. 2016;**39**(1):98-106

[74] Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunological Reviews*. 2008;**224**:166-182

[75] Garon EB, Rizvi NA, Hui RN, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *The New England Journal of Medicine*. 2015;**372**(21):2018-2028

[76] Larkin J, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma REPLY. *The New England Journal of Medicine*. 2015;**373**(13):1270-1271

[77] Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus

everolimus in advanced renal-cell carcinoma. *The New England Journal of Medicine*. 2015;**373**(19):1803-1813

[78] Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *The New England Journal of Medicine*. 2015;**372**(26):2521-2532

[79] Yang KL, Li JR, Bai CM, Sun Z, Zhao L. Efficacy of immune checkpoint inhibitors in non-small-cell lung cancer patients with different metastatic sites: A systematic review and meta-analysis. *Frontiers in Oncology*. 2020;**10**:1

[80] Tawbi HA, Forsyth PA, Algazi A, Hamid O, Hodi FS, Moschos SJ, et al. Combined nivolumab and ipilimumab in melanoma metastatic to the brain. *The New England Journal of Medicine*. 2018;**379**(8):722-730

[81] Nduom EK, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, et al. PD-L1 expression and prognostic impact in glioblastoma. *Neuro-Oncology*. 2016;**18**(2):195-205

[82] Mu LY, Long Y, Yang CL, Jin LC, Tao HP, Ge HT, et al. The IDH1 mutation-induced oncometabolite, 2-hydroxyglutarate, may affect dna methylation and expression of PD-L1 in gliomas. *Frontiers in Molecular Neuroscience*. 2018;**11**

[83] A Study of the Effectiveness and Safety of Nivolumab Compared to Bevacizumab and of Nivolumab With or Without Ipilimumab in Glioblastoma Patients (CheckMate 143). US National Library of Medicine; 2013. Available from: <https://clinicaltrials.gov/ct2/show/NCT02017717>

[84] Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The checkmate 143 phase

3 randomized clinical trial. *JAMA Oncology*. 2020;**6**(7):1003-1010

[85] An Investigational Immuno-therapy Study of Nivolumab Compared to Temozolomide, Each Given With Radiation Therapy, for Newly-diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer) (CheckMate 498). US National Library of Medicine; 2015 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02617589>

[86] Bristol-Myers Squibb Announces Phase 3 CheckMate –498 Study Did Not Meet Primary Endpoint of Overall Survival with Opdivo (nivolumab) Plus Radiation in Patients with Newly Diagnosed MGMT-Unmethylated Glioblastoma Multiforme. 2019. Available from: <https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx>

[87] An Investigational Immuno-therapy Study of Temozolomide Plus Radiation Therapy With Nivolumab or Placebo, for Newly Diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer) (CheckMate548). US National Library of Medicine; 2016 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02667587>

[88] Bristol-Myers Squibb Provides Update on Phase 3 Opdivo (nivolumab) CheckMate –548 Trial in Patients with Newly Diagnosed MGMT-Methylated Glioblastoma Multiforme. 2019. Available from: <https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx>

