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The landscape of immune checkpoint inhibitor clinical trials in glioblastoma: A systematic review

Ethan Schonfeld, John Choi, Andrew Tran, Lily H. Kim, and Michael Lim

All author affiliations are listed at the end of the article

Corresponding Author: Michael Lim, MD, Department of Neurosurgery, Stanford University School of Medicine, Stanford University, 453 Quarry Road Neurosurgery 5327, Palo Alto, CA 94304, USA (mklim@stanford.edu).

Abstract

Background. Glioblastoma is characterized by rapid tumor growth and high invasiveness. The tumor microenvironment of glioblastoma is highly immunosuppressive with both intrinsic and adaptive resistance mechanisms that result in disease recurrence despite current immunotherapeutic strategies.

Methods. In this systematic review of clinical trials involving immunotherapy for glioblastoma using ClinicalTrials.gov and PubMed databases from 2016 and onward, we explore immunotherapeutic modalities involving immune checkpoint blockade (ICB).

Results. A total of 106 clinical trials were identified, 18 with clinical outcomes. ICB in glioblastoma has failed to improve overall survival compared to the current standard of care, including those therapies inhibiting multiple checkpoints. Among all immune checkpoint trials, targets included programmed cell death protein-1 (PD-1) (35/48), PD-L1 (12/48), cytotoxic T-lymphocyte-associated protein-4 (6/48), TIGIT (2/48), B7-H3 (2/48), and TIM-3 (1/48). Preliminary results from combination immunotherapies (32.1% of all trials) demonstrated improved treatment efficacy compared to monotherapy, specifically those combining checkpoint therapy with another immunotherapy modality.

Conclusions. Clinical trials involving ICB strategies for glioblastoma have not demonstrated improved survival. Comparison of therapeutic efficacy across trials was limited due to heterogeneity in the study population and outcome operationalization. Standardization of future trials could facilitate comparison across immunotherapy modalities for robust meta-analysis. Current immunotherapy trials have shifted focus toward combination strategies; preliminary results suggest that they are more encouraging than mono-modality immunotherapies. Given the intrinsic heterogeneity of glioblastoma, the utilization of immune markers will be key for the development of future immunotherapy approaches.

Key Points

- Monotherapy immune checkpoint blockade clinical trials have limited treatment efficacy in glioblastoma. Current immunotherapy strategies in glioblastoma are now focusing on combining checkpoint therapy with other immunotherapy modalities.
- Given the heterogeneity of glioblastoma, updated standardized trial designs are needed to allow for more comprehensive profiling of specific glioblastoma subsets.
- Elucidating immune biomarkers is key to enriching subsets of patients who may have durable clinical response.

Importance of the Study

This systematic review of 106 immunotherapy clinical trials in glioblastoma since 2016 highlights the limited efficacy of immune checkpoint inhibitors alone, underscoring the rising prevalence of multimodal combinations, which comprised 32.1% of all trials. Of these multimodal strategies, some promising results came from combination therapies that targeted immune checkpoints as well as immunosuppressive myeloid populations. Despite the high volume of data that has resulted from these clinical trials, meaningful comparisons across studies were limited due to the heterogeneity of study populations and endpoints. Standardized strategies for trial design and endpoints across institutions would help uncover meta-findings from these trials. Furthermore, some studies demonstrate a small subset of patients who have durable clinical response to combination immunotherapy, emphasizing the need for reliable immune markers to guide personalized and targeted therapies. Ultimately, the goal is to develop personalized, biomarker-driven combination immune strategies to meet the unique molecular challenges that patients face with glioblastoma.

Glioblastoma is one of the most aggressive primary central nervous system (CNS) malignancies, with a median overall survival (OS) of less than 2 years[1] and a 5-year survival rate of 5%[2], despite the current standard of care involving gross total surgical resection, chemotherapy, and radiation.[3] Glioblastoma exhibits aggressive characteristics, including rapid growth,[4] high invasiveness,[5] inter- and intratumor heterogeneity,[6,7] and a high propensity for recurrence.[8] Given the poor efficacy of chemoradiation that has not improved patient outcomes since 2005 as well as recent investigations that have elucidated a highly immunosuppressive environment,[9] there has been increased interest in exploring immunotherapeutic strategies. These endeavors encompass a spectrum of approaches, including immune checkpoint blockade (ICB) or immune checkpoint inhibition, dendritic cell (DC) vaccines, adoptive cell therapies, other tumor microenvironment immunomodulators, and different combinations of these methods.

Given its positive impact on other cancers, immunotherapy has emerged as an exciting avenue for the treatment of CNS malignancies. In theory, this treatment modality targets tumors by disrupting mechanisms of immune evasion, including immune checkpoint activation (eg, activation of CD137) or blockade (eg, inhibition of programmed cell death protein-1 [PD-1]),[10] decreased trafficking or reversal of immunosuppressive myeloid cells,[11] and increasing exposure and priming of antigen-presenting cells to tumor antigens.[12] Antibody blocking of PD-1 and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) have now become standard of care in advanced metastatic melanoma and non–small cell lung cancer with durable clinical response rates.[1,13] Given this success for other cancers,[14,15] there has been a concerted effort to translate these approaches to glioblastoma. In 2014, the Phase III CheckMate 143 trial compared anti-PD-1 therapy to the standard-of-care bevacizumab for patients with recurrent glioblastoma. While CheckMate 143 did not meet its primary endpoint, it evidenced a comparable safety profile of anti-PD-1 in glioblastoma compared to other tumors, as well as similar clinical outcomes to standard of care.[16] Over the next decade, trials have evaluated immune checkpoint therapy in other glioblastoma patient populations (eg, newly diagnosed glioblastoma with different molecular markers)[17,18] as well as in combination with other immunotherapeutic strategies.

This systematic review serves as the most up-to-date compendium of clinical trials for immunotherapies involving immune checkpoint strategies in glioblastoma from 2016 through 2023, aiming to summarize recent investigations of ICB and their implications on clinical, radiographic, and biological outcomes; evaluate the safety profiles of these treatments; identify promising biomarkers for combination treatment; and provide insights to inform and guide the future development of ICB and other immunotherapy strategies in glioblastoma.

Methods

The methods of this systematic review were implemented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Search Strategy

A comprehensive literature search was performed in two databases: ClinicalTrials.gov and PubMed via pubmed.ncbi.nlm.nih.gov. ClinicalTrials.gov search strategy employed Advanced Search with the Find a Study tool: Glioblastoma (Condition or disease), Immunotherapy (Other terms), Interventional Studies (ClinicalTrials) (Study type), All studies (Study Results) From 01/01/2016 (Study Start). The query was performed on July 24, 2023. PubMed was queried with: (glioblastoma) AND (immunotherapy) OR (checkpoint) OR (vaccine) OR (adoptive cell therapy) OR (tumor microenvironment) OR (cytokine), while selecting Clinical Trial (Article Type), Humans (Species), English (Article Language). The PubMed query was performed on August 31, 2023.

