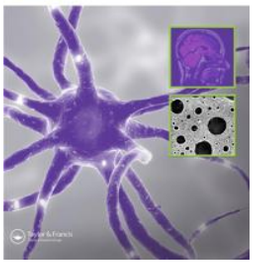
CNS Oncology



CNS Oncology

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/icns20

The challenges and clinical landscape of glioblastoma immunotherapy

Andrew Timothy Ng, Tyler Steve, Kevin T Jamouss, Abdul Arham, Sarah Kawtharani & Hazem I Assi

To cite this article: Andrew Timothy Ng, Tyler Steve, Kevin T Jamouss, Abdul Arham, Sarah Kawtharani & Hazem I Assi (2024) The challenges and clinical landscape of glioblastoma immunotherapy, CNS Oncology, 13:1, 2415878, DOI: 10.1080/20450907.2024.2415878

To link to this article: https://doi.org/10.1080/20450907.2024.2415878

Published online: 29 Oct 2024.

CNS ONCOLOGY
2024, VOL. 13, NO. 1, 2415878
https://doi.org/10.1080/20450907.2024.2415878

Taylor & Francis
Taylor & Francis Group

REVIEW

OPEN ACCESS Check for updates

The challenges and clinical landscape of glioblastoma immunotherapy

Andrew Timothy Ng*[,a], Tyler Steve[a], Kevin T Jamouss[a], Abdul Arham[a], Sarah Kawtharani[b] and Hazem I Assi**[,c]

[a]Department of Medicine, University of Massachusetts Chan Medical School – Baystate Campus, Springfield, MA 01199, USA; [b]Department of Neurosurgery, American University of Beirut Medical Center, Beirut, Lebanon; [c]Department of Hematology and Oncology, American University of Beirut Medical Center, Beirut, 1107 2020, Lebanon

ABSTRACT

Glioblastoma is associated with a dismal prognosis with the standard of care involving surgery, radiation therapy and temozolomide chemotherapy. This review investigates the features that make glioblastoma difficult to treat and the results of glioblastoma immunotherapy clinical trials so far. There have been over a hundred clinical trials involving immunotherapy in glioblastoma. We report the survival-related outcomes of every Phase III glioblastoma immunotherapy trial with online published results we could find at the time of writing. To date, the DCVax-L vaccine is the only immunotherapy shown to have statistically significant increased median survival compared with standard-of-care in a Phase III trial: 19.3 months versus 16.5 months. However, this trial used an external control group to compare with the intervention which limits its quality of evidence. In conclusion, glioblastoma immunotherapy requires further investigation to determine its significance in improving disease survival.

ARTICLE HISTORY
Received 3 February 2024
Accepted 9 October 2024

KEYWORDS
cancer vaccines; clinical research; clinical trials; drug resistance; glioblastoma; immunotherapy

1. Introduction

Glioblastoma (GB) is an intrinsic brain tumor that originates from neuroglial stem or progenitor cells [1]. It is associated with a dismal prognosis that confers a poor quality of life [2]. GB is the most aggressive and common primary brain tumor in adults and is classified as a grade 4 tumor by the World Health Organization (WHO) classification of central nervous system (CNS) tumors [3,4]. Histological features of GB include poorly differentiated astrocytic tumor cells with nuclear atypia and microvascular proliferation and necrosis [4]. Prior to 2021, greater than 90% of GB were considered isocitrate dehydrogenase IDH wild-type tumors [1]. Those that had gene mutations of isocitrate dehydrogenase (IDH) 1 or 2 were considered to have a more favorable outcome [3]. As of 2021, with the release of the 5th Edition of the WHO classification of CNS tumors, GB is restricted to only IDH wild-type tumors [4,5]. The previously classified IDH mutated GB have been reclassified as astrocytoma IDH mutated grade 4 tumors [4,5]. Additionally, a minority of GB evolve from previously diagnosed WHO grade 2 or grade 3 gliomas and are thus termed secondary glioblastomas [3].

On a molecular level, several changes occur in GB and include mutations in genes regulating P53, retinoblastoma protein (RB) signaling, receptor tyrosine kinase (RTK)/rat sarcoma (RAS)/phosphoinositide 3-kinase (PI3K) [3]. The status of MGMT promoter methylation has been identified as an important prognostic factor for patient survival, as it can predict the response to temozolomide therapy [3]. Since 2005, the standard of care has included maximal safe surgical resection followed by radiation therapy (RT) and chemotherapy with temozolomide [1,6]. Interestingly, this standard-of-care has been shown to result in a greater overall survival of 22.6 months (95% CI: 19.7–26.0) in female patients, compared with 15.9 months (95% CI: 14.0–19.4, $p = 0.0006$) in male patients [7]. Although not the focus of this review, it is important to note that sexual dimorphisms from hormones to molecular mechanisms in the disease have been documented [8].

