Tumor antigen only (TAO) vaccine platforms for glioblastoma therapeutics: a systematic review of evidence from clinical trials

Hsien-Chung Chen,[a,b] Eric T. Wong,[c,d,e] Sasmit Sarangi,[c] Heinrich Elinzano,[c] Nick A. Kuburich,[f,g] Sendurai A. Mani,[f,g] Rohaid Ali,[a] Deus Cielo,[a] Konstantina A. Svokos,[a] Prakash Sampath,[a] Athar N. Malik,[a] Christine K. Lee,[a] Curtis E. Doberstein,[a] Sean E. Lawler,[f,g] Attila Seyhan,[f,g,h,i] Wafik S. El-Deiry,[d,f,g,h,i] and Clark C. Chen[a,g,*]

[a]Department of Neurosurgery, The Warren Alpert Medical School of Brown University, Providence, RI, USA
[b]Department of Neurosurgery, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan
[c]Department of Neurology, Medicine, Neurosurgery & Radiation Oncology, Brown University Health & Rhode Island Hospital, Providence, RI, USA
[d]Division of Hematology/Oncology, Department of Medicine, Brown University Health & Rhode Island Hospital, Providence, RI, USA
[e]Brain Tumor Center & Neuro-Oncology Unit, Beth Israel Deaconess Medical Center, Boston, MA, USA
[f]Department of Pathology and Laboratory Medicine, The Warren Alpert Medical School, Brown University, Providence, RI, 02912, USA
[g]Legorreta Cancer Center, The Warren Alpert Medical School, Brown University, Providence, RI, 02912, USA
[h]Laboratory of Translational Oncology and Experimental Cancer Therapeutics, The Warren Alpert Medical School, Brown University, Providence, RI, 02903, USA
[i]The Joint Program in Cancer Biology, Brown University and Brown University Health, Providence, RI, 02903, USA

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Summary

Glioblastoma is the most common form of primary brain tumor in adults, characterized by rapid progression and poor prognosis—despite the standard of care treatment including maximal safe resection, radiotherapy, and chemotherapy. Cancer vaccination has emerged as a promising strategy to harness the patient's immune system against glioblastoma. Cancer vaccination strategies can broadly be divided into cell-based or tumor antigen only (TAO), depending on whether they incorporate the use of viable immune cells. Here, we reviewed data from clinical trials that tested TAO cancer vaccination strategies for glioblastoma treatment, including personalized vaccines. Clinical safety and efficacy profiles for each vaccination strategy are summarized. Insights gained from these clinical trials are reviewed to identify opportunities for future therapeutic advancement.

Keywords: Glioblastoma; Tumor antigen only (TAO) cancer vaccine; Clinical trials

Introduction

Glioblastoma is the most common form of primary brain cancer in adults and remains one of the deadliest human cancers.[1] Survival beyond two years from the diagnosis remains rare.[2] Recent breakthroughs in immunotherapy for other cancer types have reignited interest in its potential application for glioblastoma.[3] The concept of immunotherapy for glioblastoma is rooted in historical observations of intracranial bacterial infection, including the well-documented Coley's abscess, which preceded glioblastoma regression.[4] This principle has evolved over the past century, leading to contemporary strategies, including the development of oncolytic viruses,[5] chimeric antigen receptor-T-cells (CAR-T),[6] and cancer vaccination.[7] Among these approaches, cancer vaccination stands out as the least invasive strategy. The success of Bacillus Calmette-Guerin (BCG)[8] and Provenge[9] for bladder and prostate cancer further fueled interest in the exploration of vaccine-based approaches for glioblastoma. This article will review these efforts.

While the abbreviation "GBM" is historically used to denote glioblastoma multiforme, the World Health Organization (WHO) now refers to the disease as simply glioblastoma—the term "multiforme" was deemed non-specific.[10] In the 2021 WHO classification, gliomas that harbor an isocitrate dehydrogenase mutation (IDHm) are no longer considered glioblastomas. Despite sharing histological similarity with IDH wild-type (IDHwt) gliomas, the epidemiology, clinical course, and therapeutic response of IDHm astrocytomas differ from those of glioblastomas.[11] Many of the cancer vaccine publications reviewed here were

*Corresponding author. Department of Neurosurgery, CNS Cancer, Legorreta Cancer Center, Warren Alpert Medical School of Brown University, Translational Neuroscience, Gamma Knife Stereotactic Radiosurgery Program, Rhode Island Hospital, 593 Eddy Street - APC 6, Providence, RI, 02903, USA.
E-mail addresses: clark_chen@brown.edu, clark.chen@brown-health.org (C.C. Chen).

Research in context

Evidence before this study

Cancer vaccination strategies draw inspiration from the success of vaccines against infectious diseases. Mutations arising during carcinogenesis generate foreign antigens recognized by the immune system, providing a basis for cancer vaccination. While the scientific rationale for cancer vaccination is compelling, systematic analysis of trial outcomes is necessary to bridge the gap between concept and clinical application. To this end, a systematic PubMed search was performed, following Cochrane Collaboration and PRISMA guidelines. Peer-reviewed articles detailing cancer vaccine trial results were reviewed and integrated to generate evidence-based insights.

Added value of this study

Analysis of the clinical trial literature on cancer vaccination demonstrates that these approaches are generally safe and induce detectable vaccine-specific immune responses, though these responses may take more than 600 days to emerge. While phase I/II studies have shown encouraging feasibility and survival signals, the available phase III trials have not demonstrated a survival benefit. Factors that may limit the efficacy of cancer vaccination include insufficient cytoreduction (e.g., subtotal resection), clinical use of immunosuppressive medications (e.g., dexamethasone), and tumor-associated immune suppression.

Implications of all the available evidence

Clinical trial data on cancer vaccination support its underlying scientific premise, demonstrating safety and vaccine-specific immune responses. However, meaningful clinical efficacy has not yet been achieved. Future progress will require trial designed to address key shortfalls.

conducted before the 2021 WHO classification. In this article, the term "glioblastoma" is reserved for tumors that are IDH wild-type.

Another consideration when interpreting glioblastoma clinical trials involves the DNA repair gene, methyl-guanine methyl transferase (MGMT).[12] MGMT encodes a protein that repairs guanine nucleotides alkylated at the O^6 position.[13] This lesion arise as the dominant cytotoxic consequence of temozolomide, the standard-of-care chemotherapy for glioblastomas. Methylation of the MGMT promoter region (noted as mMGMT) suppresses MGMT transcription and is associated with a favorable temozolomide response.[14] After standard-of-care therapy, the median overall survival (mOS) for mMGMT and MGMT unmethylated (uMGMT) newly diagnosed glioblastoma are 21–26 and 12–14 months, respectively.[15] For recurrent glioblastoma, the mOS for mMGMT and uMGMT are 8–16 and 7–10 months, respectively.[16,17] The survival outcome of glioblastoma studies can vary based on the proportion of mMGMT and uMGMT tumors.

Another feature of glioblastoma is that the molecular circuitry that fuels survival and growth is notoriously redundant.[2,18] For example, multiple receptor tyrosine kinases (RTKs) drive proliferation and survival signaling in glioblastoma, and durable tumor growth suppression requires simultaneous inhibition of multiple RTKs.[19] This phenomenon contrasts with oncogene addiction in certain cancers, in which tumor viability is driven by oncogenic activation of a single RTK.[18] Thus, though inhibiting a single receptor tyrosine kinase (RTK), such as the epidermal growth factor receptor (EGFR), has proven effective in several cancer types, this approach has failed in glioblastoma in clinical trials.

Depending on whether they involve viable immune cells, cancer vaccination strategies can be broadly divided into two categories: viable cell-based or tumor antigen only (TAO). Cell-based vaccines utilize whole cells derived from the patient under treatment or from donors.[20] An example of a viable cell-based vaccination strategy involves dendritic cells (DC). In this strategy, DCs are harvested from the patient, loaded with tumor-derived antigens, and reintroduced into the patient. This type of therapy requires a significant investment in cell-processing facilities and specialized antigen-loading techniques, posing challenges for clinical translation.[7] Although cell-based vaccination strategies have demonstrated encouraging results,[21,22] the breadth and complexity of the existing literature preclude a comprehensive review within the scope of this study.

In contrast, tumor antigen only (TAO) vaccines do not rely on viable immune cells derived from patients. Instead, the vaccine consists entirely of synthetic antigen peptides, isolated tumor-derived antigen peptides, or synthetic genetic material. These vaccines are formulated as a mixture of immune adjuvants to stimulate anti-tumor immunity and are administered intradermally, subcutaneously, or intravenously. This review will focus on the clinical outcomes of the TAO vaccination therapy for high-grade gliomas or glioblastomas in the newly diagnosed and recurrent settings.