Initial Screening Using Eligibility Criteria

A manual review of all articles and trials identified from the search strategy was carried out for relevance by two reviewers. Two researchers (E.S. and A.T.) independently evaluated each manuscript or trial based on the eligibility

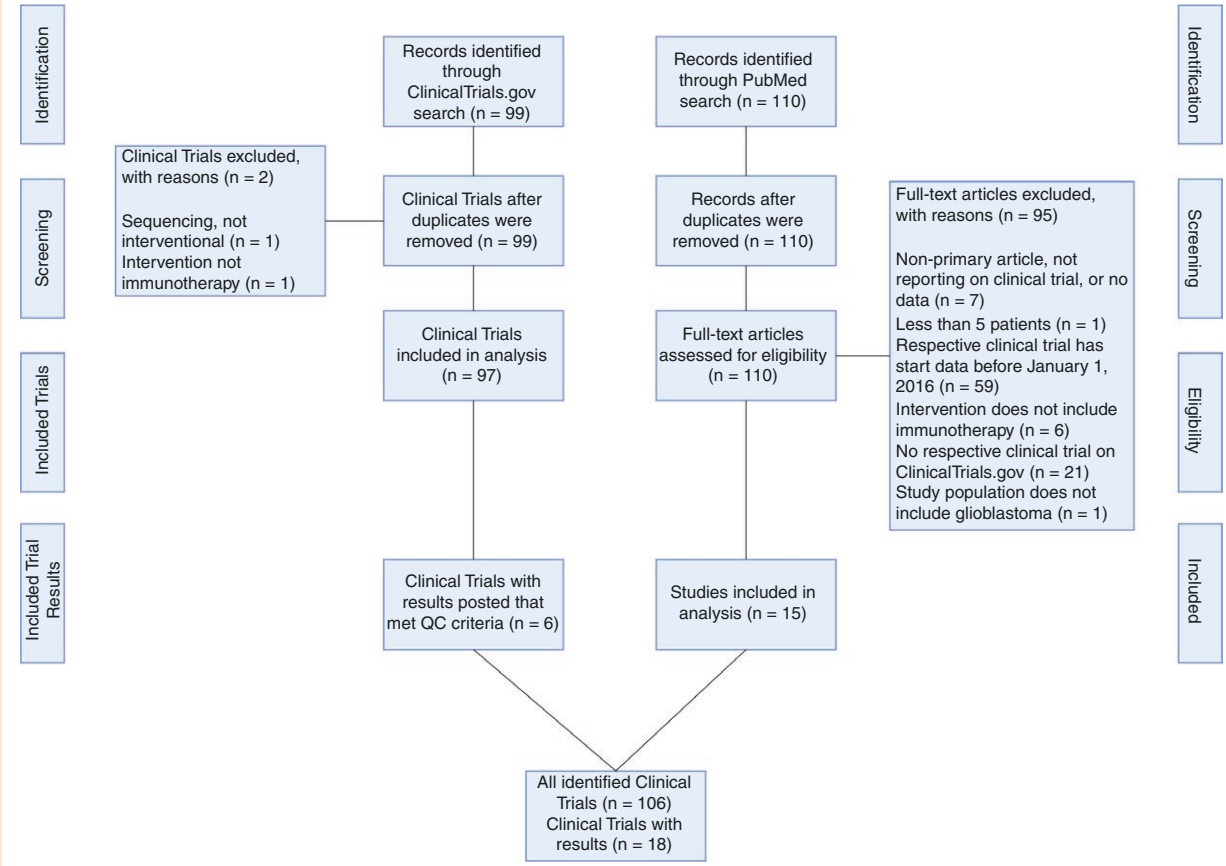

Figure 1. PRISMA flow diagram of study selection process for glioblastoma immunotherapy clinical trials, 106 clinical trials were identified from 97 clinicaltrials.gov records and 15 articles from PubMed review. Eighteen clinical trials had results available.

criteria below. Discrepancies in selection were resolved through author discussion. Eligible articles were selected for data extraction (Figure 1). No automation tools were used in the process.

Inclusion Criteria

The following inclusion criteria were employed: (1) clinical trials or manuscripts with human glioblastoma patients, (2) clinical trial start date on or after January 1, 2016. To identify relevant manuscripts, a search was conducted on PubMed for studies published on or after January 1, 2016; the queried studies were then matched up to a clinical trial with a known start date, (3) the manuscript or clinical trial reports results from a primary investigation of intervention that includes some immunotherapy.

Exclusion Criteria

Using full-text review, studies that were not registered on ClinicalTrials.gov with a National Clinical Trial number were excluded. Manuscripts featuring fewer than 5 patients, nonprimary articles (ie, review articles, editorials), non-English articles, and observational studies were also excluded.

Data Extraction and Analysis

Two researchers (E.S. and A.T.) collected the data items in parallel. One researcher (E.S.) collected all data items extracted from ClinicalTrials.gov, while the other researcher (A.T.) collected all data items from PubMed. All data items were independently reviewed by the other researcher. No data were obtained or confirmed from the study investigators, and only readily accessible data were extracted. No data collection automation tools were used.

All clinical trials registered after January 1, 2016, on ClinicalTrials.gov with data deposited that had passed quality check were included after passing quality checks. All primary and secondary outcomes included in the deposited data were collected. The most common outcome variables were used to define the data items considered for the systematic review of the PubMed database as outlined above (with specific attention paid to survival and radiographic outcomes). Outcomes of interest included OS, progression-free survival (PFS), OS rate at a set time point, all-cause mortality, serious adverse events, and objective response rate (ORR). Results from all time points for each outcome were considered. Other variables collected included: study start date, arm 1 title, arm 2 title, arm 1 number of participants, arm 2 number of participants, number of participants for each outcome, and reported statistical testing for each outcome between arms.