The goal of this review is to investigate the features of GB that make it difficult to treat and to discuss what investigations using immunotherapy for GB treatment have yielded so far. There have been over a hundred trials involving the use of immunotherapy with GB. The majority of these trials are Phase I or Phase II trials. While presenting the major immunotherapies that have been tested with GB, we report survival-related results of every Phase III GB immunotherapy trial with online

CONTACT Andrew Ng Tel.: 413 794 4373; andrew.ng@baystatehealth.org; Hazem Assi ha157@aub.edu.lb

published results we could find at the time of writing in October 2023. Our focus was on Phase III clinical trials as our primary investigative question was whether immunotherapy had been shown to have a survival benefit compared with the current standard of care. From our literature review, we have found very little evidence supporting immunotherapy increasing survival time in GB to date.

2. Glioblastoma mechanisms of treatment resistance

2.1. Cancer stem cells

GB contain cancer stem cells (CSCs) [9]. Like other stem cells, CSCs possess the ability to self-renew and differ from their parent cells in terms of metabolic and regulatory pathways, making them highly challenging to target with therapy [9]. Through the release of cytokines and chemokines, CSCs recruit immunosuppressive cells, primarily tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), T-regulatory (Treg) cells and natural killer (NK) cells. In turn, these immunosuppressive cells promote the differentiation of CSC phenotypes, leading to chemo-resistance and evasion of immune surveillance. Supporting CSCs, the extracellular matrix (ECM) of GB plays a crucial role by providing receptors for the stem cells to anchor and proliferate. Specific ECM proteins that facilitate this role are LAMA2, which is involved in CSC growth, and ITGA6, which contributes to CSC self-renewal. Further supporting the CSCs, is the hypoxic environment created from tumor cell proliferation. This hypoxic environment reduces oxygen free radical formation associated with RT and thus reduces DNA damage to the CSCs. The hypoxic environment also promotes chemoresistance by enhancing the expression of efflux ABC transporters which lead to reduced drug concentration within the cells [10].

2.2. The tumor immune microenvironment: glioma-associated microglia, myeloid-derived suppressor cells

One of the primary challenges in treating GB is its highly immunosuppressive tumor immune microenvironment. To enhance therapeutic strategies, it is imperative to gain a comprehensive understanding of the tumor immune microenvironment and the inherent heterogeneity it presents.

One of the most abundant immune cell populations in the tumor immune microenvironment (TIME) of GB consists of glioma-associated microglia (GAMs). These cells play a pivotal role in shaping the immune landscape within GB. As previously studied in different tumors, the behavior of macrophages can be influenced by a variety of cytokines and chemokines. These signaling molecules can induce a transformation of macrophages into either M1 or M2 phenotypes, with distinct functions. M1 macrophages typically exhibit tumor-suppressive functions, while M2 macrophages tend to promote tumor growth and immunosuppression. However, the complexity of GAMs in the TIME of GB goes beyond the M1/M2 classification. These GAMs often exhibit a high degree of plasticity, adapting to the specific signals and interactions within the GB microenvironment and leaning toward immunosuppressive functions. These GAMs have the ability to generate both anti-inflammatory cytokines and factors that encourage tumor growth [11]. Additionally, PD-L1 has been found on GAMs and is known to play a role in promoting angiogenesis and activating immune checkpoints [12].

Myeloid-derived suppressor cells (MDSCs) have also been observed to be present within the glioma Tumor Microenvironment. These cells employ mechanisms similar to GAMs and act by suppressing the activity of tumor-specific effector T cells. Chemoattractants released by GB cells recruit and enable tumor-associated macrophages and myeloid-derived suppressor cells (MDSCs) to bypass the blood–brain barrier (BBB) and facilitate tumor formation [13]. This process underscores the significance of the interplay between immune cells and the tumor microenvironment in glioma progression. Furthermore, a notable deficiency in the numbers of natural killer (NK) cells and T cells, which typically play a role in tumor suppression, has been observed. Apart from being deficient, both CD4 and CD8 cells have been observed to be dysfunctional. This immune cell deficit and dysfunction further contribute to the immunosuppressive nature of the glioma microenvironment [14].

2.3. Vascularity

Angiogenesis is recognized as a hallmark of tumors, and GB is particularly known for its high vascularity [4]. The rapid tumor growth of GB creates greater nutrient and oxygen demand which stimulates new blood vessel formation and thus high tumor vascularization [15]. Bevacizumab, a drug targeting VEGF, has demonstrated some improvement in progression-free survival, but not overall survival [16]. However, targeting VEGF, while potentially achieving vessel regression, can lead to hypoxia and upregulation of pro-angiogenic factors like SDF1α. This upregulation results in the recruitment of bone marrow-derived cells with the ability to promote further angiogenesis and tumor progression, ultimately leading to treatment failure. Another factor that may explain Bevacizumab's minimal efficacy in GB is the expression of the

endothelial cell-independent tube-like vascular structure called vasculogenic mimicry in GB [17]. These structures are made from tumor cells that mimic endothelial cell function through their plasticity and extracellular matrix. Vasculogenic mimicry has been associated with poor prognosis in GB and may require identification and targeting of its related signaling pathways to improve treatment [18]. Due to the highly vascular nature of GB characterized by leaky vessels and rapid infiltration into neighboring tissues, surgery has struggled to achieve tumor-free margins.