Fig. 1 shows a broad outline of the principles underlying the TAO cancer vaccination. The vaccine generally consists of tumor-associated antigens (TAAs), damage-associated molecular patterns (DAMPs), or genetic material that can be transcribed/translated into TAAs.[23] TAAs are proteins expressed on the tumor cell surface or secreted into the extracellular space that the immune system recognizes as foreign. DAMPs are endogenous

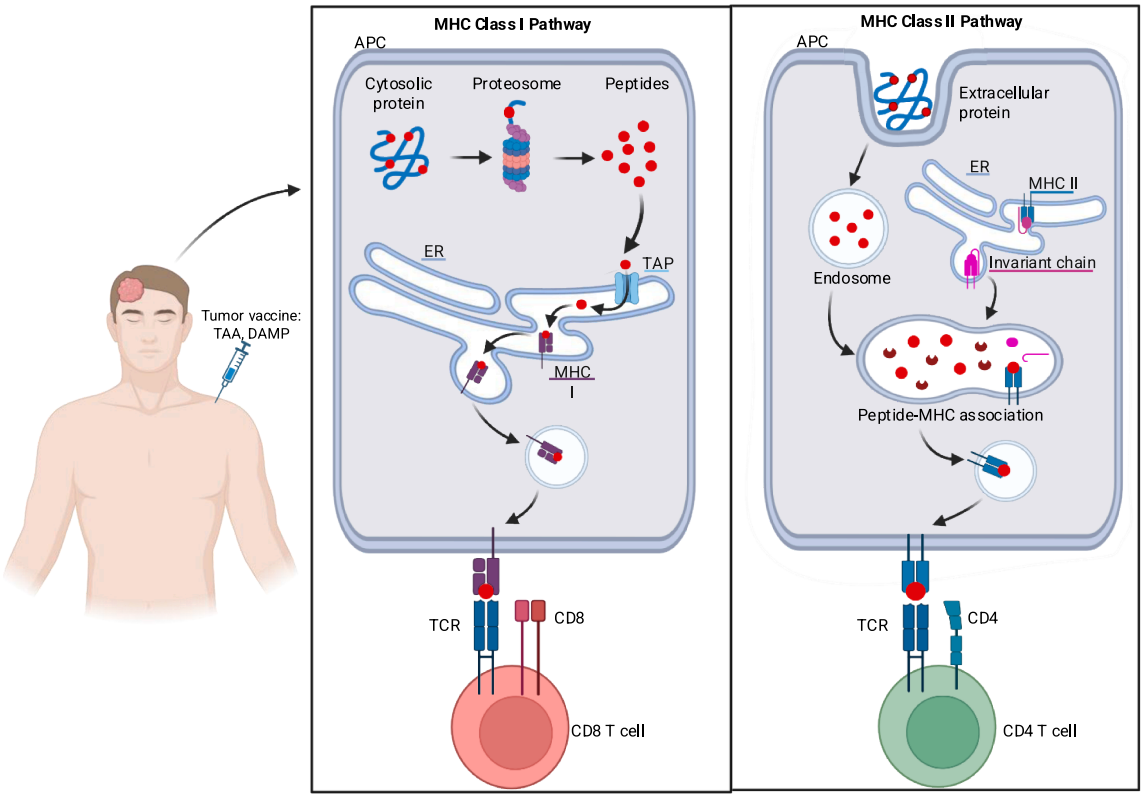

Fig. 1: Overview of the immunological principles underlying tumor antigen only (TAO) cancer vaccination. The schematic illustrates the key steps involved in antigen processing and presentation following administration of a tumor antigen only (TAO) cancer vaccine. Vaccine formulations typically contain tumor-associated antigens (TAAs), damage-associated molecular patterns (DAMPs), or genetic material encoding TAAs. After injection, TAAs are internalized by antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B cells. Cytosolic antigens are processed by proteasomes, transported into the endoplasmic reticulum via TAP, and presented on major histocompatibility complex (MHC) class I molecules to activate cytotoxic $CD8^+$ T cells. Extracellular antigens are taken up through endocytosis, processed within endosomes, and presented on MHC class II molecules to stimulate $CD4^+$ helper T cells. DAMPs bind to pattern recognition receptors (PRRs) on APCs, triggering cytokine release and amplifying the immune response.

proteins/molecules released that bind to pattern recognition receptors (PRRs) to trigger the release of cytokines that activate/augment immune responses.[24]

Upon injection into a patient, TAAs are taken up by host antigen-presenting cells (APCs), including dendritic cells (DCs), macrophages, and B cells.[25] The APCs process the TAA and present them on class I or class II major histocompatibility complexes (MHCs) to initiate immune recognition. For MHC class I molecules, TAAs are processed by cytosolic proteosomes into 8–10 amino acid peptides recognized by cytotoxic CD8+ T lymphocytes (CTLs). For MHC class II, TAAs are internalized through endocytosis and degraded into smaller peptides by proteases within the endosome. The peptide-MHC II complexes are recognized by and activate CD4+ helper T cells.[26]

Most cancer vaccine studies incorporate assessments of immune responses.[27] For cellular responses, peripheral blood mononuclear cells (PBMC) are isolated and challenged with the vaccine/control peptide. The samples are subsequently assessed using 1) clonal expansion assays, 2) tetramer assays, 3) interferon-γ secretion assays, or 4) assays that measure lysis of target cells.[28] For humoral responses, blood samples are challenged with the vaccine/control peptide, followed by quantification of antibodies against the vaccinated antigen. Immunohistochemical staining for the vaccine antigen is performed if post-vaccination tumor samples are available. More recent studies utilize single-cell T-cell receptor (TCR) sequencing to characterize the T-cell populations in the tumor microenvironment.[28]

Search strategy and selection criteria

We performed an exhaustive search of the PubMed literature with the following terms: "glioblastoma", "vaccine", "tumor-derived", "tumor-associated", "antigen", NOT "cell therapy", NOT "viral-based", NOT "oncolytic". The search was conducted on April 8, 2025. This systematic review followed the Cochrane Collaboration and the Preferred Reporting Items for Systematic

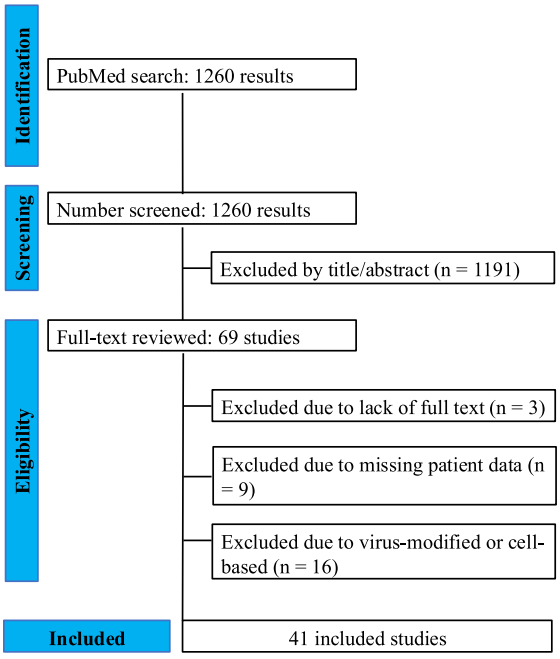

Fig. 2: PRISMA flow diagram of study selection. Flowchart illustrating the literature search and selection process for studies investigating tumor antigen only glioblastoma vaccines. A total of 41 studies met the inclusion criteria after screening and eligibility assessment.

Reviews and Meta-Analysis (PRISMA) statement guidelines. A total of 41 studies were included in the final review. The search is described in Fig. 2.

Overview of glioblastoma vaccine strategies

Broadly, the TAO cancer vaccination publications identified in our literature search can be divided into those that target i) a single epitope/pathway or ii) multiple epitopes/pathways. Strategies targeting multiple epitopes can further be subdivided into approaches that utilize i) a predetermined set of peptides irrespective of the patient's immunity or tumor mutational landscape, or ii) personalized peptides that are synthesized or selected based on the patient's immunity or tumor mutational landscape. A simplified schema of this classification scheme is shown in Fig. 3. References for these studies are shown in Supplementary Material 1. The relative merits of each therapeutic approach are summarized in Table 1.

Cancer vaccination strategies have been tested in patients with newly diagnosed and recurrent glioblastomas/high-grade gliomas. In the setting of newly diagnosed glioblastomas, priming vaccination doses are typically administered following completion of concurrent chemo-radiation. Maintenance vaccination doses are typically administered at some interval after temozolomide is given during its cycles. In the setting of recurrent glioblastomas, there is currently no standard of care. As such, TAO vaccinations are administered either with or without adjuvant chemotherapy. In the latter case, TAO is typically administered at variable times after chemotherapy. Some TAO strategies involve chemotherapeutic depletion of regulatory T-cells before vaccine administration.

Single-target tumor vaccines

Cancer vaccines targeting a single target are modeled on the success of similar vaccination strategies in infectious diseases, including vaccination against hepatitis B, influenza, and malaria.[29,30] When cross-applied to cancer biology, the strategy translates to targeting antigenic epitopes derived from proteins that are i) highly expressed in the tumor, ii) absent or minimally present in normal tissues, and iii) essential for glioblastoma proliferation or survival.

The ensuing sections review pertinent targets and the scientific rationale. Table 2 summarizes the clinical outcomes of the various trials, as presented in the section labeled "summary of clinical trials for single-target vaccines."

Epidermal growth factor receptor variant III (EGFRvIII)

Epidermal Growth Factor Receptor is highly overexpressed in glioblastoma and plays a central role in its pathogenesis.[31] EGFRvIII is an oncogenic variant of EGFR that arose from the deletion of exons 2–7 and is expressed in ~30% of glioblastomas. The resulting receptor is missing a significant portion of the extracellular ligand-binding domain,[32] creating a constitutively active receptor. Beyond driving mitogenesis and survival pathways, EGFRvIII modulates a spectrum of cellular processes required for glioblastoma viability, including DNA repair,[33,34] and the mitotic checkpoint.[32,35] The junctional sequence spanning the deletion creates a neoantigen. These properties render EGFRvIII an excellent target for cancer vaccination.[36] The EGFRvIII peptide vaccine rindopepimut (CDX-110) is a 13-amino-acid peptide spanning the EGFRvIII mutation that is chemically conjugated to keyhole limpet hemocyanin (KLH) as an immunogenic carrier. This cancer vaccine has completed Phase III testing (Table 2, Supplementary Material 1, references 1–5).