Table 1. All Immunotherapy Glioblastoma Clinical Trial Results

NCT	Title	Phase	Status	Arm(s)	All-Cause Mortality	Serious Adverse Events	OS (Months) 95% CI	OS (12 Months) 95% CI	OS (24 Months) 95% CI	PFS (Months)	PFS (Months) BICR/RANO 95% CI	Objective Response Rate (%)
NCT02667587	An Investigational Immunotherapy Study of Temozolomide Plus Radiation Therapy With Nivolumab or Placebo, for Newly Diagnosed Patients With Glioblastoma (Glioblastoma, a Malignant Brain Cancer)	3	Active	Radiotherapy, temozolomide plus nivolumab	221/355 (62.3%)	254/355 (71.6%)	28.9 (24.4–31.6); *n* = 358	82.7 (78.3–86.3); *n* = 358	55.9 (50.5–61.0); *n* = 358	14.1 (12.6–16.6); *n* = 358	10.64 (8.90–11.79); *n* = 358	–
				Radiotherapy, temozolomide plus placebo	216/354 (61.0%)	214/354 (60.5%)	32.1 (29.4–33.8); *n* = 358	87.7 (83.8–90.8); *n* = 358	63.3 (58–68.2); *n* = 358	15.2 (13.1–17.1); *n* = 358	10.32 (9.69–12.45); *n* = 358	–
NCT02617589	An Investigational Immunotherapy Study of Nivolumab Compared to Temozolomide, Each Given With Radiation Therapy, for Newly diagnosed Patients With Glioblastoma (Glioblastoma, a Malignant Brain Cancer)	3	Completed	Nivolumab + Radiation therapy	269/280 (96.1%)	206/278 (74.1%)	13.4 (12.6–14.3); *n* = 280	–	10.6 (7.3–14.6); *n* = 280	6.01 (5.65–6.21); *n* = 280	–	–
				Temozolomide + Radiation therapy	253/280 (90.4%)	141/275 (51.3%)	14.9 (13.3–16.1); *n* = 280	–	21.2 (16.5–26.3); *n* = 280	6.21 (5.98–6.90); *n* = 280	–	–
NCT03430791	Trial of Combination Tumor Treating Fields (TTF; Optune), Nivolumab Plus/Minus Ipilimumab for Recurrent Glioblastoma	2	Terminated	Nivolumab monotherapy	2/4 (50.0%)	0/4 (0.00%)	–	–	–	2.05 (1.54–2.57); *n* = 4	–	0.0% (*n* = 4)
				Nivolumab + Ipilimumab	–	–	–	–	–	–	–	–
NCT02529072	Nivolumab With DC Vaccines for Recurrent Brain Tumors	1	Completed	Nivolumab preop and Nivolumab + DC vaccine postop	3/3 (100.0%)	1/3 (33.3%)	8.0 (5.7–8.3); *n* = 3	–	–	4.3 (2.1–5.3); *n* = 3	–	–
				Nivolumab + DC pre/postop	2/3 (66.7%)	2/3 (66.7%)	15.3 (4.73–NA); *n* = 3	–	–	6.3 (4.7–10.7); *n* = 3	–	–

Table 1. Continued

NCT	Title	Phase	Status	Arm(s)	All-Cause Mortality	Serious Adverse Events	OS (Months) 95% CI	OS (12 Months) 95% CI	OS (24 Months) 95% CI	PFS (Months)	PFS (Months) BICR/RANO 95% CI	Objective Response Rate (%)
NCT02968940	Avelumab With Hypofractionated Radiation Therapy in Adults With Isocitrate Dehydrogenase (IDH) Mutant Glioblastoma	2	Completed	Avelumab and hypofractionated radiation therapy (HFRT)	4/6 (66.7%)	5/6 (83.3%)	10.1 (6.8–12); *n* = 6	–	–	4.2 (1.4–5.7); *n* = 6	–	–
NCT02858895	Convection-Enhanced Delivery (CED) of MDNA55 in Adults With Recurrent or Progressive Glioblastoma	2	Completed	MDNA55	36/47 (76.6%)	24/47 (51.1%)	11.6 (8.6–15.0); *n* = 44	–	–	3.6 (2.8–5.1); *n* = 41	–	2.4% (*n* = 41)
NCT02709616, NCT02808364	Personalized Cellular Vaccine Therapy in Treating Patients With Glioblastoma (PerCellVac)	1	Completed	Personalized dendritic cell vaccine	–	0% (0/5)	19; *n* = 5	60%; *n* = 5	40%; *n* = 5	–	–	–
NCT03636477	Protocol ATI001-102 Substudy: Evaluation of Ad-RTS-hIL-12 + Veledimex in Combination With Nivolumab in Subjects With Recurrent or Progressive Glioblastoma	1	Completed	Ad + Veledimex (VDX) + Nivolumab	15/21 (71.4%)	9/21 (42.9%)	9.8 (5.2–17.4); *n* = 21	–	–	–	–	–
NCT03400917	Phase II Trial of Autologous Dendritic Cells Loaded With Autologous Tumor-Associated Antigens (AV-Glioblastoma-1) as an Adjunctive Therapy Following Primary Surgery Plus Concurrent Chemoradiation in Patients With Newly Diagnosed Glioblastoma	2	Active	AV-Glioblastoma -1	39/60 (65.0%)	29/57 (50.9%)	16.0 (12.9–20.6); *n* = 60	70.2; *n* = 60	26.8; *n* = 60	–	10.4 (8.6–11.7); *n* = 60	–
NCT03170141	Immunogene-modified Antigen-specific T (IgT)-Cells for the Treatment of Glioblastoma Multiforme	1	Completed	Antigen-specific IgT cells	3/8 (37.5%)	1/8 (12.5%)	10 (3–24); *n* = 8	–	–	–	–	–

Table 1. Continued

NCT	Title	Phase	Status	Arm(s)	All-Cause Mortality	Serious Adverse Events	OS (Months) 95% CI	OS (12 Months) 95% CI	OS (24 Months) 95% CI	PFS (Months)	PFS (Months) BICR/RANO 95% CI	Objective Response Rate (%)
NCT03637764	A Phase I/II Open-label, Multi-Center, Safety, Preliminary Efficacy, and Pharmacokinetic (PK) Study of Isatuximab (SAR650984) in Combination With Atezolizumab or Isatuximab Alone in Patients With Advanced Malignancies	1/2	Terminated	Isatuximab + Atezolizumab	–	10/33 (30.3%)	–	–	–	–	1.30 (1.22–1.38); *n* = 33	0.0%; *n* = 33
NCT03233152	Phase I Clinical Trial on Intratumoral Ipilimumab Plus Intravenous Nivolumab Following the Resection of Recurrent Glioblastoma	1	Recruiting	Ipilimumab + Nivolumab (intracerebral + intravenous)	21/27 (78%)	–	8.75 (6.21–11.28); *n* = 27	40.7 (22–59); *n* = 27	27 (9–44); *n* = 27	2.69 (2.30–2.76); *n* = 27	–	–
NCT02798406	A Phase II, Multi-Center, Open-label Study of a Conditionally Replicative Adenovirus (DNX-2401) With Pembrolizumab (KEYTRUDA®) for Recurrent Glioblastoma or Gliosarcoma (CAPTIVE/KEYNOTE-192)	2	Completed	DNX-2401 + Pembrolizumab	–	–	12.5 (10.8–14.6); *n* = 48	52.7 (40.1–69.2); *n* = 48	–	–	–	10.4% (5/48)
NCT02937844	A Safety and Efficacy Study of Autologous Chimeric Switch Receptor-Engineered T-Cells Redirected to PD-L1 in Patients With Recurrent Glioblastoma Multiforme	1	Unknown	Anti-PD-L1 CSR T-cell + Cyclophosphamide + Fludarabine	–	–	17.0 (9.67–24.33); *n* = 14	–	–	–	–	–
NCT02669173	Targeting Myeloid-Derived Suppressor Cells in Recurrent Glioblastoma: Phase 0/1 Trial of Low Dose Capecitabine + Bevacizumab in Patients With Recurrent Glioblastoma	1	Active	Capecitabine + Bevacizumab	8/11 (72.7%)	0/11 (0.0%)	16.6 (150 mg Capecitabine) 11.5 (300 mg Capecitabine) 9.8 (450 mg Capecitabine)	–	–	–	7.2 (150 mg Capecitabine) 5.5 (300 mg Capecitabine) 7.3 (450 mg Capecitabine)	–