Lastly, Caspase 8, a protein involved in apoptosis, promotes NF-κB transcription factor activation, leading to enhanced secretion of VEGF, IL-6, IL-8, IL-1β and MCP-1. This cascade of events results in neovascularization and resistance to temozolomide [17].

2.4. The blood–brain barrier & blood–tumor barrier

The brain is a vital organ protected by the blood–brain barrier, which is highly selective in allowing substances to pass through. While this barrier is essential for safeguarding the brain in healthy individuals, it poses a significant challenge in treating GB with chemotherapeutic drugs [10]. Several studies have explored methods to enhance drug delivery to the brain, such as brain microdialysis, intracerebral implantation and intraventricular delivery. However, these approaches have often resulted in severe side effects and damage to normal brain tissue. A promising approach lies in altering the permeability of the blood–brain barrier using osmotic agents and efflux pump inhibitors [10].

Notably, the blood–brain barrier's structure and behavior are altered in the presence of brain tumors. This modified barrier is called blood–tumor barrier [19]. Examples of these structural differences between a normal blood–brain barrier and that observed in the brain-tumor barrier of GB include reduced expression of tight junction claudins in endothelial cells and thinned basement membranes [19]. These structural differences between the blood–brain barrier and blood–tumor barrier result in a leakier barrier in the blood–tumor barrier [20]. It is theorized that a better understanding of the differences between the blood–brain barrier and the blood–tumor barrier will allow for the design of therapeutics for preferential uptake into the blood–tumor barrier and lead to more favorable responses to interventions [19,20].

2.5. Repair mechanisms

GB consists of different genetic subclones and transcriptomic profiles that enable a unique DNA damage response leading to drug resistance. MGMT is a DNA repair enzyme that reverses the cytotoxic lesions generated by temozolomide. GB with highly methylated promoter sites for the *MGMT* gene have shown greater resistance compared with those with unmethylation. Another resistance mechanism to temozolomide involves the mismatch repair (MMR) system. This system typically detects temozolomide-induced mismatched pairing during DNA replication and activates signaling pathways that lead to cell cycle arrest and cell death [21]. However, it has been observed that GB with chronic exposure to temozolomide can produce clones with loss-of-function mutations of MSH6, a part of the MMR [22]. Thus, these cells with MSH6 mutations do not respond to the temozolomide-induced mismatched pairing with cell death and become resistant to temozolomide treatment [21].

Touat et al. [23] studied the relationship between MMR deficiency, temozolomide exposure and the development of hypermutation in gliomas in detail. They presented evidence that recurrent defects in the MMR pathway drive hypermutation. Additionally, while identifying the origin of MMR deficiency was challenging, their data suggested that some MMR variant defects may be induced by temozolomide. The study highlights that temozolomide treatment selectively pressures MMR-deficient cells during late stages, promoting hypermutation. This was validated through experiments using isogenic models and patient-derived xenografts, which demonstrated that MMR-deficient gliomas can exhibit resistance to temozolomide, and develop a distinct profile characterized by a lack of prominent T cell infiltrates and extensive intratumoral heterogeneity. Despite the increased mutation burden, MMR-deficient gliomas paradoxically exhibit a poor response to PD-1 blockade immunotherapy, in contrast to MMR-deficient colorectal cancers where such therapies are effective. Thus, despite the 2.5-month overall survival improvement temozolomide in glioblastoma has shown [24], these acquired deficiencies need to be considered when thinking about the treatment of the disease.

2.6. Tumor mutational burden

Tumor mutational burden (TMB) reflects the number of mutations tumor cells have. It is believed that the greater the number of mutations, the greater the number of potentially tumor-specific neoantigens (antigens arising from somatic mutations). These neoantigens could then be targeted by the immune system [25]. In non-CNS tumors, it has been shown that higher tumor mutational burden independently and positively predicted immunotherapy response [26]. However, GB has been associated with low tumor mutational burden with Hodges et al. showing only 3.5% of GB sampled

were considered to have high tumor mutational burden [27]. Interestingly, higher tumor mutational burden has been associated with decreased overall survival in diffuse gliomas except for in GB where it did not show a significant difference in overall survival [28]. One hypothesis for this was that the high proliferative activity of these non-GB diffuse gliomas with high TMB contributed to this observed phenomenon of decreased overall survival. Still, regardless of how mutational burden affects factors like proliferative activity, the low TMB associated with GB suggests that this cancer frequently has fewer neoantigens to mount an immune response to and tailor immunotherapy for.

Supporting these findings, Gromeier et al. [29] discussed the association between high TMB and the response to immune checkpoint blockade in various cancer types and highlighted gliomas as an exception. Their study showed that patients with recurrent GB and low TMB had prolonged survival following treatment with immune checkpoint inhibitors. They then demonstrated that recurrent GB tumors with lower TMB levels had enriched inflammatory gene signatures compared with those with higher TMB, but not in primary glioblastoma tumors. These findings indicate that the relationship between tumor-intrinsic inflammation and TMB emerges during the course of disease recurrence in glioblastoma. In conclusion, the study suggested that the enrichment of inflammatory signatures in combination with TMB suppression upon recurrence may explain the association between very low TMB, and prolonged survival with immune checkpoint blockade.

