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Immunologic specificity in glioblastoma: Antigen discovery and translational implications

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Abstract

Recent studies have highlighted the therapeutic potential of targeting tumor antigens (TAs) in glioblastoma (GBM). Several classes of TAs, such as tumor-associated, cancer testis, and tumor-specific antigens, have proven to be immunogenic and used safely in vaccines. Many of these vaccines have focused on tumor-associated or cancer testis antigens. However, tumor-specific antigens (TSA) present an ideal target due to the lack of tolerance and exclusive tumor expression, mitigating the risk of off-target effects. Most research on TSAs in GBM has aimed to uncover neoantigens, yet the dearth of shared neoantigens as well as the cost and labor-intensive process of identifying personal neoantigens have acted as barriers to treatment. A better understanding of the individual antigens spanning all three TA classes is important to improve the design of GBM antigen therapies and understand, fundamentally, the nature of immunologic specificity in glioma. We review the antigen classes in all cancers and how TAs are discovered. Then, we focus on the unique properties of GBM and the antigens that have been identified and used for therapy in GBM. Finally, we discuss translational considerations for future antigen-targeted treatments.

Key Points

- Numerous types of GBM antigens can be recognized by T cells, including tumor associated antigens, cancer testis antigens and tumor specific antigens.
- Therapeutic applications of antigens include vaccines and adoptive cell therapy, both of which have been shown to be safe in humans.
- Prior to targeting via therapy, antigens should be confirmed to be present in the immunopeptidome, since often algorithm-identified "neoantigens" are not displayed by the tumor.
- To combat GBM heterogeneity, antigen-focused therapies should target both MHC class I and class II restricted antigens, as well as multiple classes of antigens.

Glioblastoma (GBM) is the most common malignant central nervous system (CNS) cancer in adults.[1] With standard therapy, median survival is around 20 months,[2–4] emphasizing the need for novel therapies. Targeting tumor antigens (TAs), the peptides presented by tumors on major histocompatibility complex (MHC) molecules, either via vaccine[5,6] or adoptive T cell transfer (ACT)[7–12] has shown success in other cancers and holds promise for GBM.

As context for our discussion of TAs, it is important to briefly consider our definition for "antigen." We are exclusively referring to T cell antigens: when we use the terms "antigen," "epitope," or "peptide" we are referring to the 8–10 length or the 13–25 length amino acid sequence that is non-covalently bound to an MHC class I (HLA-A, HLA-B, or HLA-C) or MHC class II (HLA-DP, HLA-DQ, or HLA-DR) molecule,

respectively.[13,14] During class I antigen processing, proteasomes typically cleave proteins in the cytosol into shorter peptides, and during class II processing, lysosomes commonly digest uptaken extracellular proteins into shorter peptides.[13] The peptides capable of binding MHC molecules are loaded; then, the peptide-MHC complex (pMHC) is presented on the cell surface.[13] A class I pMHC can be recognized by CD8+ T cells, while a class II pMHC can be recognized by CD4+ T cells.[13,14] T cell activation requires T cell receptor (TCR) contact with amino acids from both the antigen and the MHC, as well as costimulating molecules.[15]

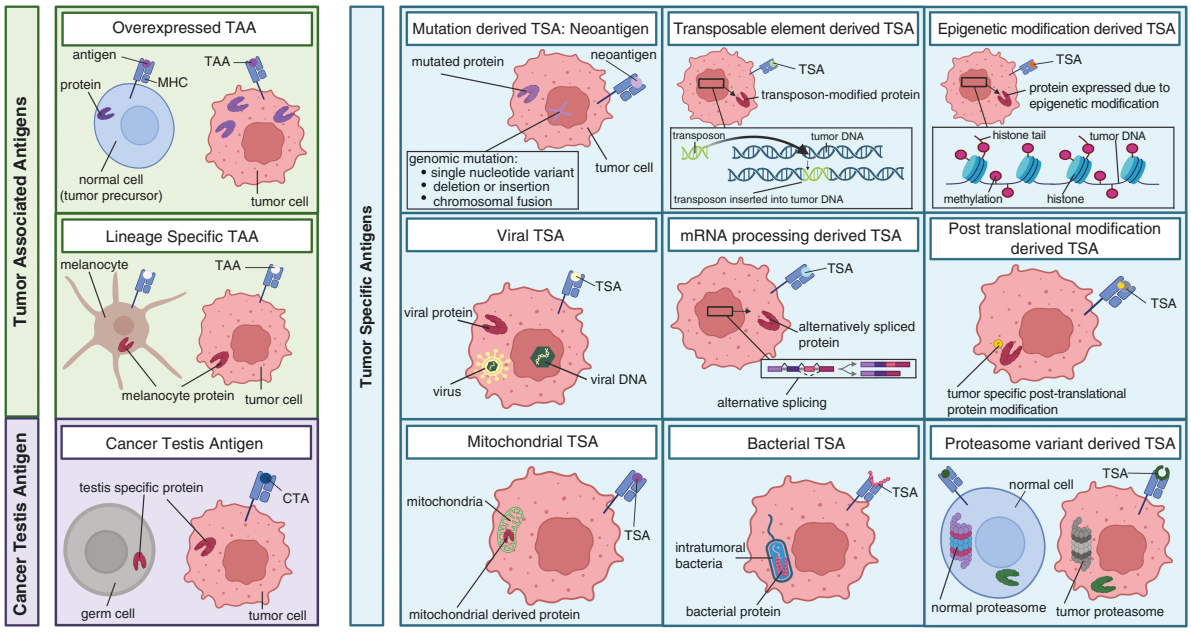
Classes of T-Cell Tumor Antigens in Cancer

Tumors can present antigens on both class I and II MHCs.[16,17] The value of targeting antigens in cancer stems from the idea that vaccinations or TCR-based therapies can augment a patient's immune response against antigens, derived from intracellular and extracellular proteins, that are presented on tumor cells. These approaches aim to induce clonal expansion of antigen-specific T cells. Overall, TAs can be classified into tumor-associated antigens (TAAs), cancer testis antigens (CTAs), and tumor-specific antigens (TSAs)[18,19] (Figure 1).

Tumor-Associated Antigens

TAAs, which are present in normal cells and tumor cells, can be further subdivided into overexpressed or lineage-specific TAAs.[19] Overexpressed TAAs are self-antigens with amplified expression in tumor cells. These include HER2 epitopes in breast cancer[20] and P53 epitopes in squamous cell carcinoma.[21] Lineage-specific TAAs are self-antigens that are normally restricted to a particular cell type. An example of this class is the melanocyte differentiation antigens (MDAs), like MART-1 epitopes,[22] which are typically only expressed by melanocytes,[23] but can also be expressed in melanoma and GBM.[19,22,24,25] Despite endogenous expression of TAAs and presumed negative selection of TCRs reactive against them, studies have shown reactivity against TAAs by cytotoxic lymphocytes (CTLs).[26–28]

Notably, Tebentafusp, a first-in-class FDA-approved treatment for metastatic uveal melanoma, is a bispecific molecule that consists of (1) an affinity-enhanced TCR specific for the HLA-A*02:01 restricted MDA gp100 peptide YLEPGPVTA



	Tumor Associated Antigens	Cancer Testis Antigens	Tumor Specific Antigens
Expression in Normal Tissue	yes	highly restricted	no
Immune Tolerance	yes	no	no