VEGFR peptide vaccine

VEGFRs (vascular endothelial growth factor receptors) play critical roles in angiogenesis, which is considered essential for glioblastoma oxygenation and growth.[37] Results from preclinical glioblastoma models suggest synergy when VEGFR1 and VEGFR2 are inhibited in suppressing angiogenesis.[38] The expression levels of VEGFR1 and VEGFR2 in glioblastomas are notably

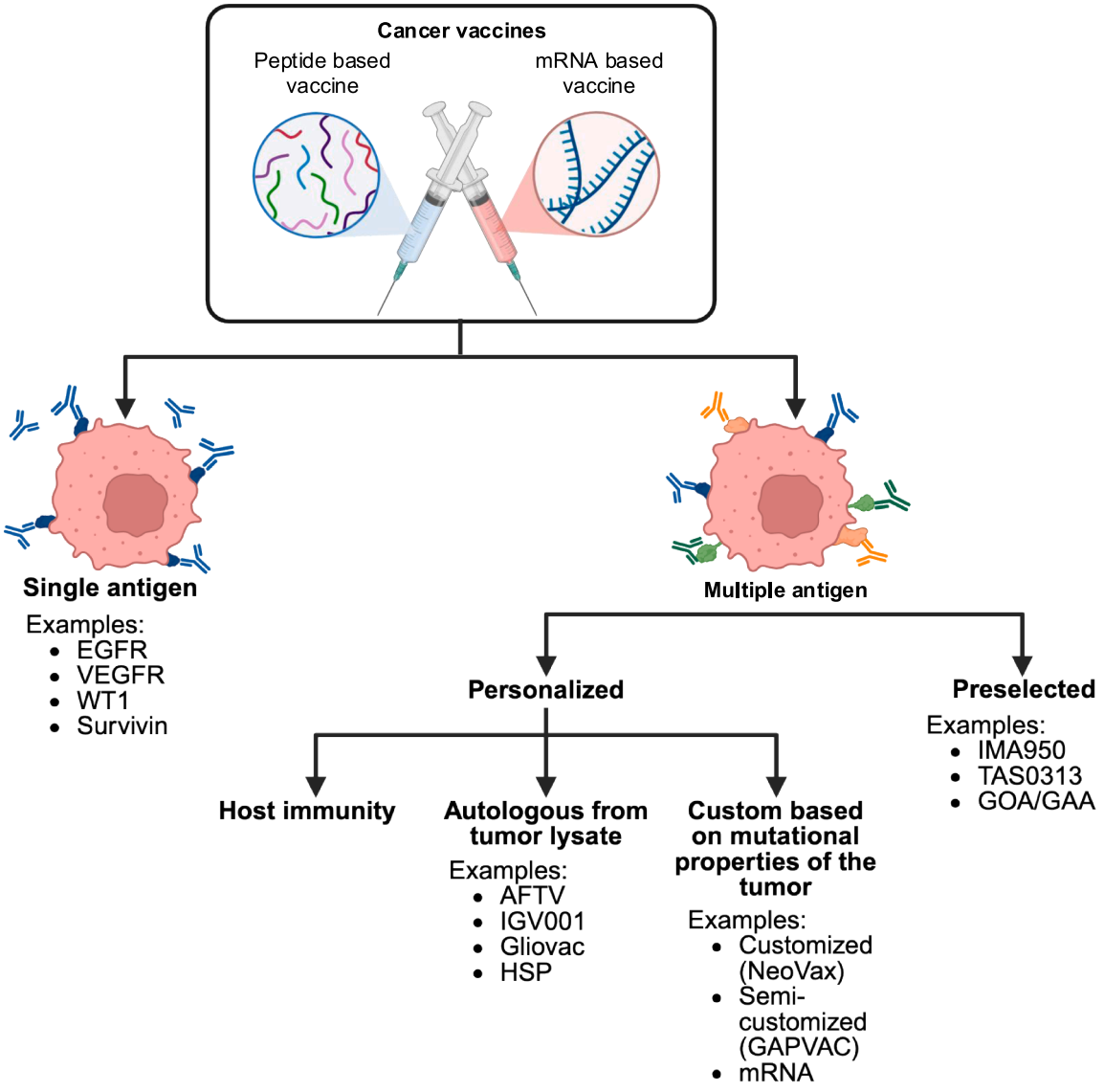

Fig. 3: Classification of tumor antigen only (TAO) vaccine strategies. The schematic summarizes the classification of TAO cancer vaccines into single-antigen and multi-antigen approaches. Single-antigen vaccines target defined epitopes such as EGFR, VEGFR, WT1, or survivin. Multi-antigen vaccines can be further divided into preselected peptide sets or personalized approaches, which include (i) vaccines tailored to host immune reactivity, (ii) autologous vaccines derived from tumor lysates, and (iii) customized vaccines developed according to tumor mutational profiles. Representative examples are listed for each category.

Vaccine strategy	Advantages	Disadvantages
Single-target vaccines	Target defined oncogenic drivers; straightforward design	Risk of immune escape; not effective against heterogeneous tumors
Multi-target vaccines	Broader immune activation; addresses tumor heterogeneity	Risk of antigenic competition; higher toxicity risk
Personalized Neoantigen vaccines	Highly specific; generates strong immune responses	Expensive and time-consuming; efficacy may lag tumor evolution
Autologous lysate vaccines	Patient-specific; broad antigen coverage	Complex logistics; immune tolerance may limit effectiveness
mRNA vaccines	Rapid and scalable manufacturing; strong immunogenicity	Requires high-end infrastructure; mRNA instability limits duration of effect

Table 1: **Relative merits of the various types of glioblastoma vaccine strategies.**

Design	NCT number	Year	Patients	Type	Treatment protocol	Vaccine protocol	Overall response	Disease control rate	OS (Median months)	PFS (Median months)	Adverse event ≥ Grade 3	Immune response
EGFRVIII												
Phase 2 (ACTIVATe)	NCT00643097	2010	18	Nd	Stand alone	Initial three vaccines given every two weeks after radiation, subsequent once a month until progression or death	NR	NR	26	14.2	None	3/17 showed positive DTH response to PEPVIII after vaccination, not correlated with OS and PFS
Phase 2 (ACTII)	Not applicable	2011	22	Nd	Vaccine + Temozolomide STD (200 mg/m^2/d per 5 days) or DI (100 mg/m^2/d per 21 days)	Initial three vaccines given every two weeks after radiation, subsequent once a month until progression or death	NR	NR	23.6	15.2	None	DI TMZ regimen produced humoral ($p = 0.037$) and DTH responses ($p = 0.036$) of greater magnitude
Phase 2 (ACTIII)	NCT00458601	2015	65	Nd	Vaccine + Temozolomide (200 mg/m^2)	Initial three vaccines given every two weeks after radiation, subsequent once a month until progression or death	NR	NR	21.8	9.2	None	Anti-EGFRvIII antibody titers increased ≥4-fold in 85% of patients
Phase 3 (ACTIV)	NCT01480479	2017	371	Nd	Vaccine + Temozolomide (150–200 mg/m^2)	Initial 2 priming doses on day 1,15 then monthly on day 22 of each Temozolomide cycle until progression	CR + PR: 31 (15%)	NR	20.1	8	TP:32 (9%) SZ:9 (2%) BE:8 (2%)[a]	Induced high anti-EGFRvIII antibody titers, with a median peak of 1:25,600
Phase 2 (ReACT)	NCT01498328	2020	36	Rec	Vaccine + Bevacizumab	Initial three vaccines given every two weeks after radiation, subsequent once a month until progression or death	CR + PR: 9 (30%)	NR	11.3	3.7	None	80% of patients developed high antibody titers, which were associated with significantly prolonged survival
VEGFR1 and 2												
Phase 1	UMIN000013381 jRCTs0311	2020	4	Nd	VEGFR1/2 Peptide Vaccine + SOC	8 times weekly then 6 times monthly after SOC	CR + PR: 3 (75%)	100%	36.8	20.8	None	CTLs specific for both VEGFR1 and 2 were induced after vaccination
WT1												
Phase 1	Not applicable	2006	10	Rec	Stand alone	weekly injected for 12 weeks intradermally	PR:1 (10%)	60%	NR	NR	None	No direct immune response data
Phase 2	Not applicable	2008	21	Rec	Stand alone	weekly injected for 12 weeks intradermally	PR:2 (9.5%)	57.1%	11.5	5	None	WT1-specific CTLs before vaccination were significantly higher in patients with GBM than in healthy controls
Phase 1	Not applicable	2015	7	Nd	Vaccine + Temozolomide (150–200 mg/m^2)	1st cohort: weekly injected for 7 weeks after CCRT 2nd cohort: biweekly injected for 3 times after CCRT	CR + PR: 6 (85.7%)	85.7%	NR	43.5	None	WT-1 specific T cells increased in early phase and decrease in late phase
Observational	Umin000002001	2016	59	Rec	Stand alone	weekly injected for 12 weeks intradermally, continue at 1- to 4-week interval if slowing or inhibition of tumor progression	2 (4%)	44.9%	8.3	2.7	None	WT1-235 IgG antibody production was significantly associated with prolonged OS and PFS
Phase 1	NCT01621542	2021	20	Rec	Vaccine with dose escalation (0.3–27 mg)	once every week for 4 weeks, then once every 2 weeks for 6 weeks and once every 4 weeks thereafter until progression	3 (20%)	5 (33.3%)	10.2	2	None	No specific immune response data
Survivin												
Phase 1	NCT01250470	2016	8	Rec	Stand alone	biweekly injected for 4 doses	2 (25%)	NR	20.2	4.1	None	Induced robust cellular and humoral response in all patients
Phase 2a	NCT02455557	2023	63	Nd	Vaccine + Temozolomide	biweekly injected for 4 doses, then every 12 weeks	NR	NR	25.9	11.4	None	Induced significant humoral and cellular immune response in nearly all patients, but T-cell response did not correlate with OS

Nd, newly diagnosed; Rec, recurrent; DI, dose intensify group; DTH, delayed-type hypersensitivity; TP, thrombocytopenia; SZ, seizure; BE, brain edema; CR, complete response; PR, partial response; NR, not reported; OS, overall survival; PFS, progression-free survival. [a]Grade 3–4 adverse events were comparable between vaccine and control groups: most common were thrombocytopenia 9%, seizure 2%, brain edema 2%, fatigue 2%. No evidence of increased immune-related cerebral toxicity. One possible treatment-related death.