3. Glioblastoma immunotherapy clinical trials

There are over a hundred clinical trials documented involving immunotherapy with GB at the time of writing. However, in our literature search, we have only identified six Phase III clinical trials (Table 1) and no Phase IV clinical trials. We have chosen to focus on reporting on the findings of these Phase III trials as they hold the most clinical relevance. The focus of our literature review was to determine if any positive mortality-related outcomes with immunotherapy had been demonstrated when compared with GB's current standard of care. Thus, markers such as progression-free survival and overall survival were most critically assessed. Progression-free survival is the length of time before a disease worsens, and overall survival is the time from initiation of investigation observation to death.

3.1. Immune checkpoint inhibitors

Immune checkpoint inhibitors have had little success in treating GB thus far. While these therapies have been beneficial in treating other malignancies, their effect on GB has been limited for reasons not completely known [30]. Several trials are ongoing involving agents that block PD-1 or PD-L1 which are involved in T-cell response to tumor cells. PD-1 is expressed on T-cells and it binds to PD-L1 on tumor cells which results in several events that benefit the tumor cell such as T-cell apoptosis, T-cell anergy, stimulation of T-regs and decrease in Natural killer cells [30]. Monoclonal antibodies that can bind to either PD-1 or PD-L1 can thus prevent the bindings and allow for the T-cell to target and destroy the tumor cell. So far, a leading theory suggests that the reason PD-1 and PD-L1 monoclonal antibodies have not shown increased survival is due to the immunosuppressive tumor microenvironment of GB. Several clinical trials have been done looking at the efficacy of these immune checkpoint inhibitors on GB and to date, none have had promising results. Many of these trials are Phase I and Phase II trials but, some Phase III have been completed.

CheckMate 143 was a Phase III randomized clinical trial comparing the effects of nivolumab, a PD-1 monoclonal antibody, against bevacizumab, a VEGF monoclonal antibody. A total of 369 subjects with recurrent GB were randomized to one of the two drugs. The median overall survival in the nivolumab arm was 9.8 months (95% CI, 8.2–11.8), and for the bevacizumab arm was 10.0 months (95% CI, 9.0–11.8) and a 12-month overall survival of 42% in both. By 27 months, every participant had died [31].

Another Phase III clinical trial, CheckMate 498 was a randomized trial comparing nivolumab + RT to the standard of care of temozolomide + RT. A total of 560 patients were randomized and results showed median overall survival was 13.4 months (95% CI, 12.6 to 14.3) with nivolumab + RT and 14.9 months (95% CI, 13.3 to 16.1) with temozolomide + RT (hazard ratio [HR], 1.31; 95% CI, 1.09 to 1.58; $p = 0.0037$) [32].

A third Phase III clinical trial, CheckMate 548, looked at nivolumab + standard of care (temozolomide + RT) against placebo + standard of care. This study randomized a total of 716 patients with newly diagnosed GB with methylated or indeterminate MGMT promoter 1:1 to these two groups. The study found that the mean overall survival for the nivolumab group was 28.9 months (95% CI, 24.4–31.6) vs the placebo group 32.1 months (95% CI, 29.4–33.8) and (HR, 1.1; 95% CI, 0.9–1.3). Thus, nivolumab did not improve survival in this trial [33].

3.2. Peptide vaccines

Vaccines have been developed as a potential therapeutic tool for GB. Peptide vaccines can be used to target different tumor-specific antigens that are found on GB. These tumor-specific antigens are specific to the tumor

Table 1. Summary of the Phase III clinical trials involving immunotherapy treatment of glioblastoma.

Type of immunotherapy	Trial comparison	Clinical trial
Immune Checkpoint inhibitors	Nivolumab versus Bevacizumab	CheckMate 143
	Nivolumab versus RT	CheckMate 498
	Nivolumab + Temozolomide + RT against placebo + Temozolomide + RT	CheckMate 548
Peptide vaccines	Rindopepimut + Temozolomide versus control injections + Temozolomide	ACT IV
	DSP-788 vaccine + Bevacizumab versus Bevacizumab alone	NCT03149003
Dendritic cell vaccines	Autologous tumor lysate-loaded dendritic cell vaccination + standard of care (surgery, radiotherapy and Temozolomide) versus standard of care alone	DCVax-L vaccine trial
CAR-T		There are no Phase III clinical trials

cells which provide an ideal target to prevent collateral damage to normal tissues [34]. To date, there have been several trials involving various tumor-specific antigens, but there are only two completed Phase III trials. One of these trials used Rindopepimut (an EGFRvIIII-based vaccine) and the other used DSP-788 (A Wilms tumor 1-based vaccine).

Rindopepimut is a vaccination that targets epidermal growth factor receptor variant III (EGFRvIII) which is found in 24–67% of GB cases where *EGFR* is overexpressed [35]. *EGFR* itself is overexpressed in approximately 40% of all GB cases [35]. This vaccine conjugates a specific peptide (PEP-3) of EGFRvIII to keyhole limpet hemocyanin (KLH), a protein that induces immune cellular response [36].