Figure 1. Tumor antigens can be ascribed to three classes: tumor-associated antigen (TAA), cancer testis antigen (CTA), and tumor-specific antigen (TSA). TAAs are self-antigens that are present in tumor cells; they include the overexpressed and the lineage specific. While the proteins from which CTAs derive are present in male germline cells and trophoblasts, antigen expression is mostly isolated to tumors. TSAs that occur secondary to mutations, like single-nucleotide variants (SNVs), insertions or deletions of amino acids (INDELs), or gene fusions, are called neoantigens. Myriad other causes of TSAs may include transposable elements, epigenetic modifications, viruses, altered mRNA processing (such as alternative splicing), post-translational modifications, mutations in mitochondrial DNA, bacterial proteins, and proteasome variants. The classes of TAs vary in their levels of expression in non-tumor tissue and in the degree of immune tolerance, factors that impact off-target effects and efficacy, respectively, of antigen-focused therapy for cancer.

and (2) an anti-CD3 single chain variable fragment.[29,30] Tebentafusp binds the gp100 antigen-HLA complexes displayed by tumor cells and recruits and activates T cells via CD3.[30] Tebentafusp demonstrates the feasibility and therapeutic potential of targeting TAAs, particularly in cancers with a low tumor mutational burden (TMB), a term that quantifies the mutations in a cancer exome,[31,32] since uveal melanoma has a median TMB of 2.1 mutations per megabase.[33]

Cancer Testis Antigens

CTAs, also known as cancer germline antigens, are highly restricted in normal tissues. They originate from proteins that are lineage-restricted to male germline cells and trophoblasts[19] and are expressed in cancers.[34] A MAGE-1 CTA was the first human TA ever identified.[35] NY-ESO, a protein from which CTAs can derive, is expressed in cancers such as ovarian, breast, bladder, prostate, and hepatocellular carcinoma.[36] While male germline cells and trophoblastic cells do not express MHC molecules,[19] potential TCRs reactive against CTAs are likely subject to some self-tolerance due to CTA protein and MHC molecule expression in the thymus.[37,38] Despite this, CTLs reactive to CTAs have been observed.[19,26] Their immunogenicity, limited presentation in normal cells, and association with oncogenicity and immune invasion,[39] make CTAs promising therapeutic targets. In fact, the FDA recently approved a TCR therapy against MAGE-A4 CTA for patients with unresectable or metastatic synovial sarcoma.[40]

Tumor-Specific Antigens

Finally, TSAs are antigens exclusive to tumor cells.[41] TSAs occur when aberrant protein expression in cancer leads to the expression of antigens novel to the immune system.[18] TSAs can arise due to genomic mutations, in which case the resulting antigen is called a neoantigen, or due to other causes.[18,42–47] Importantly, the host has not developed tolerance to TSAs, since they were not present during immunologic development.

Neoantigens—Neoantigens are usually considered the TSAs that arise due to mutations in the cancer cell genome.[18] Some neoantigens result from mutations that confer a selective advantage to cancer, termed "driver mutations, while the majority likely result from mutations that surface incidentally, termed "passenger mutations."[48,49] Neoantigens commonly occur secondary to single-nucleotide variants (SNV), in which one amino acid is substituted, resulting in a missense or nonsense mutation.[18] SNV mutations are frequent in melanoma, with a reported average of 489 SNV mutations per tumor,[50] and less frequent in pancreatic cancer, with a reported median of 48 SNVs per tumor.[51] GBM has been described as having 30–50 SNVs per tumor.[18,52] Neoantigens can also arise due to insertions or deletions of amino acids (INDELs), which can cause either frameshift or in-frame mutations, like EGFRvIII in GBM.[18] INDEL neoantigens commonly accrue in cancers that occur secondary to microsatellite instability, like colon cancer.[18,53] INDEL neoantigens often promote greater immunogenicity and may have stronger binding than SNV neoantigens.[54] In three melanoma cohorts, the number of frame-shift INDEL mutations, but neither the number of in-frame INDEL mutations nor SNV mutations, were associated with response to immune checkpoint blockade (ICB).[54] Finally, neoantigens can originate from gene fusions, like in chronic myeloid leukemia,[55] head and neck cancer,[56] or breast cancer.[57] One study determined that 21 of 92 tested GBM samples had gene fusions, suggesting that a subset of GBM patients may have gene fusion neoantigens.[58] This represents a potential vulnerability, since the immunogenicity of fusion neoantigens has been reported as greater than those from SNVs or INDELs.[54,59]

Personal vaccines targeting neoantigens have exhibited strong potential as a treatment for some cancers.[5,60,61] In a phase I clinical trial for melanoma, four of the six patients who received the personal neoantigen vaccine NeoVax, had no recurrence at 25 months.[5] Two patients with recurrence subsequently received anti-PD-1 therapy and experienced complete regression.[5] This success is likely related to melanoma having a median TMB of over 10 somatic mutations per megabase[31,62]—what the FDA considers the threshold for defining a high TMB[63]—because high TMB is associated with response to ICB in many cancers.[64,65] Since ICB's mechanism of action leverages the tumor immunity cycle,[66,67] which requires the presentation of TAs,[32] then transitively, high TMB likely corresponds to a response to antigen therapy.

However, the relationship between TMB and tumor neoantigen burden (TNB) is uncertain for two reasons. First, the TMB calculation is typically restricted to only mutations in the exome, the protein-coding regions of the genome. Hence, the TMB does not account for mutations in the non-coding regions, which comprise over 98% of the genome, and likely serve as abundant sources of TSAs.[32,68–72] Second, most mutations do not yield neoantigens,[73–76] since only a select few potential neoantigens are actually processed and presented, let alone recognized by T cells.[19] For example, less than 2% of identified somatic mutations in metastatic gastrointestinal cancers were found to have corresponding reactive T cells.[77] Still, the higher the TMB, the greater the chance of exome mutations leading to neoantigens.[75,78] For low TMB cancers like GBM, which has a median TMB of around one to two somatic mutations per megabase,[31,79] and thus, presumably, a low TNB, it may be necessary to target other types of TAs.

The lack of correlation between the existence of a mutation and the presence of its corresponding neoantigen can pose challenges to neoantigen vaccine design. The GAPVAC clinical trial for GBM sought to vaccinate patients with both off-the-shelf, or premanufactured, TAs and personal neoantigens.[52] Of the 643 identified genomic mutations from 15 patients, zero were identified by high-sensitivity mass spectrometry (M.S.) in the patients' immunopeptidome, which refers to the antigens in aggregate bound to MHC molecules.[52,80] These results did not seem to reflect a lack of sensitivity due to the successful elution of both mutated antigens from non-tumor tissues and neoantigens from a GBM patient not in the study.[52] Plus, this result corroborates other studies' findings that only a small percentage of potential neoantigens are processed and presented on MHC molecules.[73–76] As loss of antigenicity is one pathway by which tumors evade the immune system,[81] a process that can occur secondary to mutations[82] or epigenetic changes,[83,84] this lack of antigen presentation is not totally surprising and emphasizes the importance of confirming tumor presentation of neoantigens prior to targeting them.

Moreover, the GAPVAC personal vaccine only targeted SNV neoantigens.[52] As mentioned, GBM typically has only around 30–50 SNVs per tumor,[52] so the number of presented SNV neoantigens is likely much lower. Thus, future therapies for GBM and other low TMB cancers should target other sources of neoantigens, like INDELs and fusions, as well as other sources of TSAs.

Other Sources of Tumor-Specific Antigens—Although studies have focused mostly on variant neoantigens in the coding regions of DNA, it is important to recognize other TSAs, as they may serve as a reservoir of additional tumor targets. One study investigating M.S. data from multiple cancers, including GBM, discovered that for some HLA groups, non-mutation-derived TSAs may account for up to 15% of MHC class I restricted peptides.[85] A source of these alternative TSAs is altered mRNA processing. For instance, alternative splicing has been predicted to occur in cancer at over double the frequency of SNV mutations[86] and has been reported to cause TSAs in cancers like melanoma[87] or AML.[88] Another study identified alternative splicing-derived TSAs as immunogenic across multiple cancers, including GBM.[89] Notably, this study demonstrated that mRNA expression for the majority of GBM neojunctions, borne from cancer-specific splicing events, were conserved across multiple tumor regions. Thus, targeting alternative splicing TSAs may be an effective strategy for cancers like GBM with significant spatial heterogeneity.