Table 2: **Summary of clinical trials for single-target vaccines.**

higher than in normal tissues.[39] Moreover, inhibiting VEGFR1 and VEGFR2 expressions has been shown to suppress glioblastoma growth in preclinical animal models.[40] Finally, synthetic peptides derived from the coding sequences of VEGF1 and VEGFR2 can be presented by HLA-A*2402 to induce an anti-VEGFR immune response.[41] As with most other tumor vaccine strategies, synthetic peptide epitopes derived from VEGFR1 (SYGVLLWEIF) and VEGFR2 (RFVPDGNRI) are formulated with an incomplete Freund's adjuvant. While this vaccine targeted two proteins, they are considered redundant within the same signaling pathway. This strategy has completed only Phase I testing (UMIN000013381 and jRCTs031180170; Table 2; Supplementary Material 1; reference 6).

WT1

Wilms' Tumor 1 (*WT1*) encodes a zinc finger transcription factor crucial for the development of kidneys and gonads.[42] While WT1 was initially identified as a tumor suppressor, subsequent work demonstrated its oncogenic role in several cancer types, including glioblastoma.[43] WT1 is highly overexpressed in several solid cancers, including glioblastoma.[44] Its expression in gliomas correlates with higher tumor grades and poor prognosis.[45] In adult normal tissues, WT1 expression is restricted to hematopoietic stem cells, myoepithelial progenitor cells, renal podocytes, and some cells in the testis and ovary. In terms of immunogenicity, the National Cancer Institute (NCI) ranked WT1 as a top cancer antigen for vaccine development.[46] The WT1 vaccine has completed Phase II testing (Table 2, Supplementary Material 1, references 7–11).

Survivin

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and is highly expressed in malignant gliomas.[47,48] Survivin promotes glioblastoma cell survival, proliferation, and resistance to therapy.[49] It is highly expressed in glioblastoma and is minimally detected in normal tissues. The survivin-based vaccine SurVaxM is a synthetic long peptide spanning amino acids 53–67 of the survivin protein, with an M57 modification to enhance its binding affinity for HLA-A*0201. The peptide is conjugated to keyhole limpet hemocyanin (KLH) as a carrier protein and administered with sargramostim. In preclinical models, SurVaxM induces cellular and humoral immunity by stimulating CTLs and survivin-specific antibodies, leading to immune elimination of glioblastoma cells.[50] This strategy has completed Phase II testing, and the randomized Phase III study is planned (Table 2, Supplementary Material 1, references 12, 13).

Summary of clinical trials for single-target vaccines

In general, all trials demonstrated evidence of vaccine-induced immune responses. While adverse events (AEs) > grade III were noted, they were not directly attributable to the vaccine. The mOS and mPFS observed in these studies are generally within the range expected of the standard-of-care or current clinical practices. Disease Control Rate (DCR) and Objective Response Rate (ORR) are key early indicators of a treatment's anti-tumor activity and are frequently evaluated alongside OS to assess clinical benefit. ORR is defined as the proportion of patients achieving either a complete response (CR) or a partial response (PR), whereas DCR includes patients with CR, PR, or stable disease (SD). Although these metrics are valuable, their interpretation can be challenging in high-grade gliomas or glioblastoma, where therapeutic targets—such as VEGFR—may alter the tumor's appearance on MRI, complicating response assessment. Reported ORRs vary widely across studies, ranging from 9.5% to 85.7%, with the highest observed in trials involving anti-VEGFR1/2 vaccines. Similarly, DCRs span from 33.3% to 100%, with the highest rate reported for the anti-VEGFR1/2 vaccine.

Among single-target cancer vaccines, rindopepimut is the only candidate to have completed Phase III testing (Table 2). This study (NCT01480479) intended to enroll 374 newly diagnosed glioblastoma patients with minimal residual disease after surgical resection. The study was terminated early due to an interim analysis demonstrating futility. At the time of termination, the study had completed most of the planned enrollment. Analysis of the available data showed a median OS of 20.1 months in the rindopepimut group, compared with 20.0 months in the control group (HR 1.01, 95% CI 0.79–1.30; p = 0.93).

Multi-target tumor vaccines

A multi-target cancer vaccine utilizes a panel of TAA's to solicit a broad spectrum of immune responses against cancer cells. Compared to single-target vaccination strategies, a multi-target cancer vaccine potentially enhance anti-tumoral immunity by activating distinct TAA-specific T-cell clones, decreasing the likelihood of immune escape. The ensuing sections review the scientific rationale for the various multi-target vaccination strategies. Table 3 shows the clinical outcomes of these trials, which are summarized in the section labeled "summary of clinical trials for multi-target vaccines."

IMA950

The IMA950 vaccine comprises 11 synthetic tumor-associated peptides (TUMAPs). TUMAPs were identified by isolating HLA-peptide complexes derived from over 30 glioblastoma specimens. Peptide identities were characterized by mass spectrometry and ranked based on the level of expression, frequency of expression, and *in vitro* immunogenicity.[51] The IMA950 vaccine consists of the top 11 candidates (Table 4) from these studies. Nine TUMAPs bind to the human leukocyte antigen (HLA)

Design	NCT number	Year	Patients	Type	Treatment protocol	Vaccine protocol	Overall response	Disease control rate	OS (Median months)	PFS (Median months)	Adverse event ≥ Grade 3	Immune response
Preselected antigens												
IMA950												
Phase 1	NCT01222221	2016	45	Nd	Vaccine + SOC	11 intradermal injections over 24 weeks, 6 in induction phase and 5 in maintenance phase	PR:1 (2.5%)	30.8%	15.3	6-month PFS: 74.4%	2 (4%)[a]	90% had TUMAP-specific T-cell response, 50% had multi-TUMAP response
Phase 1/2	NCT01920191	2019	19	Nd	Vaccine after chemoradiotherapy	Group 1: 11 intradermal injections over 24 weeks Group 2: 9 subcutaneous or intramuscular injections over 24 weeks	CR + PR:3 (15.8%)	42.1%	19	9.5	BE:1 (6%) ISR:1 (6%)[b]	63% had CD8+ T cell responses
TAS0313												
Phase 2	Not applicable	2022	10	Rec	Stand alone	Subcutaneous injections on Days 1, 8, 15 of first 2 cycles, then Day 1 of subsequent cycles	PR:1 (10%)	50%	NR	1.7	None	CTL increased significantly with HLA-A*24 allele type
GOA/GAA												
Pilot	Not applicable	2019	10	Rec	Stand alone	Biweekly injection for up to 8 months	PR:1 (10%)	40%	9.1	4.9	None	No correlation between tumor antigen expression and antigen-specific CD8 T-cell response
Personalized antigens												
Targeting host immunity												
Phase 1	Not applicable	2005	25	Rec	Vaccine after radiotherapy and nitrosourea-based chemotherapy	Biweekly 3 mg doses, then weekly 2 mg doses per patient with max 4 peptides	PR:5 (20%)	52%	20.4	NR	None	71% patients increased in cellular or humoral responses specific to at least one fo the vaccinated peptides
Phase 1	Not applicable	2011	12	Rec	Stand alone	Weekly subcutaneous injections for 6 weeks, dose escalation trial (1–3 mg/ peptide)	PR:1 (8.3%)	66.7%	18.9	2.3	None	Both CTL and IgG responses to at least one peptide were augmented in most cases showing favorable clinical responses
Phase 3	Not applicable	2019	88	Rec	Stand alone	Weekly injections for 12 weeks, then biweekly until disease progression	PR:3 (5.3%)	42.1%	8.4	NR	None	68% CTL responses and 54% IgG boosting after vaccination,but not associated with outcomes
Autologous antigens												
AFTV												
Pilot	Not applicable	2007	12	Mixed (4 Nd, 8 Rec)	Stand alone	Weekly injection for 3 times	CR + PR:4 (33.3%)	41.7%	10.7	NR	None	Clinical responders all exhibited DTH responses
Case report	Not applicable	2011	3	Rec	AFTV + SOC	Weekly injection for 3 times	PR:2 (66.7%)	66.7%	12.4	4.8	None	Vaccine induced cellular immune response
Phase 1/2a	UMIN000001426	2014	24	Nd	AFTV + SOC	Weekly injection for 3 times	NR	NR	22.2	8.2	None	DTH response ≥10 mm after 3rd injection correlated with improved PFS
Retrospective	Not applicable	2021	277	Nd	AFTV + SOC	Weekly injection for 3 times	NR	NR	26.5	12.5	None	Not directly measured
Phase 2b	UMIN000010602	2023	63	Nd	AFTV + SOC	Weekly injection for 3 times	NR	NR	25.6	13.3	7 (23%)[c]	No significant difference in DTH response rates between vaccine and placebo
IGV001												
Pilot	Not applicable	2001	12	Rec	Stand alone	Tumor cells treated ex vivo with IGF-1R AS ODN, implanted for 24 h, then retrieved	CR + PR:8 (66.7%)	66.7%	NR	NR	None	Not directly measured

(Table 3 continues on next page)