NCT00643097 was a Phase II clinical trial that led to a Phase III trial and assessed the immune responses to adjuvant Rindopepimut in patients with newly diagnosed GB. The study had three arms. In arm 1, patients received Rindopepimut and sargramostim (GM-CSF). In arm 2, patients received 3 initial vaccinations of Rindopepimut and sargramostim + temozolomide for the first 5 days of a 28-day cycle. In arm 3, patients received 3 initial vaccinations of Rindopepimut and sargramostim + temozolomide for the first 21 days of a 28-day cycle. A total of 40 subjects were enrolled and split into the three different arms with 18, 12 and 10 subjects in each group, respectively. The primary outcome of progression-free survival for Arm 1 was 14.2 months (95% CI, 9.9–17.6), Arm 2 was 12.1 months (95% CI, 10.5–23.7) and Arm 3 11.6 months (95% CI, 8.1–12.7) [37].

A subsequent Phase II trial (ACT III) administered Rindopepimut and standard adjuvant temozolomide chemotherapy to 65 patients with newly diagnosed EGFRvIII-expressing (EGFRvIII+) GB after gross total resection and chemoradiation. Progression-free survival at 5.5 months (~8.5 mo from diagnosis) was 66%. Relative to the study entry, the median overall survival was 21.8 months (95% CI 17.9–26.5 months), and 36-month overall survival was 26%. After more than 3 months of vaccine therapy, 6 tumor samples from the participants were collected. In 4/6 of these samples, EGFRvIII protein expression was absent [38].

Finally, there was a Phase III double-blind trial (ACT IV) that randomized 745 eligible participants to either a Rindopepimut treatment group or a control group. In each group, participants would receive monthly intradermal injections with the conjugated vaccine or just the KLH antigen until disease progression or intolerance. Both groups would also be undergoing concurrent temozolomide treatment alongside the injections. Participant eligibility required GB patients to have undergone maximal surgical resection and completed standard radiation with temozolomide. With intention-to-treat analysis, the median overall survival of the Rindopepimut group was 17.4 months (95% CI 16.1–19.4 months) and 17.4 months (95% CI 16.2–18.8 months) in the control group. Thus, the trial found no significant difference in median overall survival between the two study groups. Similarly, there was no significant difference found in progression-free survival between the 2 groups with a hazard ratio of 0.94 (95% CI 0.79–1.13). Progression-free survival median time in the Rindopepimut group was 7.1 months (95% CI 5.4–7.9 months) and 5.6 months (95% CI 5.1–7.1 months) in the control group [39].

NCT03149003 was a Phase III clinical trial that tested the DSP-788 vaccine with bevacizumab versus bevacizumab alone in patients with recurrent or progressive GB following initial therapy [40]. This trial followed after the Phase I study of the DSP-788 vaccine found that the vaccine was well tolerated with no dose-limiting toxicities [41]. The DSP-788 vaccine is comprised of synthetic peptides from Wilms' tumor 1 (WT1) which is expressed in GB tumor cells [41]. In a study investigating WT1 expression in GB, 48 out of 51 samples showed immunohistochemically positive staining of WT1 protein [42]. The Phase III study enrolled 221 subjects who were randomized to receive either DSP-788 vaccine + bevacizumab or just bevacizumab. The primary outcome was overall survival starting 4 weeks after the patient was off study treatment until death or up to 24 months. The

median survival for subjects who received DSP-788 was 10.2 months (95% CI, 8.2–11.4), and for the bevacizumab group, it was 9.4 (95% CI, 7.4–10.3) [40].

3.3. Dendritic cell vaccines

A dendritic cell is an antigen-presenting cell that has the ability to activate native effector T cells which can then target and destroy tumor cells [43,44]. Being able to activate the innate and adaptive immune response is ideal when targeting a tumor such as GB that is largely protected by the blood–brain barrier and its own tumor microenvironment. It has been shown that dendritic cells are present in primary and recurrent GB and interact with other immune cells [45]. To date, there has only been one Phase III clinical trial involving dendritic cell vaccines in the treatment of GB. Fortunately, there have been several Phase II clinical trials using this treatment modality which is promising for additional Phase III trials in the future.

The only Phase III trial on dendritic cell vaccines to date tested the DCVax-L vaccine [46]. This vaccine is an autologous tumor lysate-loaded dendritic cell vaccination. The vaccine was used in patients with GB who also received standard of care (surgery, radiotherapy and temozolomide) and compared with an external control who received standard of care alone. A total of 331 subjects were enrolled in the study. In subjects who received the vaccine, the median overall survival was 19.3 months (95% CI, 17.5–21.3), compared with the external control patients who had a median overall survival of 16.5 months (95% CI, 16.0–17.5) (HR = 0.80; 98% CI, 0.00–0.94; $p = 0.002$) [46]. This was found to be both clinically significant and statistically significant improvement for these patients.