Other causes of TSAs could include changes in epigenetic regulation[90] and transposable elements (TEs), which are mobile DNA sequences that can change their location in a genome,[91,92] as in the case of ovarian cancer, breast cancer, or hepatocellular carcinoma.[93] Post-translational processing derived TSAs can arise, such as in pancreatic cancer, melanoma, and lung cancer.[94] Lastly, some studies have suggested that abnormal proteasomal processing can lead to TSAs,[44–46,95] but others have challenged this.[85]

Even mitochondria,[96,97] bacteria,[98] and virus-derived proteins may lead to TSAs. Viral TSAs occur secondary to infections like human papillomavirus (HPV),[9] Epstein–Barr virus,[99] and human T-lymphotropic virus-1[100] in squamous cell carcinoma, lymphoma, and leukemia, respectively. Vaccines that target antigens of the oncogenic viruses HPV and HBV are administered prophylactically against cervical cancer and hepatocellular carcinoma, respectively.[101] Viral antigen vaccines have also demonstrated promise as a treatment for cervical cancer.[102] Overall, alternative sources of TSAs represent potential therapy targets for cancer, especially those with low TMB, and more research is necessary to better characterize them.

Identifying and Validating Tumor Antigens in Patients

Discovery of Antigens

Two general approaches can be taken for antigen discovery: (1) a genomic approach, termed "cancer immuno genomics,"[48,103,104] that uses next-generation sequencing (NGS) to detect mutations, from which computational algorithms predict neoantigen sequences, and (2) an immunopeptidome approach that uses M.S. to detect antigens bound to MHC molecules on the cell surface.[105]

The former, which exclusively identifies neoantigens in protein-coding regions, begins with the detection of mutations via comparing whole exome sequencing (WES) of tumor DNA to normal DNA.[106,107] Multiple pipelines for "mutation calling," which is the process of identifying somatic mutations[108] are often used in conjunction to increase confidence.[18,108–111] RNA-sequencing performed in parallel to WES quantifies the expression of each mutation.[112] (However, the logic of performing this RNA sequencing is challenged by a study that compared presentation of peptide to its corresponding mRNA levels in an in vivo murine model, and found that peptides identified in the immunopeptidome had low corresponding mRNA levels.[113]) Then, mutation data and patient HLA allele information can be integrated into computational algorithms that list predicted peptides and potential peptide-HLA binding partners.[110,114,115] These algorithms often rank peptides by estimated binding affinity to respective HLA alleles, which is complex due to the highly polymorphic nature of the HLA locus.[15] The algorithms vary widely in their predictions and are typically better suited to predict class I restricted peptides.[112] Importantly, this approach does not necessarily confirm that the identified "antigens" are actually in the immunopeptidome.

Immunopeptidomics, meanwhile, can be harnessed for the identification of TAAs, CTAs or TSAs.[116] Immunopeptidomics identifies HLA-bound peptides isolated from tumor samples using M.S.[117] In this approach, MHC molecules with attached antigens are immunoprecipitated and eluted from tumor samples. Peptide sequences can be determined using protein database searching, library searching of antigens previously characterized by M.S., or de novo sequencing, which uses algorithms to predict antigen sequences directly from the mass spectra data without any references.[116,118,119] Comparisons between peptides eluted from tumor and normal tissue determine tumor specificity.

Determining the Immunogenicity of Discovered Antigens

Once identified, peptides require further testing to validate immunogenicity, since many do not elicit an immune response.[120–122] Historically, TA immunogenicity was confirmed by screening patient-derived CTLs for recognition of cells transfected with both the antigen of interest and the matched HLA.[76] At present, high-throughput screening methods for TCR reactivity to antigens can be used.[76] Several variables make this validation difficult. First, limited algorithms are available for TCR and peptide-MHC complex (pMHC) binding interactions.[18] While databases of publicly available TCRs are available for TCR comparison, the majority of these are reactive against viral antigens.[123] Second, the correlation between antigen affinity and immunogenicity is weak.[124,125] A few key components are required for antigen screening: (1) TCRs of interest, (2) antigens of interest, (3) patient-specific HLA alleles, and (4) sources of antigen-presenting cells (APCs).

Several methods are available for biased antigen screens, which focus on select antigens.[122,126–129] In brief,

healthy peripheral blood mononuclear cells (PBMCs) or immortalized T cells are transduced with TCRs of interest. APCs are transduced with patient-specific HLA alleles and pulsed with either pools of target antigens or oligonucleotides encoding target antigens. Following T cell and APC co-culture, markers of T cell activation are measured via flow cytometric analysis. Then, the cognate antigen of the target TCR can be determined through an iterative screening process.

Unbiased approaches have also been developed to identify antigen and TCR pairs, in which selected TCRs are screened against a wide range of peptides. Yeast display, for example, involves yeast which individually expresses a random peptide that is covalently linked to an HLA molecule.[130] These yeast are then co-cultured with soluble bead-multimerized TCR and iteratively enriched via affinity-based selection. After several rounds of enrichment, yeast are sequenced and the corresponding antigen sequences are determined. Thus, even though thousands of individual peptides presented by yeast are cultured together, only those that express the cognate antigen of the target TCR will be purified and eventually sequenced. Another example of an unbiased approach is the use of cytokine-capturing APCs.[131] In this system, APCs are transduced with patient-specific HLA molecules and membrane-bound antibodies that bind to either IL-2 or interferon-gamma (IFNγ). These APCs, which are also transduced with oligonucleotide pools, are co-cultured with T cells expressing target TCRs. When the target TCR binds to its cognate antigen, the T cell will release cytokines which then are "captured" by the APC. APCs with bound cytokine can be isolated and sequenced to detect the cognate antigen of the target TCR. Regardless of the screening method, confirmation of antigen immunogenicity is resource-intensive. However, the recent advent of artificial intelligence in cancer immunity research[132] has yielded tools[133] that may expedite this process.

Unique Features of Glioblastoma Relevant to Immunotherapy

As context for our discussion of TAs in GBM, it is important to briefly consider its properties that impact antigen-based therapy. In addition to the previously discussed low TMB, these include (a) heterogeneity, (b) immunological dysfunction, and (c) standard treatment.

Spatial Heterogeneity of Glioblastoma

At a genetic level, GBM remains an incredibly difficult tumor to treat due to the significant heterogeneity in transcriptional expression, as well as somatic mutations, and consequently antigens—likely a result of severe immunoediting.[134–136] Several studies have highlighted the transcriptional heterogeneity of GBM tumor samples,[79,137] with Verhaak et al. initially classifying 4 major subtypes using bulk RNA-sequencing: proneural, neural, classical, and mesenchymal.[138] Other classifications highlight the complexity, and plasticity, of GBM tumor cells.[139] Moreover, GBM tumors have been characterized as having a high frequency of subclonal mutations,[140] leading to a lack of uniform antigen expression across a tumor spatially.[141] Overall, the intratumoral and intertumoral heterogeneity of GBM tumors, at the transcriptional, mutational, and antigen level, should be considered during the design of antigen-based therapy, discussed in more detail in a later section.