Table 1. Continued

NCT	Title	Phase	Status	Arm(s)	All-Cause Mortality	Serious Adverse Events	OS (Months) 95% CI	OS (12 Months) 95% CI	OS (24 Months) 95% CI	PFS (Months)	PFS (Months) BICR/RANO 95% CI	Objective Response Rate (%)
NCT02661282	A Phase I/II Clinical Trial of Autologous CMV-Specific Cytotoxic T-Cells for Glioblastoma Patients	1/2	Completed	Temozolomide + Cytomegalovirus (CMV)-specific T-cells + Surgery	13/16 (81.3%)	–	12; *n* = 16	50 (31–82); *n* = 16	–	–	1.3 (0–8.3); *n* = 16	–
NCT03043391	Phase Ib Study of Oncolytic Polio/Rhinovirus Recombinant Against Recurrent Malignant Glioma in Children	1	Unknown	PVSRIPO	7/8 (87.5%)	3/8 (37.5%)	4.1 (1.2–10.1); *n* = 8	–	–	–	–	–
NCT03291314	Phase II Clinical Trial on the Combination of Avelumab and Axitinib for the Treatment of Patients With Recurrent Glioblastoma	2	Completed	Axitinib + Avelumab (concerted)	–	–	6.12 (4.79–7.46); *n* = 27	22.2 (6.5–37.9)	–	2.76 (1.89–3.64); *n* = 27	–	33.3%
				Axitinib + Avelumab, (staggered)	–	–	4.14 (2.88–5.41); *n* = 27	11.1 (0– 22.9)	–	2.46 (1.22–3.71); *n* = 27	–	22.2%

Metrics were defined and measured at different time points between studies. Does not include data collected after the primary completion date (*), results for the per protocol cohort, and not the intent-to-treat cohort (**), higher baseline corticosteroid dose determined the cohort, avelumab was added only after 6-week therapy if the corticosteroid dose could be tapered (***), dose escalation was used (****), survival was not the primary objective of the study and thus uncertaints are high in measurement (*****), iRANO criteria (******).

Abbreviations: DC = dendritic cell; OS = overall survival; PFS = progression-free survival.

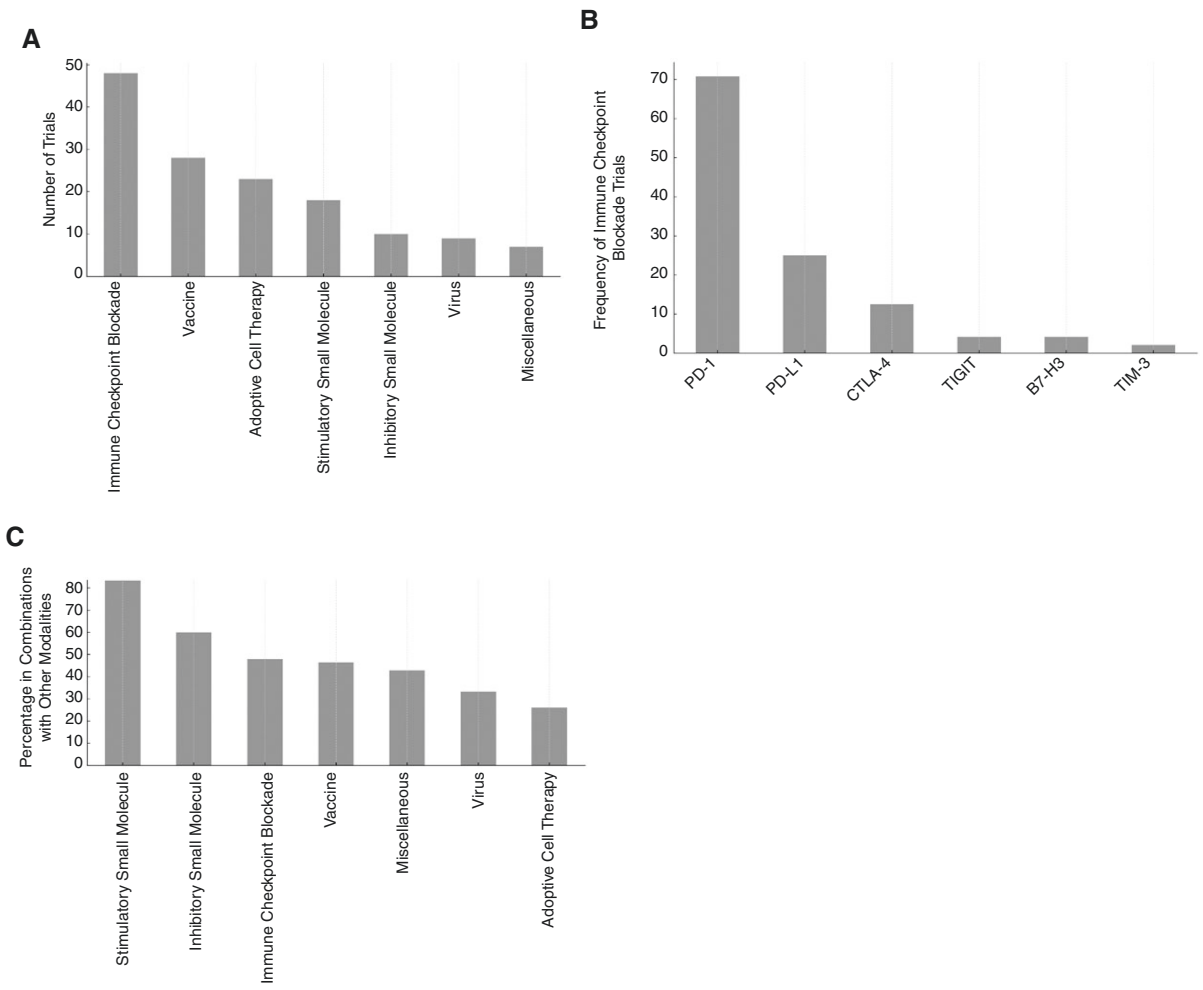

Figure 2. Distribution and characteristics of immunotherapy modalities in glioblastoma clinical trials, (a) distribution of immunotherapy modalities across all clinical trials, (b) distribution of checkpoint inhibitor targets, (c) frequency that each immunotherapy modality is combined with other modalities, demonstrating that a majority of stimulatory small molecule trials and inhibitory small molecule trials combine them with other modalities.