Design	NCT number	Year	Patients	Type	Treatment protocol	Vaccine protocol	Overall response	Disease control rate	OS (Median months)	PFS (Median months)	Adverse event ≥ Grade 3	Immune response
(Continued from previous page)												
Phase 1	NCT01550523	2018	12	Rec	Stand alone	Tumor cells incubated with IGF-1R AS ODN, encapsulated in chambers, irradiated, then implanted for 24 h	NR	NR	22.8	NR	None	CD4+ T cells were significantly lower than normal range, while CD14+ monocytes were significantly increased
Phase 1b	NCT02507583	2021	33	Nd	Stand alone	Tumor cells incubated with IGF-1R AS ODN, encapsulated in 10 or 20 chambers for 24–48 h	NR	NR	38.2	11.6	None	Induced transient increase in proinflammatory cytokines (IFN-γ, IL-12p70, IL-2) between days 7 and 42 post-treatment, but no correlate with outcomes
Phase 2b	NCT04485949	2024	93	Nd	Stand alone	Biodiffusion chambers implanted in the abdomen for 48–52 h, then removed	NR	NR	NR	NR	None	No data available
Gliovac												
Pilot	Not applicable	2015	9	Rec	Vaccine + low-dose cyclophosphamide	6 cycles of 5 intradermal injections	22.2%	100%	NR	NR	None	Local immune response in tumor bed
Case report	Not applicable	2015	1	Rec	Vaccine + low-dose cyclophosphamide	4 cycles of 5 intradermal injections	0%	100%	42	10	None	Strong local immune response
Phase II	NCT0190333	2018	5	Rec	Vaccine + low-dose cyclophosphamide + bevacizumab	Repeated cycles of 5 intradermal injections	75%	NR	12.1	7.3	GD:1 (20%) BP:1 (20%) HX:2 (40%)[d]	CD3+/CD4+ lymphocyte counts highly correlate with overall survival
HSPPC												
Phase 1	Not applicable	2013	12	Rec	Stand alone	Weekly intradermal injections until progression	91.7%	NR	11.8	NR	None	Induced robust antigen-specific cellular responses locally and systemically
Phase 2	NCT0029342	2014	41	Rec	Stand alone	Median of 6 intradermal injections post-resection	NR	NR	42.6	NR	F:1 (2.4%)[e]	No available data
Phase 2	NCT0090506	2017	46	Nd	Vaccine + SOC	Weekly vaccinations post-radiotherapy for 4 weeks, then monthly until depletion or progression	NR	NR	23.8	18	None	Low peripheral PD-L1 expression on myeloid cells correlates with better survival
Phase 1	NCT0212282	2018	20	Nd	Vaccine + SOC	6 doses administered during adjuvant TMZ	NR	NR	21	10	None	High tumor-specific immune response correlates with longer OS and PFS
Customized antigens												
Neovax												
Phase 1b	NCT0228742	2019	10	Nd	Stand alone	5 priming doses followed by booster vaccinations, divided into peptide pools	NR	NR	16.8	7.6	None	Induced robust cellular responses including polyfunctional $CD4^+$ and $CD8^+$ T cells targeting mutant neoepitopes and producing IFN-γ, IL-2, and TNF
Pilot	NCT0342209	2024	4	Nd	Stand alone	4 priming doses on Days 1, 8, 15, 22; Boosters on Day 1 of each 28-day cycle	NR	NR	15.5	12	None	A significant increase in IFN-γ producing T cells after vaccination

(Table 3 continues on next page)

Design	NCT number	Year	Patients	Type	Treatment protocol	Vaccine protocol	Overall response	Disease control rate	OS (Median months)	PFS (Median months)	Adverse event ≥ Grade 3	Immune response
(Continued from previous page)												
Cohort study	Not applicable	2024	173	Mixed (70 Nd, 103 Rec)	Stand alone	Vaccination with a median of 19 peptides per patient over multiple cycles	NR	NR	31.9	NR	4 (2.3%)[g]	Induced T-cell responses in 90% of patients, with durable antigen-specific cellular immunity, and patients with multiple vaccine-induced T-cell responses had significantly longer survival than those with absent or low responses
GAPVAC												
Phase 1	NCT02149225	2019	16	Nd	Stand alone	APVAC1 (11 vaccinations, unmutated epitopes) followed by APVAC2 (8 doses, neoepitopes)	31.3%	50%	29	NR	6 (40%)[f]	APVAC1 activate CD8+ T cells and APVAC2 elicit polyfunctional CD4+ T-cell responses
mRNA												
Phase I	NCT04573140	2024	4	Nd	Vaccine + SOC	3 biweekly priming doses followed by monthly booster injections	NR	NR	NR	NR	None	Induced potent cellular immune responses, including robust CD8+ T-cell infiltration, enhanced expression of IFN-γ

Nd, newly diagnosed; Rec, recurrent; SOC, standard of care; NR, not reported; BE, brain edema; ISR, injection site reaction; GD, gait disturbance; BP, back pain; HX, headache; F, fatigue; OS, overall survival; PFS, progression-free survival. [a]Two patients experienced vaccine-related grade 3 dose-limiting toxicity of fatigue and anaphylaxis. [b]One patient experienced vaccine-related grade 4 brain edema, but symptoms rapidly resolved after high-dose steroids. One patient experienced grade 3 injection site reaction. [c]Adverse events of grade 3 and higher occurred in 7 patients of the AFTV group (four lymphocyte, count reductions, one skin reaction, one nausea/appetite loss, and one episode of diarrhea). [d]Only grade 3 toxicities were reported (one gait disturbance/fall, one back pain, two headache). No grade 4 or 5 toxicities. [e]There were 37 serious (grade 3–5) adverse events documented, with 17 attributable to surgical resection and a single patient experienced grade 3 fatigue attributed to the vaccine. There were no grade 4 adverse events or deaths related to the vaccine. [f]There were 11 serious (grade 3–5) adverse events documented, and 6 were attributed to the vaccine (one injection site disorder, one lymphopenia, one leukopenia, two anaphylactic reaction). [g]Four patients experienced grade 3 adverse events including allergic reaction, anaphylaxis, and skin reaction. No grade 4 adverse events were observed.

Table 3: **Summary of clinical trials for multi-target vaccines.**

class I allele A*02, and two bind to various HLA class II alleles.[51] The IMA950 vaccine combines the 11 peptides and recombinant granulocyte macrophage-colony stimulating factor (GM-CSF; 75 μg). This strategy has completed Phase II testing (Table 3, Supplementary Material 1, references 14, 15).

TAS0313

TAS0313 is a multi-epitope long peptide cancer vaccine containing three long peptides (TAS0314, TAS0315, and TAS0316) totaling 12 cytotoxic T lymphocyte (CTL) epitope peptides. These epitopes were identified in a three-step process: 1) the expression of known cancer-associated antigens was examined using publicly accessible databases, 2) computational tools were used to predict potential CTL epitopes within the selected antigen, and 3) the predicted epitopes were then experimentally validated for their ability to bind to HLA molecules and stimulate CTL responses *in vitro*. The eight cancer antigens targeted by TAS0313 are shown in Table 4. The long peptide design aims to overcome the low immunogenicity of short peptides.[52] The epitope peptides were restricted by HLA-A2 and A3-supertype.[52] This strategy has completed Phase II testing (Table 3, Supplementary Material 1, reference 16).

Glioma oncoantigen/glioma angiogenesis-associated antigen (GOA/GAAA) vaccination

The GOA/GAAA (glioma oncoantigen/glioma angiogenesis-associated antigen) is a vaccine strategy that simultaneously targets antigens from both cancer cells and the tumor vasculature (Table 4). The selected epitopes are restricted to HLA-A*2402. A mixture of synthetic peptides was emulsified in Montanide ISA-51VG adjuvant (an incomplete Freund's adjuvant known to enhance immune responses) and administered subcutaneously near axillary or inguinal lymph nodes. This strategy has completed Phase I testing (Table 3, Supplementary Material 1, reference 17).

Vaccines	# of TAA	TAA
IMA950	11	Brevican (BCAN) Baculoviral IAP repeat-containing 5 (BIRC5) Chondroitin sulfate proteoglycan 4 (CSPG4) Fatty acid binding protein 7, brain (FABP7) Hepatitis B virus, core antigen (HbcAg) Insulin-like growth factor 2 mRNA binding protein 3 (IGF2BP3) Met proto-oncogene (hepatocyte growth factor receptor) (MET) Neuroligin 4, X-linked (NLGN4X) Neuronal cell adhesion molecule (NRCAM) Protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1) Tenascin C (TNC)
TAS0313	8	Epidermal growth factor receptor (EGFR) Lymphocyte-specific protein tyrosine kinase (LCK) Multi-drug resistance protein 3 (MRP3) Squamous cell carcinoma antigen recognized by T cells-2 (SART-2) Squamous cell carcinoma antigen recognized by T cells-3 (SART-3) Parathyroid hormone-related protein (PTHrP) Ubiquitin conjugating enzyme E2 V1 (UBE2V) Wolf-Hirschhorn syndrome candidate 2 (WHSC2)
GOA/GAAA	6	Lymphocyte antigen six family member K (LY6K) DEP domain containing 1 (DEPDC1) Kinesin family member 20 A (KIF20A) Forkhead box M1 (FOXM1) Vascular endothelial growth factor receptor-1 (VEGF1) Vascular endothelial growth factor receptor-2 (VEGFR2)

GOA/GAA, Glioma oncoantigen/glioma angiogenesis-associated antigen.

Table 4: **Preselected antigens targeted by multi-target vaccines.**

Personalized cancer vaccines

Personalized vaccine strategies aim to deliver immunogenic peptides based on i) host immunity, ii) antigens derived from autologous tumor lysate, and iii) targeting the mutational landscape of the tumor (Fig. 4).

i) Targeting host immunity

Building on progress in cancer vaccines with preselected target antigens, studies were designed to select patients who harbored preexisting cellular or humoral responses to the candidate vaccine peptides.[53] Two phase I/II studies that enrolled patients with recurrent high-grade gliomas demonstrated the safety and feasibility of this approach, leading to completion of a phase III study (Table 3, Supplementary Material 1, references 18–20).

ii) Targeting antigens derived from autologous tumor lysates

Autologous Formalin-Fixed Tumor Vaccine (AFTV)

AFTV is a multi-target, autologous tumor vaccine strategy that leverages the observation that the antigenicity of tumor-associated peptides is preserved after formalin fixation. While formalin fixation is associated with fragmentation, chemically modified peptides, and cross-linked peptides, these peptides are taken up by APCs, processed, and presented through MHC class I and II complexes in pre-clinical models to generate CTLs.[54] The potency and diversity of CTLs generated by antigens prepared from formalin-fixed tumor samples are comparable to those prepared from freshly isolated tumor samples.[55] Since AFTV can be prepared from formalin-fixed paraffin-embedded samples (FFPE), its application potentially expands the population of patients who may benefit from this therapy. This strategy has completed Phase IIb testing (Table 3, Supplementary Material 1, references 21–25).