Immunosuppression Characterizes Glioblastoma

Immunosuppression defines the microenvironment of GBM tumors. GBM tumors are reported as infiltrated by immunosuppressive tumor-associated macrophages (TAMs).[142–147] Microglia have also been observed to downregulate MHC class II, which would limit the presentation of class II-restricted antigen.[148–150] The accumulation of myeloid-derived suppressor cells (MDSCs) also contributes to immunosuppression.[151] Plus, T cells appear to be dysfunctional within the tumor microenvironment. Although some studies have highlighted the expression of canonical exhaustion markers on T cells derived from both human and mice tumors,[144,152–155] exhaustion does not seem to be the predominant phenotype of GBM T cells.[142,145,156,157] Specifically, studies demonstrate the lack of a strong exhaustion signature, and instead reveal the presence of *CLEC2D* expressing[142] or *GZMK* expressing T cells.[157] Also, immunosuppressive regulatory T cells (Tregs) have been shown to comprise a significant proportion of the CD4+ T cell compartment in both patients with GBM and murine models.[158,159]

GBM tumors also intrinsically contribute to immunological dysfunction. One study found that 61% of patients had at least 1% or more PD-L1-positive tumor cells, and PD-L1 expression was a negative prognostic factor.[160] Indoleamine 2,3 dioxygenase (IDO) has been shown to be expressed by GBM tumor cells and to increase the recruitment of immunosuppressive Tregs.[161–163] Expression of other proteins such as FasL[164] which induces T cell apoptosis, non-classical MHC class I molecules[165,166] which enables evasion of immune cells, and ICAM-1, which promotes immigration of myeloid cells, by GBM tumors have also been reported.[167,168] The microenvironment and tumor-intrinsic sources of immunosuppression likely contribute to the disease's limited response to immunotherapy.

Standard Treatment of Glioblastoma May Influence Response to Antigen Therapy

GBM treatments can cause further immunosuppression.[169] Some clinical trials have observed that steroid administration, commonly given to patients with GBM, was associated with no immune response to antigen therapy,[52,106] and some trials have used steroid administration as an enrollment exclusion criteria.[170,171] However, other trials have had immune responses in patients that received steroids.[172,173] Doubtlessly, timing and dose determine the effect on immune response. Radiotherapy and chemotherapy, part of the standard of care for GBM, also likely impact tumor response to immunotherapy. Radiotherapy can promote the proliferation and infiltration of Tregs, as well

as the differentiation and migration of MDSCs, fostering immunosupression.[174] Conversely, radiotherapy can cause mutations that lead to new neoantigens, providing targets for the immune system.[174] Similarly, temozolomide (TMZ) has been observed to cause immunosuppression, and in particular, lymphopenia.[175] However, TMZ can also cause a hypermutation phenomenon,[176] which presumably leads to the presentation of more antigens novel to the immune system. In fact, hypermutation has been observed to be associated with increased levels of CD8+T cell infiltration.[139] The impact of treatments like corticosteroids, radiation, and chemotherapy on response to antigen therapy requires further investigation.

Tumor Antigens in Glioblastoma

Glioblastoma Tumor Antigens

GBM TAAs have been used in several vaccines (Supplementary Table S1). GBM CTAs (Supplementary Table S2) and shared TSAs (Supplementary Table S3) have also been used in vaccines or shown to be immunogenic in vitro. Table 1 lists clinical trials that target TAs in GBM via vaccine.

As depicted in the "Antigen Discovery" section earlier, for an epitope to be a true TA, two criteria must be met: (1) the epitope is presented endogenously on HLA molecules by tumor cells and (2) T cells can bind the pMHC and elicit an immune response.[194] For criterion one, antigen presentation can be confirmed by eluting antigens from tumor cells via M.S. An indirect way to confirm criterion one, while simultaneously confirming criterion two, occurs when CTLs that are specific for a particular antigen lyse tumor cells because this process requires tumor display of the antigen. IFNγ ELISpot assays, tetramer assays, or antigen screens can confirm criterion two (antigen immunogenicity) but not criterion one.

A caveat: while almost every TA listed in the supplementary tables have been shown to be immunogenic, not all the antigens listed are necessarily presented by MHC molecules on GBM cells, despite the expression of the protein from which the antigen is derived. While this is in part due to HLA restriction of peptides,[140] it does not fully account for such discrepancies. One study highlighted this phenomenon when different antigens with the same MHC restriction and originating from the same source protein were differentially expressed in tumor and normal brain.[195] Normal brain and tumor cells both expressed the mRNA for the protein PTPRZ1.[195] However, while one HLA-A*02-restricted PTPRZ1-derived antigen was exclusively presented in tumor but not the normal brain, another HLA-A*02-restricted PTPRZ1-derived antigen was expressed in both tumor and normal brain.[195] In a separate study, TAs with the same HLA restriction from the same source protein were not always simultaneously presented by HLA-matched GBM.[196] It is possible that technical artifact could contribute to the observed lack of concordance between protein expression and antigen presentation. However, other biological reasons might include different expressions of proteasomes, different binding strength to MHCs, preferential display of more immunogenic peptides, or the opposite: MHC downregulation to facilitate immune escape.[197] Further investigation should be done in preclinical models to interrogate the mechanisms underpinning tissue or tumor specificity of the immunopeptidome. This can be explored via an in vivo murine model that allows for the tagging of MHC I complexes from defined cell populations.[113] Moreover, the differential display of antigens by tumor tissue underscores the importance of directly confirming MHC presentation of antigens on tumor cells before targeting them with therapy.

Tumor-Associated Antigens in Glioblastoma

Many identified GBM TAs belong to the TAA class (Supplementary Table S1). Overexpressed TAAs in GBM include peptides derived from ARF4L,[198] GALT3,[198] AIM-2,[199] HER-2,[22] EphA2,[22,200] tyrosinase,[22] Sox2,[201] Sox11,[202] and EphB6v.[203] Lineage-specific TAAs include MDAs from TRP-2,[25] Mart-1,[22] and gp100.[22] While not tumor-specific, GBM TAAs have successfully induced immune responses from T cells either in vitro,[22] ex vivo,[198,200–203] endogenously without intervention,[52] or endogenously after vaccination.[25,204] Although targeting these TAAs with an exogenous intervention risks deadly autoimmune reactions,[205] many GBM TAAs have been used safely in vaccines (Table 1).

HER2 antigens—Human Epidermal Growth Factor Receptor 2 (HER2) is often overexpressed in GBM, including in glioblastoma stem cells (GSCs),[206] and is associated with primary GBM[207] and worse survival.[208] The HER2 antigen KIFGSLAFL has been shown to be immunogenic, as antigen-specific T cells lysed HLA-matched glioma cells ex vivo.[22] A phase I clinical trial of a dendritic cell (DC) based vaccine that included this antigen showed that some patients developed antigen-specific CD8+T cells.[187] However, the presence of CD8+T cells to the vaccine TAs did not correlate with improved survival.[187] In phase I of the ICT-107 trial, which included the HER2 antigen VMAGVGSPYV, HER2 was shown to be downregulated in recurrent tumors, suggesting either successful targeting of HER2-expressing cells or a degree of immunoediting.[209] However, HER2 mRNA is expressed in normal brain,[210] so prior to targeting any HER2 antigens in the future, MHC display of HER2 antigens should be evaluated both on GBM and on normal brain.