Results from the ClinicalTrials.gov and PubMed were synthesized, if applicable, to identify all National Clinical Trial (NCT)-registered clinical trials and provide the most recent study outcomes for each clinical trial. Eligibility for synthesis and clinical summation required the studies to have matching NCT numbers, with preference given to the most recent result for each outcome. The number of available studies, the number of participants, differing patient populations (eg, recurrent glioblastoma, isocitrate dehydrogenase [IDH] wild-type glioblastoma, newly diagnosed, glioblastoma), and heterogenous time points used to define the clinical metrics did not allow for a sufficiently powered meta-analysis.

The major bias for results being left out of this systematic review is the failure to consider negative results being reported in non-peer-reviewed media such as news articles or posts. To counter this potential bias, we additionally employed the PubMed review to detect all results, not solely those reported to ClinicalTrials.gov, and limited the subsequent analysis to only allow for conclusions related to the results that were submitted. Certainty was assessed by restricting results collected to those that met quality control criteria from ClinicalTrials.gov or passed peer review.

Ethics Statement

This study did not require any involvement with human research subjects necessitating the use of an institutional review board or ethics committee. All data were procured through publically available deidentified databases on clinicaltrials.gov and pubmed.ncbi.nlm.nih.gov. No animal studies were performed for this study.

Results

Search Results

Using the search strategy as detailed above, we identified 99 Clinical Trial records and 110 PubMed citations that investigated immunotherapeutic interventions in human

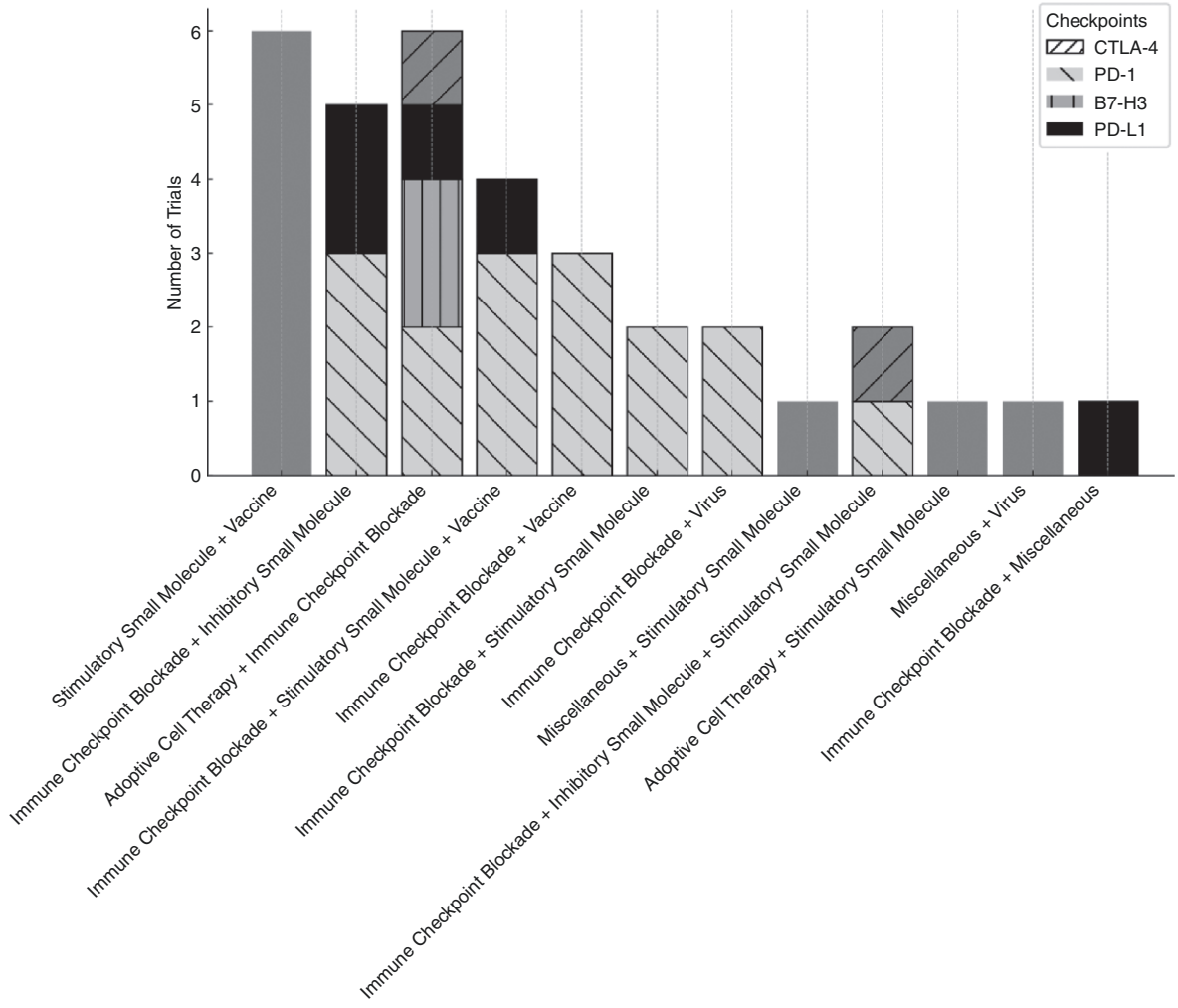

Figure 3. Combinatorial immunotherapy approaches and checkpoint targets in glioblastoma trials, all combinations of immunotherapy modalities are included, where for any combination that includes immune checkpoint blockade, the bar is segmented to represent the proportions of immune checkpoint targets, where targets are represented by patterns detailed in the figure key (top right).

patients with glioblastoma. Following exclusion criteria and screening, 97 Clinical Trial records and 15 PubMed citations were included in the final analysis, resulting in a total of 106 identified Clinical Trials (Figure 1). Two clinical trials were excluded, one being a profiling program with the intervention mainly involving collection of blood and tumor samples rather than the administration of immunotherapy[19] and the other being a combination chemotherapy with anti-angiogenic therapy but not including immunotherapy in the groups outlined below.[20] Of those included, 6 of the Clinical Trial records and 15 PubMed citations had outcome results that were then merged together for final review and analysis (Table 1). Of the 106 Clinical Trials identified (Supplementary Table 1), each trial was categorized according to its immunotherapy type as (1) ICB (48/106), (2) vaccine (28/106), (3) adoptive cell therapy (23/106), (4) stimulatory small molecule (18/106), (5) inhibitory small molecule (10/106), (6) virus (9/106), and (7) miscellaneous (7/106). Some trials were placed in multiple classes. Although DC vaccines are a subtype of adoptive cell therapy, they were included in the vaccine class given their frequent usage and the need to distinguish them from the infusion of activated lymphocytes. Cytokines, polymers (eg, Poly-ICLC), and protein ligands were included in small molecule classes.