- [89] Keenan TE, Burke KP, Van Allen EM. Genomic correlates of response to immune checkpoint blockade. *Nature Medicine*. 2019;**25**(3):389-402
- [90] Chongsathidkiet P, Jackson C, Koyama S, Loebel F, Cui XY, Farber SH, et al. Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nature Medicine*. 2018;**24**(9):1459-+
- [91] Marin-Acevedo JA, Kimbrough EO, Lou YY. Next generation of immune checkpoint inhibitors and beyond. *Journal of Hematology & Oncology*. 2021;**14**(1):45
- [92] Rayner AA, Grimm EA, Lotze MT, Chu EW, Rosenberg SA. Lymphokine-activated killer (LAK) cells. Analysis of factors relevant to the immunotherapy of human cancer. *Cancer*. 1985;**55**(6): 1327-1333
- [93] Hayes RL, Koslow M, Hiesiger EM, Hymes KB, Hochster HS, Moore EJ, et al. Improved long term survival after intracavitary interleukin-2 and lymphokine-activated killer cells for adults with recurrent malignant glioma. *Cancer*. 1995;**76**(5):840-852
- [94] Dillman RO, Duma CM, Schiltz PM, DePriest C, Ellis RA, Okamoto K, et al. Intracavitary placement of autologous lymphokine-activated killer (LAK) cells after resection of recurrent glioblastoma. *Journal of Immunotherapy*. 2004;**27**(5):398-404
- [95] Dillman RO, Duma CM, Ellis RA, Cornforth AN, Schiltz PM, Sharp SL, et al. Intralesional lymphokine-activated killer cells as adjuvant therapy for primary glioblastoma. *Journal of Immunotherapy*. 2009;**32**(9):914-919
- [96] Wang J, Shen F, Yao Y, Wang LL, Zhu Y, Hu J. Adoptive cell therapy: A novel and potential immunotherapy for glioblastoma. *Frontiers in Oncology*. 2020;**10**:59
- [97] Law TM, Motzer RJ, Mazumdar M, Sell KW, Walther PJ, Oconnell M, et al. Phase-III randomized trial of interleukin-2 with or without lymphokine-activated killer-cells in the treatment of patients with advanced renal-cell carcinoma. *Cancer*. 1995;**76**(5):824-832
- [98] Burger MC, Zhang C, Harter PN, Romanski A, Strassheimer F, Senft C, et al. CAR-engineered nk cells for the treatment of glioblastoma: Turning innate effectors into precision tools for cancer immunotherapy. *Frontiers in Immunology*. 2019;**10**:2683
- [99] Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nature Reviews. Immunology*. 2018;**18**(11): 671-688
- [100] Ishikawa E, Tsuboi K, Saijo K, Harada H, Takano S, Nose T, et al. Autologous natural killer cell therapy for human recurrent malignant glioma. *Anticancer Research*. 2004;**24**(3b):1861-1871
- [101] Campbell KS, Hasegawa J. Natural killer cell biology: An update and future directions. *The Journal of Allergy and Clinical Immunology*. 2013;**132**(3):536-544
- [102] Cornel AM, Mimpen IL, Nierkens S. MHC class i downregulation in cancer: Underlying mechanisms and potential targets for cancer immunotherapy. *Cancers (Basel)*. 2020;**12**(7):536
- [103] Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer immune evasion through loss of mhc class i antigen presentation. *Frontiers in Immunology*. 2021;**12**:1
- [104] Zagzag D, Salnikow K, Chiriboga L, Yee H, Lan L, Ali MA, et al. Downregulation of major histocompatibility complex antigens in

invading glioma cells: Stealth invasion of the brain. *Laboratory Investigation*. 2005;**85**(3):328-341

[105] Intracranial Injection of NK-92/5.28.z Cells in Patients With Recurrent HER2-positive Glioblastoma (CAR2BRAIN). US National Library of Medicine; 2017 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT03383978>

[106] Natural Killer Cell (CYNK-001) IV Infusion or IT Administration in Adults With Recurrent GBM (CYNK001GBM01). US National Library of Medicine; 2020. Available from: <https://clinicaltrials.gov/ct2/show/NCT04489420>

[107] Intra-tumoral Injection of Natural Killer Cells in High-Grade Gliomas (NK HGG). 2020 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT04254419>

[108] Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**:14639-14645

[109] Maxwell R, Luksik AS, Garzon-Muvdi T, Lim M. The potential of cellular- and viral-based immunotherapies for malignant glioma-dendritic cell vaccines, adoptive cell transfer, and oncolytic viruses. *Current Neurology and Neuroscience Reports*. 2017;**17**(6):50

[110] Berghoff AS, Kiesel B, Widhalm G, Rajky O, Ricken G, Wohrer A, et al. Programmed death ligand 1 (PD-L1) expression and tumor infiltrating lymphocytes in diffuse and anaplastic gliomas. *European Journal of Cancer*. 2015;**51**:S593-S

[111] Schuessler A, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, et al.