Cancer Testis Antigens in Glioblastoma

CTAs in GBM have been reported as immunogenic both in vivo and in vitro (Table S2). The first TA discovered in brain cancer was the CTA SART1(259)690-698 peptide (EYRGFTQDF). The antigen was originally identified in squamous cell carcinoma[211] and was subsequently demonstrated in glioma cell lines, as CTLs specific for the antigen could lyse HLA-matched glioma cells expressing Sart1(259).[212] Other GBM CTAs include peptides derived from Sart-3, IL-13Rα2, Mage1, MageC2, and Survivin. CTAs have been used in many vaccines for GBM.[171,183,188,192,193,204,209,213–216]

Table 1. T Cell Tumor Antigen Vaccine Clinical Trials in GBM

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
"off the shelf" antigens												
VEGFR-2												
VXM01 Bacterial vaccine encoding VEGFR-2	NCT02718443	VEGFR-2 antigens	*Salmonella typhi* Ty21a carrying a DNA plasmid encoding vascular endothelial growth factor receptor (VEGFR)-2[177]	oral	nivolumab	Vaximm GmbH	None listed	I	2016	14	C	NR; SG
VXM01 Bacterial vaccine encoding VEGFR-2	NCT03750071	VEGFR-2 antigens	Same as above	oral	Avelumab	Vaximm GmbH	None listed	I/II	2018	28	A, not Rec	NR; SG
EGFRvIII												
Peptide Vaccine(ZAP IT)	NCT00626015	EGFRvIII neoantigen (LEEKKGNYVVTDHC)	Rindopepimut is a 14-amino acid peptide, PEP-VIII LEEKKGNYVVTDHC[178] chemically conjugated to keyhole limpet he-mocyanin (KLH)[179]	i.d.	daclizumab/ basiliximab, temozolomide (TMZ)	Duke univer-sity	None listed	I	2007	6	C	NR; SG
Peptide Vaccine(ACT II)	NCT00643097	EGFRvIII neoantigen (LEEKKGNYVVTDHC)	Rindopepimut	i.d	GM-CSF, dose-intensified (DI)TMZ	Celldex Therapeutics	None listed	II	2007	40	C	NR; P
Peptide Vaccine(ACT III)	NCT00458601	EGFRvIII neoantigen (LEEKKGNYVVTDHC)	Rindopepimut	i.d.	GM-CSF	Celldex Therapeutics	None listed	II	2007	65	C	NR; SG
Peptide Vaccine(ACT IV)	NCT01480479	EGFRvIII neoantigen (LEEKKGNYVVTDHC)	Rindopepimut	i.d.	GM-CSF	Celldex Therapeutics	None listed	III	2011	745	C	NR; P
CMV antigens												
mRNA loaded autologous DC vaccine(ATTAC)	NCT00639639	cytomegalovirus (CMV) phospho-protein 65 (pp65) antigens	Autologous dendritic cells pulsed with mRNA of pp65 fused to LAMP	i.d.	human cyto-megalovirus (CMV)-autologous lymphocyte transfer or GM-CSF, + unpulsed DCs or td toxoid preconditioning, DI TMZ[170]	Duke Univer-sity	None listed	I	2006	23	C	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
mRNA loaded autologous DC vaccine (REGU-LATe)	NCT00626483	CMV pp65 antigens	Autologous dendritic cells pulsed with mRNA of pp65 fused to LAMP	i.d.	GM-CSF, Basiliximab	Duke Univer-sity	None listed	I	2007	34	C	NR; SG
mRNA loaded autologous DC vaccine (ELE-VATE)	NCT02366728	CMV pp65 antigens	Autologous dendritic cells pulsed with mRNA of pp65 fused to LAMP	i.d.	unpulsed DCs or td toxoid preconditioning, basiliximab	Duke Univer-sity	None listed	II	2015	64	C	R; P
mRNA loaded autologous DC vaccine(AVERT)	NCT02529072	CMV pp65 antigens	Autologous dendritic cells pulsed with mRNA of pp65 fused to LAMP	i.d.	nivolumab, td toxoid preconditioning	Duke Univer-sity	None listed	I	2016	6	C	R; P
mRNA loaded au-tologous DC vac-cine (ATTAC-II)	NCT02465268	CMV pp65 antigens	autologous DCs pulsed with either short-length or full-length pp65-LAMP mRNA[180]	i.d.	TdToxoid, GM-CSF	University of Florida	None listed	II	2016	175	C	R; P
mRNA loaded DC vaccine(ATTAC-P)	NCT03615404	CMV pp65 antigens	pp65-LAMP mRNA-pulsed autologous DCs	i.d.	TdToxoid, GM-CSF, DITMZ	Duke Univer-sity	None listed	I	2018	11	C	NR; SG
mRNA loaded DC vaccine(I-ATTAC)	NCT03927222	CMV pp65 antigens	pp65-LAMP mRNA-pulsed autologous DCs	i.d.	TdToxoid, GM-CSF, DITMZ	Duke Univer-sity	None listed	II	2019	6	T	NR; SG
mRNA loaded DC vaccine(DERIVe)	NCT03688178	CMV pp65 antigens	pp65-LAMP mRNA-pulsed autologous DCs	i.d.	TdToxoid, unpulsed DCs, Varlilumab	Duke Univer-sity	None listed	II	2020	43	A, not Rec	R; P
mRNA DC vac-cine	NCT04963413	CMV pp65 antigens	Full-length pp65-LAMP mRNA-pulsed autologous DCs	i.d.	GM-CSF	University of Florida	None listed	I	2022	6	T	NR; SG
VBI-1901 en-veloped virus-like particle vaccine[181]	NCT03382977	CMV pp65 and glycoprotein B (gB) antigens	a gB/pp65 enveloped virus-like particle[181]	i.d. or i.m	GM-CSF or AS01B	VBI Vaccines Inc.	None listed	I/II	2017	98	Rec	NR; S
PNOC020 RNA Lipid Particle (RNA-LP) Vaccine	NCT04573140	CMV pp65 antigens	Autologous total tumor mRNA and full-length pp65-LAMP mRNA loaded DOTAP liposome vaccine	i.v.	none	University of Florida	None listed	I	2021	52	Rec	NR; P