3.4. CAR-T

Chimeric antigen receptors (CARs) are chimeric molecules that are synthetic antigen-specific receptors placed on T-cells [30]. These molecules are composed of three components including a targeting moiety, a transmembrane domain and an intracellular domain [30,47]. The engineered targeting moiety attached to the T-cell is used to identify and attach to the tumor cell allowing the T-cell to destroy it. To date, there have been three primary targets of CAR-T therapy for GB, which include IL-13 Rα2, HER2 and EGFRvIII [48]. IL-13 Rα2 is expressed in 75% of GB and linked to poor prognosis. HER2 is a heavily researched receptor that is involved in many other cancers, most notably breast cancer. HER2 has been considered an ideal tumor-associated antigen for [48]. As mentioned previously, *EGFRvIII* is an oncogene that is found in approximately 40% of the 24–67% GB that over-express *EGFR* [48]. Most trials to date on CAR-T therapy for GB are Phase I trials, however, there have been some phase two trials. These trials have been focused on EGFRvIII and one on B7H3 which is expressed in 70% of GB and not expressed in normal tissues which makes it an ideal target antigen. To date, there have been no Phase III clinic trials on CAR-T therapy for GB.

4. Conclusion & future perspective

At the time of writing, immunotherapy is not part of the standard of care for GB. With over a hundred clinical trials involving immunotherapy in GB, there is a clear interest in determining the role of these treatment modalities in GB management. The only clinical trial that has shown statistically significant survival benefit when compared with standard of care is that using the DCVax-L vaccine.

This DCVax-L clinical trial's methodology is discussed in detail by Gatto et al. [49], but it is essential to note that the significant increase in overall survival statistic was generated by comparing the DCVax-L vaccine group to an external control group. The trial's crossover design where participants were allowed to cross over to start receiving the vaccine after tumor recurrence led to most of the control group being given the DCVax-L treatment. Because of this, a new external control group was created.

This external control group was created from five Phase III GB studies with a control arm treated with RT and a temozolomide regimen. This methodology leads to limitations in its applicability. One of these issues was that the treatment group in the trial only included patients with gross or near total resection of their tumor with the disease confined to one hemisphere. This inclusion criterion was not present in the studies the external control group was formed from. Thus, there were characteristic differences between the treatment group and the control group they were compared with. In summary, this post-hoc retrospective analysis that compares the DCVax-L intervention arm to the external control group encourages the need for a true randomized control trial using this technology rather than providing definitive evidence of populations that can benefit from this therapy.

Although not the focus of our review, in general, immunotherapy is well tolerated in the trials that have been published so far. So, while there are many Phase I and Phase II trials that support the safety of immunotherapy in GB, there is a paucity of data that supports the effectiveness of immunotherapy in treating GB. Taking note of current effect sizes, we have not found any evidence that immunotherapy could be offered as a curative option for GB at present.

From the low tumor mutational burden to the tumor microenvironment, there are several factors that make

GB both very pro-tumorigenic and resistant to treatment. Yet, with further elucidation of the biology of GB, advancement of immunotherapy and many clinical trials, significant improvements in glioblastoma treatment with immunotherapy appear possible. Evidently, much further study will be required if this possibility does exist. To summarize this literature review, the present clinical landscape of glioblastoma immunotherapy is one requiring further investigation to determine if it has a significant role in improving survival with the disease.

Article highlights

- Glioblastoma is an aggressive, grade 4 brain tumor originating from neuroglial stem or progenitor cells, associated with poor prognosis and quality of life.
- The current standard of care is a maximal safe surgical resection followed by radiation therapy and chemotherapy with temozolomide.
- This review investigates the features that make glioblastoma difficult to treat and the results of glioblastoma immunotherapy clinical trials so far.

Glioblastoma mechanisms of treatment resistance

- Many aspects of glioblastoma create treatment resistance including a blood–tumor barrier, high vascularity, repair mechanisms and an immunosuppressive tumor immune microenvironment.

Glioblastoma immunotherapy clinical trials

- There have been Phase III clinical trials using checkpoint inhibitors, and peptide vaccines, dendritic cell vaccines in glioblastoma.
- To date, the DCVax-L dendritic cell vaccine is the only immunotherapy treatment with a Phase III trial showing statistically significant survival benefit, but with methodological limitations including using an external control group for comparisons.

Conclusion

- There are over a hundred clinical trials involving the use of immunotherapy with glioblastoma suggesting interest and investigation into its utility.
- The present clinical landscape of glioblastoma immunotherapy is one requiring further investigation to determine if it has a significant role in improving survival with the disease.

Competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Writing disclosure

No funded writing assistance was utilized in the production of this manuscript.

ORCID

Andrew Timothy Ng https://orcid.org/0000-0002-5490-3251
Tyler Steve https://orcid.org/0009-0005-5543-3991
Kevin T Jamouss https://orcid.org/0000-0001-5039-6013
Sarah Kawtharani https://orcid.org/0009-0009-4310-4655
Hazem I Assi https://orcid.org/0000-0002-8483-0938