Autologous t-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Research*. 2014;**74**(13):3466-3476

[112] Evaluation of Recovery From Drug-Induced Lymphopenia Using Cytomegalovirus-specific T-cell Adoptive Transfer (ERaDICAte). US National Library of Medicine; 2008 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00693095>

[113] Reap EA, Suryadevara CM, Batich KA, Sanchez-Perez L, Archer GE, Schmittling RJ, et al. Dendritic cells enhance polyfunctionality of adoptively transferred t cells that target cytomegalovirus in glioblastoma. *Cancer Research*. 2018;**78**(1):256-264

[114] Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood*. 2014;**123**(17):2625-2635

[115] Autologous T Cells Redirected to EGFRVIII-With a Chimeric Antigen Receptor in Patients With EGFRVIII+ Glioblastoma. US National Library of Medicine; 2014 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02209376>

[116] O'Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrisette JJD, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Science Translational Medicine*. 2017;**9**(399):1-15

[117] Zhang JG, Eguchi J, Kruse CA, Gomez GG, Fakhrai H, Schroter S, et al. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. *Clinical Cancer Research*. 2007;**13**(2 Pt 1):566-575

- [118] Schlegel J, Stumm G, Brandle K, Merdes A, Mechttersheimer G, Hynes NE, et al. Amplification and differential expression of members of the erbB-gene family in human glioblastoma. *Journal of Neuro-Oncology*. 1994;22(3):201-207
- [119] Koka V, Potti A, Forseen SE, Pervez H, Fraiman GN, Koch M, et al. Role of Her-2/neu overexpression and clinical determinants of early mortality in glioblastoma multiforme. *American Journal of Clinical Oncology*. 2003;26(4):332-335
- [120] Andersson U, Guo D, Malmer B, Bergenheim AT, Brannstrom T, Hedman H, et al. Epidermal growth factor receptor family (EGFR, ErbB2-4) in gliomas and meningiomas. *Acta Neuropathologica*. 2004;108(2):135-142
- [121] CMV-specific Cytotoxic T Lymphocytes Expressing CAR Targeting HER2 in Patients With GBM (HERT-GBM). US National Library of Medicine; 2010 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01109095>
- [122] Ahmed N, Brawley V, Hegde M, Bielamowicz K, Kalra M, Landi D, et al. HER2-specific chimeric antigen receptor-modified virus-specific t cells for progressive glioblastoma a phase 1 dose-escalation trial. *JAMA Oncology*. 2017;3(8):1094-1101
- [123] Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): A randomised controlled phase 2 trial. *The Lancet Oncology*. 2014;15(9):943-953
- [124] Debinski W, Gibo DM. Molecular expression analysis of restrictive receptor for interleukin 13, a brain tumor-associated cancer/testis antigen. *Molecular Medicine*. 2000;6(5):440-449
- [125] Thaci B, Brown CE, Binello E, Werbaneth K, Sampath P, Sengupta S. Significance of interleukin-13 receptor alpha 2-targeted glioblastoma therapy. *Neuro-Oncology*. 2014;16(10):1304-1312
- [126] Brown CE, Badie B, Barish ME, Weng L, Ostberg JR, Chang WC, et al. Bioactivity and safety of IL13Ralpha2-redredirected chimeric antigen receptor CD8+ T cells in patients with recurrent glioblastoma. *Clinical Cancer Research*. 2015;21(18):4062-4072
- [127] Cellular Adoptive Immunotherapy Using Genetically Modified T-Lymphocytes in Treating Patients With Recurrent or Refractory High-Grade Malignant Glioma. US National Library of Medicine; 2008 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT00730613>
- [128] Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma. US National Library of Medicine; 2014 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02208362>
- [129] Brown CE, Alizadeh D, Starr R, Weng LH, Wagner JR, Naranjo A, et al. Regression of glioblastoma after chimeric antigen receptor t-cell therapy. *The New England Journal of Medicine*. 2016;375(26):2561-2569
- [130] Liu GN, Rui W, Zhao XQ, Lin X. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. *Cellular & Molecular Immunology*. 2021;18(5):1085-1095
- [131] Vora P, Venugopal C, Salim SK, Tatari N, Bakhshinyan D, Singh M, et al. The rational development of CD133-targeting immunotherapies for glioblastoma. *Cell Stem Cell*. 2020;26(6):832-844, e6