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
RNA-LP vaccine	NCT06389591	CMV pp65 antigens	pp65 RNA-LPs, autolo-gous tumor mRNA, and full-length pp65-LAMP mRNA loaded DOTAP liposome vaccine	i.v.	none	University of Florida	None listed	I	2024	24	Not yet Rec.	R; P
ITI-1001 DNA vaccine	NCT05698199	CMV IE-1, pp65 and gB antigens	2 DNA plasmids. 1st encodes IE-1 and pp65 antigens fused to LAMP1. 2nd encodes gB antigen fused to LAMP1.	i.m.	TdToxoid	Immunomic Thera-peutics, Inc.	None listed	I	2023	10	A, not Rec	NR; SG
Peptide vaccine (PERFORMANCE)	NCT02864368	CMV pp65 and gB antigens	PEP-CMV Component A (26 amino acids encoding multiple po-tential class I, class II, and antibody epitopes of CMV pp65 across several haplotypes)[182] and PEP-CMV Compo-nent B (a neutralizing antibody epitope from human CMV glyco-protein B conjugated to Keyhole Limpet Hemocyanin)	i.d.	TdToxoid, GM-CSF, Montanide ISA-51, DITMZ	Duke Univer-sity	None listed	I	2016	27	T	R; P
Peptide vaccine (INTERROGATE-GBM)	NCT06132438	CMV pp65 antigens	long synthetic peptide of 26 amino acids de-rived from CMV pp65 that has both MHC class I and II epitopes		TdToxoid	Charlotte Lemech (The University of New South Wales)	None listed	I	2023	26	Not yet Rec.	NR; SG
Survivin (BIRC5)												
SurVaxM Peptide vaccine	NCT01250470	Survivin 53-67/M57	SurVaxM = SVN53-67/M57-KLH: synthetic peptide of survivin 53-67, with cysteine to methionine substitu-tion at amino acid 57, conjugated to keyhole limpet hemocyanin (KLH)[183]	s.c.	Montanide ISA-51, GM-CSF,	Roswell Park Cancer Insti-tute	A*02, A*03	I	2012	9	C	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
SurVaxM Peptide vaccine	NCT02455557	Survivin 53-67/M57	SVN53-67/M57-KLH	s.c.	Montanide ISA-51, GM-CSF	Roswell Park Cancer Insti-tute	A*02, A*03, A*11, A*24	II	2015	66	A, not Rec	NR; SG
SurVaxM Peptide vaccine (SUR-VIVE)	NCT05163080	Survivin 53-67/M57	SVN53-67/M57-KLH	s.c.	Montanide ISA-51 or GM-CSF, Temozolomide	MimiVax, LLC	None listed	II	2021	247	A, not Rec	R; P
Wilms' tumor gene 1 (WT1)												
WT2725 Peptide vaccine[184]	NCT01621542	WT1 126-134 antigen	Peptide in water-in-oil emulsions	s.c	none	Sumitomo Pharma America, Inc.	A*0201+, A*0206+	I	2012	62	C	NR; SG
DSP-7888 or Ombipepimut-S (adegramotide/ nelatimotide) Peptide Vaccine	NCT02498665	Nelatimotide: synthetic pep-tide of $WT1_{126-134}$ (RMFPNAPYL) and a modified $WT1_{235-243}$ (CYTWNQMNL). Adegramotide: ($WT1_{34-51}$: WAPVLD FAPPGASAYGSL), which includes HLA-A*02:01 restricted WT1 37-45 (VLDFAPPGA)[185]	Peptides in water-in-oil emulsions mixed with Montanide ISA-51[185]	i.d. or s.c.	Montanide ISA-51	Sumitomo Pharma America, Inc.	A*02:01, A*02:06, A*24:02	I	2015	24	C	NR: P
DSP-7888 or Ombipepimut-S (adegramotide/ nelatimotide) Peptide Vaccine (WIZARD 201G)	NCT03149003	Same as above	Peptides in water-in-oil emulsions mixed with Montanide ISA-51[185]	i.d.	Montanide ISA-51, Bevacizumab	Sumitomo Pharma America, Inc.	A*02:01, A*02:06, A*24:02	III	2017	221	C	R: P
autologous WT1 mRNA-transfected DC Vaccine	NCT01291420	WT1 antigens	mRNA-electroporated Autologous Dendritic Cell	i.d.	none	University Hospital, Antwerp	None listed	I	2010	48	C	NR; SG
Autolo-gous mRNA loaded DCs vaccine(ADDIT-GLIO)	NCT02649582	WT1	Autologous WT1 mRNA-loaded den-dritic cell vaccine	i.d.	none	University Hospital, Antwerp		I/II	2015	20	Rec	NR:SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
Telomerase (TERT)												
Peptide vaccine	NCT00069940	TERT: 540-548	Peptide vaccine	s.c.	GM-CSF	Dana-Farber Cancer Insti-tute	A*0201	I	2000		C	NR:SG
Peptide vaccine(UCPVax-Glio)	NCT04280848	TERT antigens	Montanide ISA-51 VG	s.c.	none	Centre Hospitalier Universitaire de Besancon	None listed	II	2020	56	A, not Rec	NR: P
CD133												
ICT-121 Peptide Loaded Autolo-gous DC Vaccine	NCT02049489	CD133 antigens	Autologous DCs pulsed with CD133 peptides	i.d	none	Precision Life Sciences Group	A2	I	2013	20	C	NR; SG
NY-ESO-1												
Peptide vaccine(DC205-NY-ESO-1)(CDX-1401)	NCT01522820	NY-ESO-1	Monoclonal antibody specific for DEC-205 fused to NY-ESO-1 peptide[186]	intranodally	Sirolimus	Roswell Park Cancer Insti-tute	A2	I	2012	18	C	NR; P
multiple protein sources												
peptide-pulsed autologous den-dritic cell vaccine	NCT00612001	TRP-2, gp100, her-2/neu, survivin antigens[187]	Peptide-pulsed autolo-gous DC vaccine	i.d.[187]	GM-CSF or IL-4	Jonsson Comprehen-sive Cancer Center, UCLA	A*201	I	2006	8	C	NR; SG
Peptide loaded DC vaccine	NCT00766753	IL-13Rα2 345-353:1A9V, EphA2 883-891, YKL-40 202-211, gp100 209-217	Autologous type-1 alpha-DCs loaded with peptides	intranodally	Poly-ICLC	University of Pittsburgh	A2	I/II	2006	22	C	NR; SG
mRNA trans-fected DC vaccine	NCT00846456	hTERT and survivin antigens	Autologous dendritic cells transfected with hTERT and Survivin mRNA	i.d.	none	Oslo Univer-sity Hospital	None listed	I/II	2009	20	C	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
DC vaccine(ICT-107)	NCT01280552	MAGE1(161) EADPTGHSY, AIM-2(14) RSDSGQQARY, TRP-2(180) SVYDFFVWL, gp100(210M) IMDQVPFSV, HER2(773) VMAGVGSPYV and IL13Ra2 (345) WLPFGFILI	Autologous dendritic cells pulsed with antigens	i.d.	ICT107	Precision Life Sciences Group	A1, A2	II	2011	124	C	R; P
Peptide vaccine(SL-701)	NCT02078648	Survivin 95-104 with T to M substitu-tion, IL-13Rα2345-353:1A9V, EphA2883-891[188]	Multi-peptide cocktail	s.c.	poly-ICLC + GM-CSF, Bevacizumab, Imiquimod	Stemline Thera-peutics, Inc.	A2	I/II	2014	74	C	R; SG
Peptide vaccine (IMA950)	NCT01222221	9 HLA-02-restricted glioblastoma-associated peptides and 2 HLA class II (2 HLA DR) binding peptides) and HBV antigen: BCAN 478-486, CSPG 421-29, FABP7 118-126, PTPRZ1 195-203, IGF2BP3 552-560, NLGN4X 131-139, NRCAM 692-700, PTPRZ1 1347-1355, TNC 3-11, MET 651-667 and BIRC5 97-111 HBV 18-27,	Multi-peptide cocktail	i.d.	GM-CSF	Cancer Re-search UK	A*02	I	2010	45	C	NR; SG
Peptide vaccine (IMA950)	NCT01403285	Same as above	Multi-peptide cocktail	Not speci-fied	GM-CSF,Imiquimod, Cyclophosphamide	Immatics Biotechnolo-gies GmbH	A*02	I	2011	6	T	NR; SG
Peptide vaccine (IMA950)	NCT01920191	Same as above	Multi-peptide cocktail	i.d. or s.c.	Poly-ICLC	University Hospital, Geneva	A2	I/II	2013	19	C	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
Peptide vaccine (IMA950)	NCT03665545	Same as above	Multi-peptide cocktail	s.c.	Poly-ICLC, pembrolizumab	University Hospital, Geneva	A*0201	I/II	2018	18	A, not Rec	R; P
ADU-623 Bac-terial vaccine encoding antigens	NCT01967758	EGFRvIII neoantigen and NY-ESO-1 CTA	Live-attenuated Lis-teria Monocytogenes Strain (ΔactA/ΔinlB) encoding EGFRvIII-NY-ESO-1[189]	i.v	amoxicillin or tri-methoprim/sulfa-methoxazole	Providence Health & Services	None listed	I	2014	11	C	NR; SG
Peptide vaccine (ROSALIE)	NCT04116658	Gut bacteria origins peptides containing 3 CD8 HLA-A2 epitopes with mimicry to GB-TAAs (IL13Rα2, BIRC5, and FOXM1) and the CD4 epitope UCP2(E2401)	Multi-peptide cocktail	s.c.	Nivolumab, Bevacizumab	Enterome	A2	I/II	2020	100	A, not Rec	NR; S
mRNA Vaccine(P30-EPS)	NCT05938387	8 HLA02 and HLADR restricted glioblastoma-associated antigens(CVGBM)	mRNA with unmodi-fied nucleotides formulated with lipid nanoparticles[190]	i.m.	none	CureVac	A*02:01[190]	I	2023	16	Rec	NR; S
P30-EPS Peptide vaccine(ETAPA I)	NCT05283109	EphA2, CMV pp65, and survivin antigens	P30-linked EphA2, CMV pp65, and survivin peptides	Not speci-fied	Poly-ICLC	Mustafa Khasraw, (Duke Uni-versity)	A*0201	I	2023	36	Rec	NR; SG
INO-5401 DNA vaccine	NCT03491683	WT1, prostate-specific membrane antigen (PSMA), and TERT antigens	INO-5401: Vaccine comprised of 3 sep-arate DNA plasmids encoding WT1, PSMA, and TERT	i.m.	DNA plasmid encoding interleukin-12 (INO-9012), Cemiplimab, electroporation via CELLECTRA® 2000 EP device	Inovio Phar-maceuticals	None listed	I/II	2018	52	C	NR; P
Personalized antigens												
Neoantigen-based												