References

1. Le Rhun E, Preusser M, Roth P, et al. Molecular targeted therapy of glioblastoma. Cancer Treat Rev. 2019;80:101896. doi:10.1016/j.ctrv.2019.101896
2. Omuro A. Glioblastoma and other malignant gliomas. JAMA. 2013;310(17):1842. doi:10.1001/jama.2013.280319
3. Wirsching H-G, Galanis E, Weller M. Glioblastoma. Handb Clin Neurol. 2016;134:381–397. doi:10.1016/B978-0-12-802997-8.00023-2
4. WHO Classification of Tumours Editorial Board. Central Nervous System Tumours. 5th ed. Vol 6. Lyon, France: IARC; 2021.
5. Stoyanov GS, Lyutfi E, Georgieva R, et al. Reclassification of Glioblastoma Multiforme According to the 2021 World Health Organization Classification of Central Nervous System Tumors: A Single Institution Report and Practical Significance. Cureus. 2022;14(2):e21822. doi:10.7759/cureus.21822
6. National Comprehensive Cancer Network. 'Central Nervous System Cancers (Version 1.2023)'. 2023. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf
7. Ostrom QT, Rubin JB, Lathia JD, Berens ME, Barnholtz-Sloan JS. Females have the survival advantage in glioblastoma. Neuro Oncol. 2018;20(4):576–577. doi:10.1093/neuonc/noy002
8. Carrano A, Juarez JJ, Incontri D, Ibarra A, Guerrero Cazares H. Sex-specific differences in glioblastoma. Cells. 2021;10(7):1783. doi:10.3390/cells10071783
9. Gimple RC, Bhargava S, Dixit D, Rich JN. Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. Genes Dev. 2019;33(11–12):591–609. doi:10.1101/gad.324301.119
10. Da Ros M, De Gregorio V, Iorio AL, et al. Glioblastoma chemoresistance: the double play by microenvironment and blood–brain barrier. Int J Mol Sci. 2018;19(10):2879. doi:10.3390/ijms19102879
11. Sharma P, Aaroe A, Liang J, Puduvalli VK. Tumor microenvironment in glioblastoma: current and emerging concepts. Neurooncol Adv. 2023;5(1):vdad009. doi:10.1093/noajnl/vdad009
12. Andersen RS, Anand A, Harwood DSL, Kristensen BW. Tumor-associated microglia and macrophages in the glioblastoma microenvironment and their implications for therapy. Cancers (Basel). 2021;13(17):4255. doi:10.3390/cancers13174255
13. Yuan B, Wang G, Tang X, Tong A, Zhou L. Immunotherapy of glioblastoma: recent advances and future prospects. Hum Vaccin Immunother. 2022;18(5):2055417. doi:10.1080/21645515.2022.2055417
14. Chen Z, Hambardzumyan D. Immune microenvironment in glioblastoma subtypes. Front Immunol. 2018;9:1004. doi:10.3389/fimmu.2018.01004
15. Pacheco C, Martins C, Monteiro J, Baltazar F, Costa BM, Sarmento B. Glioblastoma vasculature: from its critical role in tumor survival to relevant *in vitro* modelling. Frontiers in Drug Delivery. 2022;2:823412. doi:10.3389/fddev.2022.823412