Assessment of Heterogeneity

The identified studies were clinically and methodologically heterogeneous. Sources of heterogeneity included the number of patients, types of patients, patient treatment profiles both pre- and peri-study period, types of clinical outcomes, as well as the length of follow-up for clinical outcomes. The study populations were highly heterogeneous as some trials restricted cohorts to patients with newly diagnosed glioblastoma (4/18), recurrent or progressive glioblastoma (10/18), and IDH mutant-only glioblastoma (1/18). Furthermore, some studies included in their inclusion or exclusion criteria patients' past treatment or

resection and IDH mutation status, among other factors. Finally, some studies included other brain tumor pathologies (4/18), including those that comprised multiple grades of glioma in addition to glioblastoma (2/18).

Risk of Biases of Included Studies

Selection bias.—Included study results are at high risk of bias based on PRISMA criteria.[21] The cause for bias is from heterogeneous patient populations. As discussed in the assessment of heterogeneity, some trials included only patients who had newly diagnosed glioblastoma, while others included recurrent glioblastoma. Similarly, some trials had restricted their patient population to glioblastoma patients only, while others included glioblastoma among other gliomas, which could result in a substantial difference in the OS, PFS, and all-cause mortality. Some studies included only MGMT methylated or indeterminate tumor subtype patients,[22] where MGMT promoter methylation is known to be a positive prognostic factor for improved survival in glioblastoma.[23] A meta-analysis was not performed due to the variable effects of OS and PFS across the studies from patient selection bias. Conclusions were drawn only as they pertain to the specific patient populations included in the study, and a comparison between different study arms was performed to analyze study results when available.

Comparability

All studies reported were interventional immunotherapy trials. Control arms between trials considerably differed, as some control arms consisted of surgical, radiotherapy (RT), and temozolomide (TMZ) management, whereas others included anti-PD-1[24] or DC vaccines.[24] Other studies used historical controls, which usually involved the current standard of care per the Stupp protocol.

Outcomes

Risk of bias was evaluated for (1) the follow-up timeframe of reported outcomes and (2) operationalization of outcome metrics. We assessed all-cause mortality, serious adverse events, OS, OS Frequency (12 months and 24 months), PFS, PFS Blinded Independent Central Review/Response Assessment in Neuro-Oncology (BICR/RANO), and ORR. While OS and PFS were clearly defined across the studies, some studies did not report OS (2/18) or PFS (6/18). Furthermore, there was substantial heterogeneity in the length of follow-up for clinical outcomes (OS: 1 year to 4.5 years, PFS: 6 months to 6 years). PFS had a high degree of heterogeneity in its operationalization across studies, being evaluated as study investigator assessed, by BICR/RANO criteria, or iRANO criteria that factors in immune responses to evaluate tumor progression.[25] All-cause mortality (14/18) and serious adverse events (13/18) were clearly defined and evaluated in most studies, while ORR was not evaluated in most studies (5/18). To address these biases, specifically in regard to the inconsistent operationalization of PFS and the high heterogeneity of study patient populations, a meta-analysis was not performed, and the results are organized in a narrative form.

Summary of Trial Results

The final merged results ($n = 18$) included studies from all phases: Phase III (2/18), Phase II (6/18), Phase I/II (2/18), and Phase I (8/18). The majority of included trial results were from completed trials (11/18); however, studies with a status of active (2/18), recruiting (1/18), terminated (2/18), and unknown (2/18) were also included due to having important clinical outcomes. A total of 1637 patients were included across all studies with results, whose sample sizes ranged from 4 to 709 patients. Five of the 18 trials had 2 arms, but one of these studies was terminated before the second arm received treatment. Of the 106 clinical trials from 2016 and onward that investigated immunotherapy in glioblastoma (Supplementary Table 1), the most common class of immunotherapy explored was ICB (48/106) (Figure 2). Among all immune checkpoint trials, targets included PD-1 (35/48), PD-L1 (12/48), CTLA-4 (6/48), TIGIT (2/48), B7-H3 (2/48), and TIM-3 (1/48) (Figure 3).

Checkpoint Monotherapy: Phase III Trial Results

The summarized trial results (Table 1) show limited clinical efficacy for immune checkpoint monotherapy in glioblastoma. Two large randomized control trials (RCTs) demonstrated no clinical benefit using anti-PD-1 as monoimmunotherapy for newly diagnosed glioblastoma patients. The first trial, CheckMate 548 (NCT02667587[22]), examined the efficacy of combining anti-PD-1 with standard-of-care RT and TMZ versus standard of care alone in patients with methylated MGMT promoter status (~350 patients in each arm). The second trial, CheckMate 498 (NCT02617589[26]), examined the efficacy of anti-PD-1 with radiation in patients with unmethylated MGMT promoters compared to standard of care (anti-PD-1 + RT versus TMZ + RT). Both studies did not meet their primary endpoint of improved OS, and the results of CheckMate 498 actually demonstrated decreased OS with anti-PD-1 + RT compared to standard of care with TMZ + RT (CheckMate 548: $P = .34$, Cox Proportional Hazard 1.12 [0.87–1.43], CheckMate 498: $P = .0037$, Cox Proportional Hazard 1.31 [1.09–1.58]). Additionally, correlative studies from both trials demonstrated that PD-L1 expression in glioblastoma does not significantly predict response to anti-PD-1.[17,18]

Combination Therapy With Multiple Checkpoint Inhibitors

One of the adaptive responses that tumors demonstrate in recurrence and checkpoint monotherapy is the upregulation of other checkpoint molecules.[27] As such, combination strategies with multiple immune checkpoints were explored in subsequent trials. Early Phase (I and II) clinical trials found that the combination of certain checkpoint inhibitors did not have increased clinical benefit. The trial GlitIpNi (NCT03233152[28]) investigated intratumoral anti-CTLA-4 with systemic anti-PD-1 administration

following resection of recurrent glioblastoma; this strategy did not demonstrate any significant difference in PFS when compared to a historical control group of recurrent glioblastoma patients treated with axitinib, avelumab, and lomustine.[29] The GlitIpNi trial found an OS frequency at 24 months of 27% (9%–44%) and an OS at the 1-year time point of 8.75 months.[29] Notably, low tumoral expression of B7-H3, an immune checkpoint, was a marker for improved response to anti-PD-1 and anti-CTLA-4 therapy.[29] A follow-up investigation (NCT03430791[30]) that examined the efficacy of combining tumor treating fields and anti-PD-1 with and without anti-CTLA-4 therapy for recurrent glioblastoma additionally failed to improve clinical outcomes and was terminated after an ORR of 0.0% ($n = 4$) and PFS of 2.05 months (1.54–2.57) within a 2-year time frame.