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
Peptide vaccines (GAPVAC-101)	NCT02149225	Off the shelf antigens (APVAC1) and personal-ized neoantigens (APVAC2)	Multipeptide cocktail	i.d.	Poly-ICLC, GM-CSF	Immatics Biotechnolo-gies GmbH, Germany	A*02:01, A*24:02	I	2014	16	C	NR; SG
Peptide vaccine(NeoPep)	NCT05557240	Off the shelf CMV antigens (NPVAC1), person-alized neoantigens (NPVAC2)	Multipeptide cocktail	N.S.	Poly-ICLC	Shanghai 10th People's Hospital	None listed	I	2022	10	Rec	NR; SG
Peptide vaccine(NeoVax)	NCT02287428	Personalized neoantigens	Multipeptide cocktail	s.c.[106]	Pembrolizumab	Dana-Farber Cancer Insti-tute	None listed	I	2014	56	Rec	R; P
Peptide vaccine	NCT02510950	Personalized neoantigens	Multipeptide cocktail	s.c[236]	Poly-ICLC	Washington University School of Medicine	None listed	I	2015	1	T	NR; SG
Peptide vaccine(NeoVax)	NCT03422094	Personalized neoantigens	Multipeptide cocktail	s.c.	Nivolumab, Ipilimumab, Poly-ICLC	Washington University School of Medicine	None listed	I	2018	3	T	NR; S
Peptide vaccine	NCT03223103	Personalized neoantigens	Multipeptide cocktail		Neoantigen peptides vaccine, Poly-ICLC, TumorTreatment Fields	Albert Ein-stein College of Medicine	None listed	I	2018	13	A, not Rec	NR; SG
GNOS-PV01 DNA vaccine	NCT04015700	Personalized neoantigens	DNA plasmid vector expressing tumor-specific antigens		DNA plasmid encoding interleukin-12 (INO-9012), electropora-tion via CELLECTRA® 2000 EP device	Washington University School of Medicine	None listed	I	2020	9	A, not Rec	NR; SG
DNA vaccine	NCT05743595	Personalized neoantigens	Personalized neoantigen DNA vaccine	i.m.	Retifanlimab,TDS-IM v 2.0 electroporation device	Washington University School of Medicine	None listed	I	2023	12	Rec	NR; S
peptide pulsed DC Vaccine	NCT04968366	Personalized neoantigens	autologous dendritic cells pulsed with multiple neoantigen peptides	i.d.	none	Beijing Tiantan Hos-pital	None listed	I	2021	11	A, not Rec	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
Peptide loaded DC vaccine (ZSNeo-DC1.1)	NCT06253234	Personalized neoantigens	autologous dendritic cells loaded with multiple neoantigen peptides	N.S.	Personalized neoantigen pulsed DC vaccine(ZSNeo-DC1.1)	Beijing Tiantan Hos-pital	None listed	I	2024	12	Rec	NR; SG
personalized multiple tumor antigens												
peptide-pulsed autologous den-dritic cell vaccine	NCT00612001	TRP-2, gp100, her-2/neu, survivin antigens[187]	Peptide-pulsed autolo-gous DC vaccine	i.d.[187]	GM-CSF or IL-4	Jonsson Comprehen-sive Cancer Center, UCLA	A*201	I	2006	8	C	NR; SG
mRNA pulsed DC vaccine (PERCELLVAC)	NCT02709616	Multiple personalized tumor-associated antigens[191]	mRNA-TAA pulsed au-tologous DC vaccine	i.d.+ i.v.	low-dose cyclo-phosphamide, poly I:C, imiquimod and anti-PD-1 antibody[191]	Guangdong 999 Brain Hospital	None listed	I	2016	10	C	NR; SG
mRNA pulsed DC vaccine (PerCellVac2)	NCT02808364	Multiple personalized tumor associated antigens[191]	mRNA-TAA pulsed au-tologous DC vaccine	i.d.+ i.v.	low-dose cyclo-phosphamide, poly I:C, imiquimod and anti-PD-1 antibody[191]	Guangdong 999 Brain Hospital		I	2016	10	C	NR; SG
Peptide vaccine(ITK1)[192]	JPRN-UMIN000001243	EGFR 800-809 (DYVREHKDNI), Lck-486-494 (TFDYLRSVL), Lck-488-497 (DYLRSVLEDF), MRP3-503-511 (LYAWEPSFL), MRP3-1293-1302 (NYSVRYRPGL), PAP-213-221 (LYCESVHNF), PSA-248-257 (HYRKWIKDTI), PTHrP-102-111 (RYLTQETNKV), SART2-93-101 (DYSARWNEI), SART2-161-169 (AYDFLYNYL), SART3-109-118 (VYDYNCHVDL)	Multipeptide vaccine	s.c.	none	Kurume University School of Medicine	A*24[192]	I	2006	12	C	NR; SG

Table 1. Continued

Vaccine type	NCT number	Target antigen(s)	Delivery platform	Deli-very route	Adjuvant/Combina-tion interventions	Sponsor	HLA Enroll-ment Restric-tion	Phase	Start Year	Enroll-ment	Status	Design
Peptide vaccine(ITK1)[192]	JPRN-UMIN000006970	Same as above	Multipeptide vaccine	s.c.	none	Kurume University School of Medicine	A*24	III	2012	88	C	R: P
Peptide vaccine(TAS0313)	JapicCTI-183824	SART2 p93-101 (DYSARWNEI), SART3 p734-742 (QIRPIFSNR), SART3 p109-118 (VYDYNCHVDL), SART3 p302-310 (LLQAEAPRL), EGFR p800–809 (DYVREHKDNI), PTHRP p102-111 (RYLTQETNKV), LCK p246-254 (KLVERLGAA), LCK p90-99 (ILEQSGEWWK), MRP3 p503-511 (LYAWEPSFL), TMEM189 p43-51 (RLQEWCSVI), LCK p488-497 (DYLRSVLEDF), WHSC2 p103-111 (ASLDSDPWV)	multi-epitope long peptide vaccine	s.c.	Montanide™ ISA 51 VG[193]	Taiho Pharmaceutical	A*02:01, A*02:06, A*02:07, A*11:01, A*24:02, A*31:01, A*33:03[193]	I/II	2019	17	C	NR; S

N.S. = not specified; DC = Dendritic cells; i.d. = intradermal; i.v. = intravenous; i.m. = intramuscular; C = Completed; A = Active; Rec = Recruiting; Not Rec = Not Recruiting; T = Terminated; NR = Non-Randomized; R = Randomized; SG = Single Group; P = Parallel; S = Sequential.