16. Fu M, Zhou Z, Huang X, et al. Use of Bevacizumab in recurrent glioblastoma: a scoping review and evidence map. BMC Cancer. 2023;23(1):544. doi:10.1186/s12885-023-11043-6
17. Ahir BK, Engelhard HH, Lakka SS. Tumor development and angiogenesis in adult brain tumor: glioblastoma. Mol Neurobiol. 2020;57(5):2461–2478. doi:10.1007/s12035-020-01892-8
18. Mao J-M, Liu J, Guo G, Mao X-G, Li C-X. Glioblastoma vasculogenic mimicry: signaling pathways progression and potential anti-angiogenesis targets. Biomark Res. 2015;3:8. doi:10.1186/s40364-015-0034-3
19. Steeg PS. The blood-tumour barrier in cancer biology and therapy. Nat Rev Clin Oncol. 2021;18(11):696–714. doi:10.1038/s41571-021-00529-6
20. Marcucci F, Corti A, Ferreri AJM. Breaching the blood–brain tumor barrier for tumor therapy. Cancers (Basel). 2021;13(10):2391. doi:10.3390/cancers13102391
21. Jiapaer S, Furuta T, Tanaka S, Kitabayashi T, Nakada M. Potential strategies overcoming the temozolomide resistance for glioblastoma. Neurol Med Chir (Tokyo). 2018;58(10):405–421. doi:10.2176/nmc.ra.2018-0141
22. Cahill DP, Levine KK, Betensky RA, et al. Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. Clin Cancer Res. 2007;13(7):2038–2045. doi:10.1158/1078-0432.CCR-06-2149
23. Touat M, Li YY, Boynton AN, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. Nature. 2020;580(7804):517–523. doi:10.1038/s41586-020-2209-9
24. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352(10):987–996. doi:10.1056/NEJMoa043330
25. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The challenges of tumor mutational burden as an immunotherapy biomarker. Cancer Cell. 2021;39(2):154–173. doi:10.1016/j.ccell.2020.10.001
26. Goodman AM, Kato S, Bazhenova L, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. Mol Cancer Ther. 2017;16(11):2598–2608. doi:10.1158/1535-7163.MCT-17-0386
27. Hodges TR, Ott M, Xiu J, et al. Mutational burden, immune checkpoint expression, and mismatch repair in glioma: implications for immune checkpoint immunotherapy. Neuro Oncol. 2017;19(8):1047–1057. doi:10.1093/neuonc/nox026
28. Wang L, Ge J, Lan Y, et al. Tumor mutational burden is associated with poor outcomes in diffuse glioma. BMC Cancer. 2020;20(1):213. doi:10.1186/s12885-020-6658-1
29. Gromeier M, Brown MC, Zhang G, et al. Very low mutation burden is a feature of inflamed recurrent glioblastomas responsive to cancer immunotherapy. Nat Commun. 2021;12(1):352. doi:10.1038/s41467-020-20469-6
30. Mahmoud AB, Ajina R, Aref S, et al. Advances in immunotherapy for glioblastoma multiforme. Front Immunol. 2022;13:944452. doi:10.3389/fimmu.2022.944452
31. Reardon DA, Brandes AA, Omuro A, et al. Effect of Nivolumab vs bevacizumab in patients with recurrent glioblastoma. JAMA Oncol. 2020;6(7):1003. doi:10.1001/jamaoncol.2020.1024
32. Omuro A, Brandes AA, Carpentier AF, et al. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated *MGMT* promoter: an international randomized Phase III trial. Neuro Oncol. 2023;25(1):123–134. doi:10.1093/neuonc/noac099
33. Lim M, Weller M, Idbaih A, et al. Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated *MGMT* promoter. Neuro Oncol. 2022;24(11):1935–1949. doi:10.1093/neuonc/noac116
34. Huang B, Li X, Li Y, Zhang J, Zong Z, Zhang H. Current Immunotherapies for Glioblastoma Multiforme. Front Immunol. 2021;11:603911. doi:10.3389/fimmu.2020.603911
35. Heimberger AB, Suki D, Yang D, Shi W, Aldape K. The natural history of EGFR and EGFRvIII in glioblastoma patients. J Transl Med. 2005;3:38. doi:10.1186/1479-5876-3-38
36. Heimberger AB, Sampson JH. The PEPvIII-KLH (CDX-110) vaccine in glioblastoma multiforme patients. Expert Opin Biol Ther. 2009;9(8):1087–1098. doi:10.1517/14712590903124346
37. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol. 2010;28(31):4722–4729. doi:10.1200/JCO.2010.28.6963
38. Schuster J, Lai RK, Recht LD, et al. A Phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro Oncol. 2015;17(6):854–861. doi:10.1093/neuonc/nou348
39. Weller M, Butowski N, Tran DD, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international Phase III trial. Lancet Oncol. 2017;18(10):1373–1385. doi:10.1016/S1470-2045(17)30517-X
40. A Study of DSP-7888 Dosing Emulsion in Combination With Bevacizumab in Patients With Recurrent or Progressive Glioblastoma Following Initial Therapy. ClinicalTrials.gov identifier: NCT03149003. Updated November 15, 2023. Accessed 25 October 2024. https://clinicaltrials.gov/study/NCT03149003
41. Spira A, Hansen AR, Harb WA, et al. Multicenter, Open-Label, Phase I Study of DSP-7888 Dosing Emulsion in Patients with Advanced Malignancies. Target Oncol. 2021;16(4):461–469. doi:10.1007/s11523-021-00813-6
42. Nakahara Y, Okamoto H, Mineta T, Tabuchi K. Expression of the Wilms' tumor gene product WT1 in glioblastomas and medulloblastomas. Brain Tumor Pathol. 2004;21:113–116. doi:10.1007/BF02482185
43. Calmeiro J, Carrascal MA, Tavares AR, et al. Dendritic cell vaccines for cancer immunotherapy: the role of human conventional type 1 dendritic cells. Pharmaceutics. 2020;12(2):158. doi:10.3390/pharmaceutics12020158

44. Li L, Zhou J, Dong X, Liao Q, Zhou D, Zhou Y. Dendritic cell vaccines for glioblastoma fail to complete clinical translation: bottlenecks and potential countermeasures. Int Immunopharmacol. 2022;109:108929. doi:10.1016/j.intimp.2022.108929
45. Friedrich M, Hahn M, Michel J, et al. Dysfunctional dendritic cells limit antigen-specific T cell response in glioma. Neuro Oncol. 2023;25(2):263–276. doi:10.1093/neuonc/noac138
46. Liau LM, Ashkan K, Brem S, et al. Association of autologous tumor lysate-loaded dendritic cell vaccination with extension of survival among patients with newly diagnosed and recurrent glioblastoma. JAMA Oncol. 2023;9(1):112. doi:10.1001/jamaoncol.2022.5370
47. Lin Y-J, Mashouf LA, Lim M. CAR T cell therapy in primary brain tumors: current investigations and the future. Front Immunol. 2022;13:817296. doi:10.3389/fimmu.2022.817296
48. Bagley SJ, Desai AS, Linette GP, June CH, O'Rourke DM. CAR T-cell therapy for glioblastoma: recent clinical advances and future challenges. Neuro Oncol. 2018;20(11):1429–1438. doi:10.1093/neuonc/noy032
49. Gatto L, Di Nunno V, Tosoni A, Bartolini S, Ranieri L, Franceschi E. DCVax-L vaccination in patients with glioblastoma: real promise or negative trial? The debate is open. Cancers (Basel). 2023;15(12):3251. doi:10.3390/cancers15123251