- [132] Shaim H, Shanley M, Basar R, Daher M, Gumin J, Zamler DB, et al. Targeting the alphav integrin/TGF-beta axis improves natural killer cell function against glioblastoma stem cells. *The Journal of Clinical Investigation*. 2021;**131**(14):1
- [133] Majzner RG, Mackall CL. Clinical lessons learned from the first leg of the CAR T cell journey. *Nature Medicine*. 2019;**25**(9):1341-1355
- [134] Sterner RC, Sterner RM. CAR-T cell therapy: Current limitations and potential strategies. *Blood Cancer Journal*. 2021;**11**(4):69
- [135] Bielamowicz K, Fousek K, Byrd TT, Samaha H, Mukherjee M, Aware N, et al. Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma. *Neuro-Oncology*. 2018;**20**(4):506-518
- [136] Krenciute G, Prinzing BL, Yi Z, Wu MF, Liu H, Dotti G, et al. Transgenic expression of IL15 improves antiglioma activity of IL13Ralpha2-CAR T cells but results in antigen loss variants. *Cancer Immunology Research*. 2017;**5**(7):571-581
- [137] Nazha B, Inal C, Owonikoko TK. Disialoganglioside GD2 expression in solid tumors and role as a target for cancer therapy. *Frontiers in Oncology*. 2020;**10**:1000
- [138] 3rd Generation GD-2 Chimeric Antigen Receptor and iCaspase Suicide Safety Switch, Neuroblastoma, GRAIN (GRAIN). US National Library of Medicine; 2013 [cited 2021]. Available from: <https://clinicaltrials.gov/ct2/show/NCT01822652>
- [139] Heczey A, Louis CU, Savoldo B, Dakhova O, Durett A, Grilley B, et al. CAR T cells administered in combination with lymphodepletion and PD-1 inhibition to patients with neuroblastoma. *Molecular Therapy*. 2017;**25**(9):2214-2224
- [140] Moghimi B, Muthugounder S, Jambon S, Tibbetts R, Hung L, Bassiri H, et al. Preclinical assessment of the efficacy and specificity of GD2-B7H3 SynNotch CAR-T in metastatic neuroblastoma. *Nature Communications*. 2021;**12**(1):511
- [141] Jin L, Ge H, Long Y, Yang C, Chang YE, Mu L, et al. CD70, a novel target of CAR T-cell therapy for gliomas. *Neuro-Oncology*. 2018;**20**(1):55-65
- [142] Jin L, Tao H, Karachi A, Long Y, Hou AY, Na M, et al. CXCR1- or CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors. *Nature Communications*. 2019;**10**(1):4016
- [143] Weber EW, Parker KR, Sotillo E, Lynn RC, Anbunathan H, Lattin J, et al. Transient rest restores functionality in exhausted CAR-T cells through epigenetic remodeling. *Science*. 2021;**372**(6537):49
- [144] Raychaudhuri B, Rayman P, Ireland J, Ko J, Rini B, Borden EC, et al. Myeloid-derived suppressor cell accumulation and function in patients with newly diagnosed glioblastoma. *Neuro-Oncology*. 2011;**13**(6):591-599
- [145] Alban TJ, Bayik D, Otvos B, Rabljenovic A, Leng L, Jia-Shiun L, et al. Glioblastoma myeloid-derived suppressor cell subsets express differential macrophage migration inhibitory factor receptor profiles that can be targeted to reduce immune suppression. *Frontiers in Immunology*. 2020;**11**:1191
- [146] Wightman SM, Alban TJ, Chen X, Lathia JD, Wang Y, Stark GR. Bazedoxifene inhibits sustained STAT3 activation and increases survival in GBM. *Translational Oncology*. 2021;**14**(11):101192
- [147] Alghamri MS, McClellan BL, Avvari RP, Thalla R, Carney S, Hartlage MS, et al. G-CSF secreted by

mutant IDH1 glioma stem cells abolishes myeloid cell immunosuppression and enhances the efficacy of immunotherapy. *Science Advances*. 2021;7(40):eabh3243