IGV-001

IGV-001 is another platform for autologous cancer vaccine therapy. The underlying premise is to induce tumor-specific antigen release via immunogenic cell death (ICD). ICD is achieved by inhibiting the insulin-like growth factor 1 receptor (IGF-1R), a tyrosine kinase receptor that promotes glioblastoma proliferation and

survival.[56] To achieve ICD, tumor-derived glioblastoma cells are co-incubated with an 18-mer antisense oligonucleotide targeting IGF1R (termed IMV-001) in a biodiffusing chamber (BCD) designed to release small subcellular particles through a 0.1 μm filter. A key aspect of IGV-001 involves implanting this BCD into the abdomen, thereby bypassing the immunosuppressive glioblastoma microenvironment. Because of intrinsic immune surveillance and lymphatics in this compartment, released tumor antigens are expected to be captured by antigen-presenting cells, initiating the cascade that ultimately results in anti-glioblastoma immunity. This strategy has completed Phase IIb, with results still under evaluation (Table 3, Supplementary Material 1, references 26–29).

ERC1671 (Gliovac)

ERC1671 (Gliovac) is another mult-target cancer vaccine strategy that combines autologous and allogeneic tumor lysates. The vaccine strategy involves cycles of subcutaneous injections using lysates derived from three irradiated, three donor patient-derived glioblastoma cell lines (noted as ERC A, B, and C) and a fourth lysate derived from tumors isolated from the patient under treatment (ERC D). This strategy's key premise is that glioblastoma patients may have developed immune tolerance to autologous tumor antigens.[57] The inclusion of foreign antigens from the donor tumor lines is thought to disrupt this immune tolerance. The inclusion of autologous antigens enables the reactivated immune system to mount T-cell responses that specifically target these antigens. This strategy is currently in Phase II evaluation (Table 3, Supplementary Material 1, references 30–32).

Heat Shock Protein (HSP) peptide vaccination

The heat shock protein (HSP) peptide cancer vaccine strategy leverages the observation that HSPs can deliver tumor antigens and facilitate the development of antitumor immunity. The HSP gp-96 can simultaneously bind tumor proteins and the CD91 receptor of APCs. Upon binding CD91, the tumor protein-HSP gp-96 (HSPPC-96) complex is internalized by APC, leading to the cleavage and presentation of antigenic peptides on MHC molecules. Thus, by purifying HSPPC-96-tumor complexes from resected glioblastoma specimens, the polyvalent vaccine is isolated for subsequent administration. In addition to facilitating antigen uptake and presentation, HSPPC-96 triggers local immune activation by activating natural killer (NK) cells.[58] This strategy

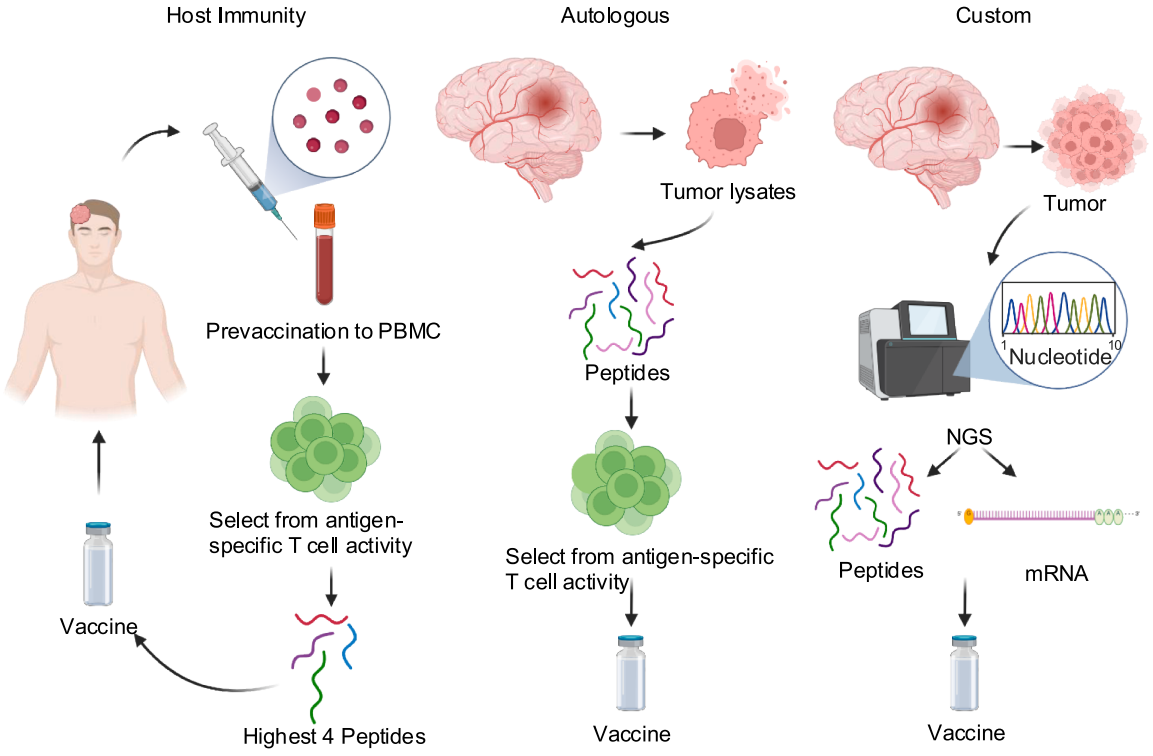

Fig. 4: Personalized tumor antigen only (TAO) vaccine strategies. The schematic illustrates three main approaches to personalized TAO vaccine development: (i) selection of immunogenic peptides based on host immunity using peripheral blood mononuclear cells (PBMCs), (ii) peptides derived from autologous tumor lysates, and (iii) identification of neoantigens through next-generation sequencing (NGS) of tumor-specific mutations for customized peptide or mRNA vaccine synthesis.

has completed Phase II testing (Table 3, Supplementary Material 1, references 33–36).

iii) Targeting the genetic landscape of the tumor

Neovax

The Neovax approach utilizes next-generation sequencing (NGS) and transcriptomic analyses to identify patient-specific mutations and computationally predict those likely to encode neoantigen epitopes presented by class I HLA. Immunizing peptides targeting neo-antigens are selected and synthesized based on the patient's HLA and computational predictions. The peptides are typically formulated and mixed in pools containing various forms of immune adjuvants (including ICLC and GM-CSF). These formulations are then administered subcutaneously. This strategy has completed Phase I/Ib testing (Table 3, Supplementary Material 1, references 37–38).

The European experience

Using an approach analogous to the Neovax strategy, in which synthetic peptides were generated from genomic information derived from the patient's tumor specimen, a "real-world" observational study was reported (Table 3, Supplementary Material 1, reference 39).

GAPVAC

The GAPVAC (Glioma Actively Personalized Vaccine Consortium) also uses next-generation sequencing (NGS) and transcriptomic analyses to characterize tumor samples. However, instead of synthesizing the epitope peptide for each patient, the information was used to select from a library of 59 HLA class I-binding peptides and three HLA class II-binding peptides that are frequently overexpressed in glioblastomas (APVAC1). This selection is augmented with one or two peptides that are *de novo* synthesized for each patient (APVAC2).[59] The strategy aims to vaccinate the patient with a mixture of 20 peptides. The vaccination course was integrated with standard glioblastoma treatment.[60] This strategy has completed Phase I testing (Table 3, Supplementary Material 1, reference 40).

mRNA-based approach

The success of the mRNA vaccine against SARS-CoV-2 has paved the way for mRNA-based cancer vaccines.[61] Advances in lipid particle formulation that mimic viral aggregates and immune activation strategies further provide the foundation for personalized mRNA vaccines. Manufacturing mRNA cancer vaccines involves *in vitro* transcription of tumor-derived mRNA, purification of the transcripts, and formulation into lipid particle aggregates (LPAs). In addition to tumor-specific mRNAs, mRNA encoding known glioblastoma-associated antigens can be incorporated into the RNA-LPA particle. As an illustration example, Mendez-Gomez et al. (Supplementary Material 1, reference 41), assembled RNA-LPA containing tumor-specific mRNA and mRNA encoding pp65 (which is over-expressed in glioblastomas) and showed that this RNA-LPA elicited rapid cytokine/chemokine release, immune activation/trafficking, and glioma-specific immune responses in glioblastoma patients. This strategy has completed Phase I testing (Table 3, Supplementary Material 1, reference 41).

Summary of clinical trials for multi-target vaccines

The schedules of multi-target vaccine administration are similar to those reported for single-target vaccines. All multi-target vaccination studies demonstrate evidence of vaccine-induced immune responses. In contrast to single-target vaccination studies, AEs > grade III have been noted in multi-target vaccination studies (Table 3), including anaphylaxis requiring medical treatment. The mOS and mPFS observed in select studies have exceeded results achieved with the standard of care or current clinical practices. However, some of these reports included post-hoc analysis (e.g., IGV-001), inclusion of IDHm astrocytoma patients (the European experience), or vaccine treatment before tumor progression was detected (the European experience).

As in single-target vaccine trials, both Objective Response Rates (ORRs) and Disease Control Rates (DCRs) exhibit considerable variability across studies. Reported ORRs range from 2.5% to 66.7%, with the HSPPC vaccine demonstrating the highest ORR. DCRs span from 40% to 100%, with AFTV, IGV-001, with Gliovac achieving the highest rates of disease control. Notably, overall survival (OS) outcomes differ significantly even among studies with comparable ORR and DCR values, suggesting that early radiographic or clinical responses may not fully capture the long-term therapeutic impact.