Combining Immune Checkpoint Inhibition With Other Immunotherapy Modalities

Of the 18 trials that included outcomes for any immunotherapeutic intervention for glioblastoma, combination immunotherapy (defined here as multiple immunotherapeutic strategies) was investigated in 6 of the studies, while the remaining studies typically included mono-immunotherapy with or without conventional chemoradiation (Table 1). Among all multimodal immunotherapy trials regardless of data reporting, immune checkpoint therapy was most often combined with stimulatory small molecules (8/31), vaccines (7/31), inhibitory small molecules (7/31), and adoptive cell therapy (6/31). The highest combination of modalities was 3 immunotherapeutic strategies and involved ICB with small molecule immunomodulating agents (Figure 3). Targets within the IDO1-kynurenine pathway were featured as the most frequent target of inhibitory small molecule therapies (5/10).

While the majority of trials that featured combination immune checkpoint inhibition with other immunotherapeutic modalities had limited power and scale, several of these studies demonstrated improved clinical outcomes compared to studies that examined checkpoint blockade alone (Table 1). The Phase I trial AVERT (NCT02529072[24]) used a 2-arm RCT to compare neoadjuvant anti-PD-1 and DC vaccine combination against neoadjuvant anti-PD-1 alone in recurrent glioblastoma, with both arms having postoperative anti-PD-1 and DC vaccine therapy. The arm receiving neoadjuvant anti-PD-1 and DC vaccine combination displayed improved OS (15.3 months vs. 8 months) and improved PFS (6.3–4.3 months) compared to the neoadjuvant anti-PD-1 arm alone. It should be noted, however, that this trial only had 3 patients in each of the 2 arms.

In the trial NCT03636477,[31] IL-12 administration in combination with anti-PD-1 resulted in an OS of 9.8 months (5.2–17.4) for patients with recurrent or progressive glioblastoma.[32] The results of this safety study led to a subsequent Phase II clinical trial (NCT04006119).[32] Moreover, the Phase II CAPTIVE trial (NCT02798406[33]) combined an oncolytic adenovirus with anti-PD-1 for recurrent glioblastoma. Achieving an OS of 12.5 months (9.7–24.3) in a cohort of 48 patients, 56.2% of patients demonstrated clinical benefit compared to historical controls.[34] Objective responders to this therapy had moderate PD-1 expression on their immune cells and an inflammatory microenvironment signature pretherapy, which indicate that inflammatory signatures such as those involving interferon-related genes may serve as a biomarker for patients who are more likely to respond to immunotherapy. Furthermore, correlative studies noted posttreatment downregulation of ARG2 and NOS2 in partial responders.[34]

Targeting Myeloid-Derived Suppressor Cells

Given that glioblastoma is rich in infiltrating myeloid cells, a series of Phase I trials examined the impact of modulating this immunosuppressive population. Most frequently, myeloid-derived suppressor cell (MDSC) populations were targeted (Supplementary Table 1). In a Phase 0/I trial with 11 recurrent glioblastoma patients, exposure to low-dose capecitabine achieved an OS of 16.6 months and a reduction in peripheral MDSC levels postresection (NCT02669173).[35,36] This trial further demonstrated that while CTLA-4 was reduced upon exposure to capecitabine, PD-1 levels on CD45 + immune cells were increased, which lends support to future combination therapy strategies featuring anti-PD-1.[36] Additional trials have targeted the myeloid compartment. In a Phase IIb trial, the IL4 receptor (IL4R) was targeted via the toxin MDNA55 (NCT02858895[37]). IL4R is highly expressed in glioblastoma cells, tumor associated macrophages (TAMs), and MDSCs. In a population of recurrent glioblastoma patients, an OS of 11.6 months was achieved, with a subgroup of patients who had high IL4R expression experiencing the greatest clinical benefit (OS 15 months, OS-12 55%).[38] Finally, oncolytic viruses such as those featured in the Phase I trial (NCT03152318) have demonstrated decreased representation of immunosuppressive MDSC populations and increased infiltration of antitumoral T cells in a study that examined 41 patients with recurrent glioblastoma.[39] Median OS of patients with IDH wild-type glioblastoma was 11.6 months (7.8–14.9) after treatment, and a durable clinical effect was linked to seronegativity after administration of oncolytic Herpes Simplex Virus treatments.[39]

Discussion

In recent decades, the clinical application of PD-1 blockade has galvanized the field of cancer immunotherapy.[40] This systematic review assessed the landscape of immunotherapy trials for glioblastoma from 2016 to 2023, with a particular focus on ICB strategies. The analysis of 106 clinical trials highlighted that thus far immune checkpoint inhibition has not worked for glioblastoma; however, combination studies involving immune checkpoints and other immunomodulating agents suggest promising directions and important considerations for future clinical trials. While the definitive anti-PD-1 trials in glioblastoma have been negative, the success of anti-PD-1 therapy in specific colorectal cancer patient populations (patients with microsatellite instability [MSI]-high/mismatch repair-deficient) highlights the opportunity for other modalities of

immunotherapy to create a glioblastoma tumor microenvironment that may be susceptible to checkpoint therapy.[41] The results of this review show a trend in trials favoring approaches that target the immunosuppressive myeloid compartment as well as reversing T-cell exhaustion with immune checkpoint inhibition strategies.

Heterogeneity is a consequential barrier for new glioblastoma therapies. Given the sizeable inter-tumoral (between patients) as well as intratumoral (within the same patient) heterogeneity in glioblastoma, there is a necessity for refining biomarkers for individuals who may show response to specific immune-based strategies. Such characterization is limited by the overall low numbers of responders from these trials and biomarkers within the target axis have shown limited roles for predicting response. CheckMate 548, CAPTIVE, and GlitIpNi trials have shown that PD-L1 expression does not correlate with survival outcomes,[17,34] although the lattermost study did uncover that high B7-H3 expression was correlated with a negative response. The results of the CAPTIVE trial suggest that a higher resolution of phenotyping involving cell type and cytokine distributions, degree of inflammatory gene signatures, neoantigen/MSI, and metabolite markers may provide a more refined look for biomarkers that predict response.