[148] Voloshin T, Kaynan N, Davidi S, Porat Y, Shteingauz A, Schneiderman RS, et al. Tumor-treating fields (TTFields) induce immunogenic cell death resulting in enhanced antitumor efficacy when combined with anti-PD-1 therapy. *Cancer Immunology, Immunotherapy*. 2020;69(7):1191-1204

[149] Flores CT, Wildes TJ, Drake JA, Moore GL, Dean BD, Abraham RS, et al. Lin(−)CCR2(+) hematopoietic stem and progenitor cells overcome resistance to PD-1 blockade. *Nature Communications*. 2018;9(1):4313

[150] Flores-Toro JA, Luo D, Gopinath A, Sarkisian MR, Campbell JJ, Charo IF, et al. CCR2 inhibition reduces tumor myeloid cells and unmasks a checkpoint inhibitor effect to slow progression of resistant murine gliomas. *Proceedings of the National Academy of Sciences of the United States of America*. 2020;117(2):1129-1138

[151] Sabbagh A, Beccaria K, Ling X, Marisetty A, Ott M, Caruso H, et al. Opening of the blood-brain barrier using low-intensity pulsed ultrasound enhances responses to immunotherapy in preclinical glioma models. *Clinical Cancer Research*. 2021;27(15):4325-4337

[152] Srivastava S, Riddell SR. Chimeric antigen receptor T cell therapy: Challenges to bench-to-bedside efficacy. *Journal of Immunology*. 2018;200(2):459-468

[153] van der Burg SH, Arens R, Ossendorp F, van Hall T, Melief CJ. Vaccines for established cancer: Overcoming the challenges posed by immune evasion. *Nature Reviews. Cancer*. 2016;16(4):219-233

[154] van der Burg SH, Bijker MS, Welters MJ, Offringa R, Melief CJ.

Improved peptide vaccine strategies, creating synthetic artificial infections to maximize immune efficacy. *Advanced Drug Delivery Reviews*. 2006;58(8):916-930

[155] Zom GG, Khan S, Filippov DV, Ossendorp F. TLR ligand-peptide conjugate vaccines: Toward clinical application. *Advances in Immunology*. 2012;114:177-201

[156] Wen Y, Collier JH. Supramolecular peptide vaccines: Tuning adaptive immunity. *Current Opinion in Immunology*. 2015;35:73-79

[157] Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS, et al. TanCAR: A novel bispecific chimeric antigen receptor for cancer immunotherapy. *Molecular Therapy: Nucleic Acids*. 2013;2:e105

[158] Collins JM, Redman JM, Gulley JL. Combining vaccines and immune checkpoint inhibitors to prime, expand, and facilitate effective tumor immunotherapy. *Expert Review of Vaccines*. 2018;17(8):697-705

[159] Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. *Clinical Cancer Research*. 2017;23(9):2255-2266

[160] Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. *Scientific Reports*. 2017;7(1):737

[161] Suarez ER, de Change K, Sun J, Sui J, Freeman GJ, Signoretti S, et al. Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model. *Oncotarget*. 2016;7(23):34341-34355

[162] Liu X, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, et al. A chimeric

switch-receptor targeting PD1 augments the efficacy of second-generation CAR T cells in advanced solid tumors. *Cancer Research*. 2016;**76**(6):1578-1590

[163] Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC. Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. *Molecular Immunology*. 2012;**51**(3-4):263-272

[164] Salehi A, Paturu MR, Patel B, Cain MD, Mahlokozera T, Yang AB, et al. Therapeutic enhancement of blood-brain and blood-tumor barriers permeability by laser interstitial thermal therapy. *Neuro-Oncology Advances*. 2020;**2**(1):vdaa071

[165] Leuthardt EC, Duan C, Kim MJ, Campian JL, Kim AH, Miller-Thomas MM, et al. Hyperthermic laser ablation of recurrent glioblastoma leads to temporary disruption of the peritumoral blood brain barrier. *PLoS One*. 2016;**11**(2):e0148613