Two randomized trials explored the multi-target vaccination strategy, but neither met the primary endpoint. The first study selected patients who harbored pre-existing immune responses to the administered vaccine peptides. This study randomized 88 HLA–A24–positive, recurrent high-grade glioma patients (including 18 IDHm grade IV astrocytomas) to receive peptides selected based on the patient's pre-existing peptide-specific immunoglobulin G levels or placebo (randomized 2:1). mOS for the treatment and placebo-treated groups were 8.4 and 8.0 months, respectively ($p = 0.621$) (Supplementary Material 1, reference 20). The second (UMIN000010602) enrolled 63 newly diagnosed IDH wild-type glioblastoma patients; 32 received AFTV, and 31 received placebo. The mOS for the treatment group was 25.6 months, which was not significantly different from the placebo group (Supplementary Material 1, reference 25).

Discussion

While Phase I and II cancer vaccine studies show evidence of immune responses and potentially promising survival outcomes, the Phase III studies for rindopepimut, AFTV, and multi-target vaccine targeting pre-existing immunity showed no difference between treatment and control groups. In retrospect, the survival reported by the various phase I and II studies fell within ranges of mMGMT glioblastomas or the IDHm astrocytoma survival. While survival beyond 3 years is observed in patient subsets (e.g., AFTV-treated patients who underwent gross total resection), interpretation of a post hoc analysis derived from such a small sample size (n = 11) warrants caution.

Despite these observations, the consistent documentation of vaccine safety and tumor-specific responses warrants some optimism. Results from single-cell T cell receptor sequencing analysis demonstrated neo-antigen-specific T cells[62] in the glioblastoma tumor infiltrate, and the absence of vaccine target proteins in the post-treatment clinical specimens[63] provides proof-of-principle. However, the modest survival outcome of the published studies and the finding that anti-tumoral immunity pre-exists before cancer vaccination in glioblastoma patients[64] raised questions on the potency of anti-glioblastoma immunity. Moreover, the time from vaccine administration to first detection of vaccine-induced immune response can be > 600 days,[65] which may exceed the survival expectations for some glioblastoma patients. While likely incremental in significance, advances in surgery, chemotherapy, and radiation therapy may afford the time required to mount an anti-glioblastoma immune response.

The notable redundancy in the molecular circuitry that fuels glioblastoma growth[66] and the intratumoral genetic and epigenetic heterogeneity[67] suggest that single-target vaccine therapy is unlikely to significantly prolong survival, unless the target is essential and functionally non-redundant. With multiple target vaccination therapeutic strategies potentially address the challenges associated with redundant circuitry and intratumoral heterogeneity, antigenic competition[68] can lead to a less focused and weaker immune response against the tumor. Further, as demonstrated by the IMA950 and other multi-target vaccine trials, multi-target vaccination potentially increases the risk of serious adverse events.[69] Harvesting available data to identify effective anti-tumoral antigens and targeting them may serve as a middle-ground approach in cancer vaccination therapies.

Improving gene landscape analysis and pathology assessments is crucial for developing personalized and effective therapies. To overcome the limitations of traditional bulk genomic analyses, which fail to capture the profound heterogeneity of glioblastomas, researchers now use advanced multi-omics techniques, including single-cell RNA sequencing (scRNA-seq) and spatial genomics, which detail the transcriptional profile of individual cells within the tumor microenvironment (TME).[70] T-cell receptor (TCR) sequencing enables a precise understanding of the T-cell immune response, tracks vaccine effectiveness, and uncovers potential predictive biomarkers.[71] Non-invasive liquid biopsies, which analyze cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) from blood or cerebrospinal fluid, enable monitoring of tumor evolution, thereby enabling adaptation of vaccine strategies.[72] In addition to these genomic tools, the 2021 World Health Organization (WHO) classification for CNS tumors provides improved pathology classification, enabling trial designs that select for more comparable patients to strengthen study outcomes.

Beyond target selection, the immune-suppressive tumor microenvironment and corticosteroid-associated immune suppression remain significant therapeutic barriers for cancer vaccination strategies. The question of whether bevacizumab should replace corticosteroids in glioblastoma patients undergoing immunotherapy remains an open question. Emerging treatment paradigms,[73] including laser thermal ablation[74] and immune modulation through tumor-treating fields offer potential strategies for tackling these barriers. For instance, the acute, local inflammation provoked by laser thermal ablation or oncolytic viruses[5] can counteract the immunosuppressive glioblastoma microenvironment and amplify anti-tumor immunity.[75] These strategies warrant consideration in the next stage of cancer vaccine strategies as a glioblastoma treatment platform.

Contributors

CC and HC planned and designed the study, developed the search strategy, screened potential studies, extracted/accessed/verified the data, performed the analysis, and reviewed/interpreted the identified studies. NK, SM, and AS provided methodological support. RA, DC, KS, PS, AM, CL, CD, SL, and WE contributed to data interpretation and manuscript preparation. All authors approved the final version of the manuscript.

Declaration of interests

CCC reports receiving consulting fees from Medtronic and EF Biotech, as well as meeting support from GT Medical. These companies had no involvement in the study design, data interpretation, or reporting of this work.

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SAM is the founder and advisor of Iylon Precision Oncology and Sathgen Therapeutics, and serves as an advisor to Xelcis Bio. None of these companies had any involvement in the study design, data interpretation, or reporting of this work.

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WSE is the founder and shareholder of Resurrect Therapeutics; the founder and shareholder (SRA) of SMURF-Therapeutics; and the founder and shareholder of P53 Therapeutics, with no associated research funding. WSE is also the founder of Oncoceutics and a shareholder of Oncoceutics/Chimerix (SRA from Chimerix), and holds stock in Jazz Pharmaceuticals. Additional roles include service on the ACS BrightEdge Advisory Board and the Ocean Biomedical Scientific Advisory Board; Chair of the WIN Consortium; Editor-in-Chief of Oncotarget; Co-Chair of Carls Life Sciences; member of the Executive Committee for the Precision Oncology Alliance; and equity holder in Global Cancer Technology. None of these entities had any role in the study's design, data interpretation, or reporting.

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Appendix A. Supplementary data

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References

1 Schaff LR, Mellinghoff IK. Glioblastoma and other primary brain malignancies in adults: a review. *JAMA*. 2023;329(7):574–587.
2 Pouyan A, Ghorbanlo M, Eslami M, et al. Glioblastoma multiforme: insights into pathogenesis, key signaling pathways, and therapeutic strategies. *Mol Cancer*. 2025;24(1):58.
3 Hu A, Sun L, Lin H, Liao Y, Yang H, Mao Y. Harnessing innate immune pathways for therapeutic advancement in cancer. *Signal Transduct Target Ther*. 2024;9(1):68.
4 Devaraja K, Singh M, Sharan K, Aggarwal S. Coley's toxin to first approved therapeutic Vaccine-A brief historical account in the progression of immunobiology-based cancer treatment. *Biomedicines*. 2024;12(12):2746.
5 Hamad A, Yusubalieva GM, Baklaushev VP, Chumakov PM, Lipatova AV. Recent developments in glioblastoma therapy: oncolytic viruses and emerging future strategies. *Viruses*. 2023;15(2):547.
6 Park S, Maus MV, Choi BD. CAR-T cell therapy for the treatment of adult high-grade gliomas. *NPJ Precis Oncol*. 2024;8(1):279.
7 Xiong Z, Raphael I, Olin M, Okada H, Li X, Kohanbash G. Glioblastoma vaccines: past, present, and opportunities. *eBioMedicine*. 2024;100:104963.
8 Hawgood BJ. Albert Calmette (1863-1933) and Camille Guerin (1872-1961): the C and G of BCG vaccine. *J Med Biogr*. 2007;15(3):139–146.
9 Plosker GL. Sipuleucel-T: in metastatic castration-resistant prostate cancer. *Drugs*. 2011;71(1):101–108.
10 Stoyanov GS, Dzhenkov DL. On the concepts and history of glioblastoma Multiforme - morphology, genetics and epigenetics. *Folia Med (Plovdiv)*. 2018;60(1):48–66.
11 Fares J, Wan Y, Mair R, Price SJ. Molecular diversity in isocitrate dehydrogenase-wild-type glioblastoma. *Brain Commun*. 2024;6(2):fcae108.
12 Pegg AE. Mammalian O6-alkylguanine-DNA alkyltransferase: regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res*. 1990;50(19):6119–6129.
13 Butler M, Pongor L, Su YT, et al. MGMT status as a clinical biomarker in glioblastoma. *Trends Cancer*. 2020;6(5):380–391.
14 Szopa W, Burley TA, Kramer-Marek G, Kaspera W. Diagnostic and therapeutic biomarkers in glioblastoma: current status and future perspectives. *Biomed Res Int*. 2017;2017:8013575.
15 Caccese M, Simonelli M, Villani V, et al. Definition of the prognostic role of MGMT promoter methylation value by pyrosequencing in newly diagnosed IDH wild-type glioblastoma patients treated with radiochemotherapy: a large multicenter study. *Cancers (Basel)*. 2022;14(10):2425.
16 Draaisma K, Chatzipli A, Taphoorn M, et al. Molecular evolution of IDH wild-type glioblastomas treated with standard of care affects survival and design of precision medicine trials: a report from the EORTC 1542 Study. *J Clin Oncol*. 2020;38(1):81–99.
17 Wick W, Gorlia T, Bendszus M, et al. Lomustine and Bevacizumab in progressive glioblastoma. *N Engl J Med*. 2017;377(20):1954–1963.
18 Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. 2007;318(5848):287–290.
19 Tilak M, Holborn J, New LA, Lalonde J, Jones N. Receptor tyrosine kinase signaling and targeting in glioblastoma multiforme. *Int J Mol Sci*. 2021;22(4):1831.
20 Thomas AA, Fisher JL, Ernstoff MS, Fadul CE. Vaccine-based immunotherapy for glioblastoma. *CNS Oncol*. 2013;2(4):331–349.
21 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: a phase 3 prospective externally controlled cohort trial. *JAMA Oncol*. 2023;9(1):112–121.
22 Shah S, Nag A, Lucke-Wold B. Autologous tumor lysate-loaded dendritic cell vaccination in glioblastoma patients: a systematic review of literature. *Clin Transl Oncol*. 2025;27(7):2889–2903.
23 Kaczmarek M, Poznanska J, Fechner F, et al. Cancer vaccine therapeutics: limitations and Effectiveness-A literature review. *Cells*. 2023;12(17).
24 Chen F, Tang H, Cai X, et al. DAMPs in immunosenescence and cancer. *Semin Cancer Biol*. 2024;106–107:123–142.
25 Wheeler CJ, Black KL, Liu G, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res*. 2008;68(14):5955–5964.
26 Harryvan TJ, de Lange S, Hawinkels L, Verdegaal EME. The ABCs of antigen presentation by stromal non-professional antigen-presenting cells. *Int J Mol Sci*. 2021;23(1):137.
27 Fan T, Zhang M, Yang J, Zhu Z, Cao W, Dong C. Therapeutic cancer vaccines: advancements, challenges, and prospects. *Signal Transduct Target Ther*. 2023;8(1):450.
28 Lim KP, Zainal NS. Monitoring T cells responses mounted by therapeutic cancer vaccines. *Front Mol Biosci*. 2021;8:623475.
29 Mahmood F, Xu R, Awan MUN, et al. HBV vaccines: advances and development. *Vaccines (Basel)*. 2023;11(12):1862.
30 Duffy PE, Patrick Gorres J. Patrick Gorres J. Malaria vaccines since 2000: progress, priorities, products. *NPJ Vaccines*. 2020;5(1):48.
31 Xu H, Zong H, Ma C, et al. Epidermal growth factor receptor in glioblastoma. *Oncol Lett*. 2017;14(1):512–516.
32 An Z, Aksoy O, Zheng T, Fan QW, Weiss WA. Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. *Oncogene*. 2018;37(12):1561–1575.
33 Struve N, Binder ZA, Stead LF, et al. EGFRvIII upregulates DNA mismatch repair resulting in increased temozolomide sensitivity of MGMT promoter methylated glioblastoma. *Oncogene*. 2020;39(15):3041–3055.
34 Nitta M, Kozono D, Kennedy R, et al. Targeting EGFR induced oxidative stress by PARP1 inhibition in glioblastoma therapy. *PLoS One*. 2010;5(5):e10767.
35 Shen Y, Li J, Nitta M, et al. Orthogonal targeting of EGFRvIII expressing glioblastomas through simultaneous EGFR and PLK1 inhibition. *Oncotarget*. 2015;6(14):11751–11767.
36 Choi BD, Archer GE, Mitchell DA, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. *Brain Pathol*. 2009;19(4):713–723.
37 Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG, Batchelor TT. Angiogenesis in brain tumours. *Nat Rev Neurosci*. 2007;8(8):610–622.
38 Szabo E, Schneider H, Seystahl K, et al. Autocrine VEGFR1 and VEGFR2 signaling promotes survival in human glioblastoma models in vitro and in vivo. *Neuro Oncol*. 2016;18(9):1242–1252.
39 Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer*. 2013;13(12):871–882.
40 Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature*. 1993;362(6423):841–844.
41 Shibao S, Ueda R, Saito K, et al. A pilot study of peptide vaccines for VEGF receptor 1 and 2 in patients with recurrent/progressive high grade glioma. *Oncotarget*. 2018;9(30):21569–21579.
42 Toska E, Roberts SG. Mechanisms of transcriptional regulation by WT1 (Wilms' tumour 1). *Biochem J*. 2014;461(1):15–32.