The 2 largest studies reviewed, CheckMate 548 (NCT02667587[22]) and CheckMate 498 (NCT02617589[26]), did not use a combination of immunotherapies but instead investigated anti-PD-1 compared to standard of care across patients with methylated (CheckMate 548) and unmethylated (CheckMate 498) MGMT promoters. Several factors were thought to contribute to the limited efficacy of anti-PD-1 in glioblastoma in these trials: (1) an overall low tumor mutational burden, which would in turn impact T-cell recognition of tumor cells, (2) relatively low number of infiltrating T cells, (3) a higher proportion of infiltrating immunosuppressive myeloid cells, (4) systemic lymphodepletion from TMZ exposure, and (5) high steroid exposure, which while helping with mass effect symptoms from tumor-associated vasogenic edema can also have immunosuppressive effects on checkpoint blockade.[42] Given that TMZ results in lymphodepletion and has greater efficacy in patients with methylated MGMT promoters, CheckMate 498 tried to assess the efficacy of replacing TMZ with anti-PD-1 in patients with unmethylated MGMT promoters but similarly did not show improved survival outcomes.

The next generation of ICB trials for glioblastoma represents a shift from the mono-immunotherapy paradigm, with 32.1% of the trials covered in this study featuring some combination of immunotherapeutic strategies. However, many of these trials lack the scale and scope that were demonstrated in the aforementioned Phase III trials and can only offer limited conclusions. Certain trends are gleaned from these studies though, including increased support for targeting MDSC populations that promote glioblastoma-mediated immunosuppression and tumor aggressiveness.[43–45] A trial combining capecitabine with bevacizumab for patients with recurrent glioblastoma demonstrated improved PFS of 7.3 months along with significant reduction of peripheral MDSC levels following surgical resection, both with and without an untreated reference cohort.[35,36] Studies have since demonstrated that capecitabine conversion to 5-FU selectively kills MDSCs and also synergizes with anti-PD-L1.[46] Future work may seek to incorporate similar MDSC-targeting approaches with immunotherapies of other modalities.

It should be noted that efforts toward targeting MDSC populations have been concurrent with exploring the potential of ICB. While not included in the purview of our study as it was conducted several years prior to the start of our search criteria (2016 to present), the Phase II trial examining oral anti-CSF1R therapy for recurrent glioblastoma targeted MDSC infiltration into the tumor microenvironment.[47] Despite promising preclinical results involving this therapeutic strategy, this trial failed to meet its primary endpoint of 6-month PFS for patients. Since then, alternative combination immunotherapies involving ICB have been explored for targeting the myeloid compartment. While the potential of combining oncolytic viral therapy with anti-PD-1[34] demonstrated changes in T-cell infiltration, there are also several implications for this combination strategy targeting MDSCs as well. In a study examining the efficacy of oncolytic viral therapy with PD-L1 blockade in pancreatic ductal adenocarcinoma, this strategy was able to reverse the immunosuppressive tumor microenvironment by suppressing MDSC accumulation in preclinical models.[48]

As discussed earlier in our analysis of risk of biases and assessment of heterogeneity, this systematic review had several limitations, including heterogeneous patient definitions across glioblastoma cohorts and varying operationalization of clinical outcomes across trials. Additionally, many trials lacked multiple arms to facilitate direct evaluation of the immunotherapy's success. Furthermore, there was molecular heterogeneity, as MGMT and IDH statuses were not defined across every trial. As a result, a meta-analysis of the results was not possible. As future trials investigate immunotherapies for glioblastoma, meta-analyses will be critical to inform the most efficacious combination of therapies. This highlights the importance of standardized trial designs to facilitate comparisons across studies. The need for improved glioblastoma immunotherapy trial design is further motivated by the potential of eliciting new molecular biomarkers that may predict clinical outcomes. In many trials, small cohorts of patients show durable clinical response. Checkpoint inhibition has been found particularly efficacious in specific patient populations. Patients with Lynch syndrome, a hereditary condition associated with MSI,[49] were found to exhibit an improved response to ICB.[50] The characterization of high MSI as a patient biomarker for ICB response led to the FDA originally approving anti-PD-1 for MSI-high colorectal cancer. In glioblastoma, patient subsets with similar biomarkers may be identified via studies that incorporate multiple time points for peripheral blood and tissue collection for serial immunophenotyping, which could ultimately contribute to a compendium of data that would enrich for candidate biomarkers of patients who are responders and nonresponders to immunotherapy. It should be noted that the glioblastoma AGILE trial[51] is a promising example of a study that utilizes biomarkers to define patient responders for later-phase therapies. However, its requirement for larger arms makes it inapplicable to the constraints of the many smaller Phase I trials featured in this review.

Our review provides the most up-to-date compendium of immunotherapy trials for glioblastoma in the literature.

While the success of PD-1 checkpoint inhibitors in other cancers has sparked cautious optimism, the multiple mechanisms of immune evasion featured in glioblastoma demand a multifaceted approach. Preliminary findings regarding combination checkpoint therapy with other immunomodulating agents show promise, but future larger trials will be needed to not only confirm these results but also uncover biomarkers for patients who demonstrate durable clinical response. Given that responders to certain therapies (eg, oncolytic virus) tend to share similar pretreatment immune profiles (eg, PD-1 expression), personalized biomarker-driven combination immunotherapies that target multiple immune populations emerge as a promising avenue for the treatment of this intrinsically and adaptively resistant cancer.

Conclusions

Immune checkpoint inhibition for glioblastoma has thus far not seen the same success as many other solid tumors. Comparison of therapeutic efficacy across trials was limited due to heterogeneity in the study population and outcome operationalization, suggesting the utility of standardization for future trials to facilitate comparison across immunotherapy modalities. This review underscores growing evidence that checkpoint therapy combined with other immunomodulating agents is more encouraging than mono-modality immunotherapies. Furthermore, the utilization of biomarkers will be vital for the development of personalized combination immunotherapy strategies.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology Advances* (https://academic.oup.com/noa).

Keywords

clinical trial | combination therapy | glioblastoma | immune marker | immunotherapy

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Data Availability

This paper will be available on pubmed.ncbi.nlm.nih.gov and any interested persons who would like a copy of the manuscript and its associated data can request it via email to the corresponding author.

Affiliations

Stanford University School of Medicine, Stanford University, Stanford, California, USA (E.S.); Department of Neurosurgery, Stanford University School of Medicine, Stanford University, Stanford, California, USA (J.C., A.T., L.H.K., M.L.)

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