43 Yang L, Han Y, Suarez Saiz F, Minden MD. A tumor suppressor and oncogene: the WT1 story. *Leukemia*. 2007;21(5):868–876.
44 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(3):113–116.
45 Rauscher J, Beschorner R, Gierke M, et al. WT1 expression increases with malignancy and indicates unfavourable outcome in astrocytoma. *J Clin Pathol*. 2014;67(7):556–561.
46 Van Driessche A, Berneman ZN, Van Tendeloo VF. Active specific immunotherapy targeting the Wilms' tumor protein 1 (WT1) for patients with hematological malignancies and solid tumors: lessons from early clinical trials. *Oncologist*. 2012;17(2):250–259.
47 Li F, Ambrosini G, Chu EY, et al. Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature*. 1998;396(6711):580–584.
48 Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*. 1997;3(8):917–921.
49 Kajiwara Y, Yamasaki F, Hama S, et al. Expression of survivin in astrocytic tumors: correlation with malignant grade and prognosis. *Cancer*. 2003;97(4):1077–1083.
50 Ciesielski MJ, Kozbor D, Castanaro CA, Barone TA, Fenstermaker RA. Therapeutic effect of a T helper cell supported CTL response induced by a survivin peptide vaccine against murine cerebral glioma. *Cancer Immunol Immunother*. 2008;57(12):1827–1835.
51 Dutoit V, Herold-Mende C, Hilf N, et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. *Brain*. 2012;135(Pt 4):1042–1054.
52 Kondo S, Shimizu T, Koyama T, et al. First-in-human study of the cancer peptide vaccine TAS0313 in patients with advanced solid tumors. *Cancer Sci*. 2021;112(4):1514–1523.
53 Mine T, Sato Y, Noguchi M, et al. Humoral responses to peptides correlate with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing, peptide-specific cellular responses. *Clin Cancer Res*. 2004;10(3):929–937.
54 Tsurushima H, Liu SQ, Tuboi K, et al. Reduction of end-stage malignant glioma by injection with autologous cytotoxic T lymphocytes. *Jpn J Cancer Res*. 1999;90(5):536–545.
55 Liu SQ, Saijo K, Todoroki T, Ohno T. Induction of human autologous cytotoxic T lymphocytes on formalin-fixed and paraffin-embedded tumour sections. *Nat Med*. 1995;1(3):267–271.
56 Andrews DW, Resnicoff M, Flanders AE, et al. Results of a pilot study involving the use of an antisense oligodeoxynucleotide directed against the insulin-like growth factor type I receptor in malignant astrocytomas. *J Clin Oncol*. 2001;19(8):2189–2200.
57 Bota DA, Alexandru-Abrams D, Pretto C, et al. Use of ERC-1671 vaccine in a patient with recurrent glioblastoma multiforme after progression during bevacizumab therapy: first published report. *Perm J*. 2015;19(2):41–46.
58 Yang I, Fang S, Parsa AT. Heat shock proteins in glioblastomas. *Neurosurg Clin N Am*. 2010;21(1):111–123.
59 Kreiter S, Vormehr M, van de Roemer N, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature*. 2015;520(7549):692–696.
60 Morse MA, Chapman R, Powderly J, et al. Phase I study utilizing a novel antigen-presenting cell-targeted vaccine with toll-like receptor stimulation to induce immunity to self-antigens in cancer patients. *Clin Cancer Res*. 2011;17(14):4844–4853.
61 Bettini E, Locci M. SARS-CoV-2 mRNA vaccines: immunological mechanism and beyond. *Vaccines (Basel)*. 2021;9(2):147.
62 Paria BC, Levin N, Lowery FJ, et al. Rapid identification and evaluation of neoantigen-reactive T-Cell receptors from single cells. *J Immunother*. 2021;44(1):1–8.
63 Keskin DB, Anandappa AJ, Sun J, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature*. 2019;565(7738):234–239.
64 Segura-Collar B, Hiller-Vallina S, de Dios O, et al. Advanced immunotherapies for glioblastoma: tumor neoantigen vaccines in combination with immunomodulators. *Acta Neuropathol Commun*. 2023;11(1):79.
65 Latzer P, Zelba H, Battke F, et al. A real-world observation of patients with glioblastoma treated with a personalized peptide vaccine. *Nat Commun*. 2024;15(1):6870.
66 Garcia JH, Jain S, Aghi MK. Metabolic drivers of invasion in glioblastoma. *Front Cell Dev Biol*. 2021;9:683276.
67 Parker NR, Hudson AL, Khong P, et al. Intratumoral heterogeneity identified at the epigenetic, genetic and transcriptional level in glioblastoma. *Sci Rep*. 2016;6:22477.
68 Eidinger D, Khan SA, Millar KG. The effect of antigenic competition on various manifestations of humoral antibody formation and cellular immunity. *J Exp Med*. 1968;128(5):1183–1200.
69 Migliorini D, Dutoit V, Allard M, et al. Phase I/II trial testing safety and immunogenicity of the multipeptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients. *Neuro Oncol*. 2019;21(7):923–933.
70 Ren Y, Huang Z, Zhou L, et al. Spatial transcriptomics reveals niche-specific enrichment and vulnerabilities of radial glial stem-like cells in malignant gliomas. *Nat Commun*. 2023;14(1):1028.
71 Hsu M, Sedighim S, Wang T, et al. TCR sequencing can identify and track glioma-infiltrating T cells after DC vaccination. *Cancer Immunol Res*. 2016;4(5):412–418.
72 Iorgulescu JB, Blewett T, Xiong K, et al. Impact of higher cell-free DNA yields on liquid biopsy testing in glioblastoma patients. *Clin Chem*. 2025;71(1):215–225.
73 Sharma P, Aaroe A, Liang J, Puduvalli VK. Tumor microenvironment in glioblastoma: current and emerging concepts. *Neurooncol Adv*. 2023;5(1):vdad009.
74 de Groot JF, Kim AH, Prabhu S, et al. Efficacy of laser interstitial thermal therapy (LITT) for newly diagnosed and recurrent IDH wild-type glioblastoma. *Neurooncol Adv*. 2022;4(1):vdac040.
75 Chandar JS, Bhatia S, Ingle S, et al. Laser interstitial thermal therapy induces robust local immune response for newly diagnosed glioblastoma with long-term survival and disease control. *J Immunother*. 2023;46(9):351–354.