IL-13Rα2 antigens—Sixty-seven percent to ninety-six percent[217,218] of GBM tumors express IL-13Rα2, an IL13 receptor subunit, which is implicated in GBM invasion[219,220] and cooperates with EGFRvIII to promote GBM proliferation.[220] Multiple GBM clinical trials have used antigens from IL-13Rα2.[204,215,221] A clinical trial using peptide-pulsed DCs included a variant of an endogenous IL-13Rα antigen.[204,222] In this trial, 10 of 19 patients who received the vaccine were found to have an immune response to the antigen.[204] This same IL-13Rα2 antigen variant was included in SL-701 vaccine; in a clinical trial for this vaccine, patients' T cell response to the vaccine did not correlate with survival.[223] In phase I of ICT-107, which also vaccinated with an IL-13Rα2 antigen, IL-13Rα2 was shown to be downregulated in recurrent tumors after vacination.[209] Moreover, in phase II of the ICT-107 trial, vaccination with a cocktail of antigens showed therapeutic benefit with an increased OS of 1.6 months in treated compared to untreated groups. However, it is unclear which of the vaccine peptides, individually or in combination, may have conferred the survival benefit. Overall, antigens from IL-13Rα2 represent potential therapeutic targets in GBM due to their minimal expression in normal tissue, role in tumor progression and proliferation, and confirmed immunogenicity in vaccinated GBM patients. IL-13Rα2 antigens' therapeutic application for GBM is under investigation in another clinical trial.[224]

Survivin antigens—Survivin (also known as BIRC5) inhibits apoptosis[225] and is expressed in 80-90% of GBM,[22,226] including GSCs.[227] Survivin expression in GBM correlates with worse prognosis.[228–230] Notably, survivin has been shown to have low intratumoral heterogeneity and high expression across GBM tumor samples.[140] A phase I clinical trial vaccinated seven newly diagnosed GBM patients with DCs transfected with mRNA encoding survivin and hTERT.[171] Strikingly, the median progression-free survival (PFS) for the treated group was 694 days, which was 2.9 times longer than the median PFS of 236 days in the control.[171] Separately, the IMA950 vaccine included the class II HLA-DR-restricted survivin 97-111 antigen, and in a phase I trial with the vaccine in combination with poly-ICLC, 11 of 16 treated patients developed peptide-specific CD4+T cells.[173] However, no tumor infiltrating vaccine-specific T cells were detected.[173] Additionally, there was no association between patient T cell response to TAs and survival in an IMA-950 vaccine trial that used granulocyte-macrophage colony-stimulating factor (GM-CSF) as an adjuvant.[231] Finally, the SurvmaxM vaccine includes a peptide that contains multiple HLA-restricted survivin epitopes, and it induced antigen-specific CD8+T cells in patients.[183] Overall, survivin antigens represent a potentially promising target for GBM therapy due to their high expression, low variance across samples, and immunogenicity. A phase II trial including SurvmaxM is currently underway (NCT05163080), and another active clinical trial includes a survivin antigen (NCT05283109).

Tumor-Specific Antigens in Glioblastoma

GBM shared neoantigen: EGFRVIII (LEEKKGNY VVTDHC)—Besides the IDH1 neoantigen,[232] which is more common in lower grade glioma and secondary GBM,[233] the only shared neoantigen discovered to date in GBM is one from EGFRvIII,[234] a constitutively active variant of EGFR with a mutated extracellular domain, resulting from an in-frame deletion of EGFR exons 2-7.[235,236] The EGFRvIII mutation has been shown in vitro to promote cell proliferation, angiogenesis, and invasion,[237] and studies indicate that it is expressed in 17-64% of GBM tumors.[218,235] However, the presence of the mutation in GBM has an equivocal association with survival.[238,239] The administration of rindopepimut, the EGFRvIII neoantigen (LEEKKGNYVVTDHC) conjugated to keyhole limpet hemocyanin (KLH),[240] did not show survival benefit in a phase III trial.[3] However, this may be because rindopepimut was not designed as a *T cell* neoantigen therapy. While KLH can serve as an adjuvant for induction of antigen-specific CD8+T cells,[183] KLH primarily activates an antibody response.[241] In fact, in one preclinical study of rindopepimut, vaccinated mice did not develop significant cytotoxic responses, but instead developed increased antibody titers.[242] Moreover, the EGFRvIII neoantigen in rindopepimut is HLA restricted.[179,234] Yet, clinical trials did not restrict enrollment based on HLA.[3,243–245] Thus, a vaccine trial designed to augment the T cell response against a EGFRvIII neoantigen has yet to be developed. Importantly, CTLs specific for the EGFRvIII neoantigen have been able to lyse glioma cells expressing the mutated protein in vitro in an HLA-restricted manner.[234] As such, this shared neoantigen is still a promising target for future therapies. The recent results of a Chimeric Antigen Receptor (CAR) therapy against both EGFRvIII and EGFR[246] provide further reason to be cautiously optimistic about the therapeutic potential of the EGFRvIII neoantigen.

GBM personal neoantigens—In contrast to shared neoantigens, which are mutated TSAs present in multiple patients with GBM, personal neoantigens are mutated TSAs presented only by one or a very minimal subset of patients. In GBM neoantigens identified in personal vaccines[247,248] and the Cancer Antigen Atlas,[249] the majority are personal, likely a reflection of their status as passenger mutations. While personal neoantigens will not be comprehensively covered here, they are still incredibly relevant to therapy.[103] Neoantigen vaccines for GBM have been demonstrated to induce neoantigen-specific T cells in patients,[106,172,247] with one study showing that vaccination can induce neoantigen reactive TILs.[106] Encouragingly, a recent clinical trial demonstrated that in 173 GBM patients vaccinated with a median of 19 personal neoantigens, patients who had T cell responses to multiple peptides had a median survival of 53 months compared to 27 months in those with a response to one or zero.[172] These studies represent the promise that antigen-based therapies have for improving outcomes for GBM patients.

TSA of unknown etiology in glioblastoma: BCAN478-486—Some nonmutant glioblastoma antigens have been

identified as tumor-specific; however, the reason for their selective presentation by tumor cells is unknown. Their tumor-exclusive presentation could be due to any of the previously mentioned causes, including differential mRNA processing, post-translational modification, and/or proteasome processing.[95,250] The TSAs of unknown etiology include a group of HLA-A*02 restricted neoantigens identified by Dutoit et al.[195] Of these, BCAN478-486 is promising, since brevican (encoded by *BCAN)* is a brain-specific extracellular matrix molecule involved in tumor invasion and expressed by GSCs.[195] Brevican has also been implicated in migration and metastasis, and over-expression is linked to decreased survival in GBM.[195] When tested, eight out of eight GBM patients' T cells mounted an immune response ex vivo to BCAN478-486 in the original Dutoit et al. study,[195] and the subsequent use of this antigen in vaccines induced immune responses in patients.[52,173] GBM samples taken before and after vaccination with IMA950, which included the peptide, and Poly-ICLC showed a lower percentage of BCAN positive cells, indicating that vaccination may have resulted either in successful targeting of the cells with the antigen, or in antigen downregulation.[173] However, as previously mentioned, no association between T cell response to any IMA950 peptides and survival was seen in the IMA950 plus GM-CSF trial.[231] Overall, BCA478-486 has potential as an antigen target for GBM due to brevican's potential role in oncogenicity, its association with decreased survival, and its demonstrated immunogenicity in vaccines.

TSA of unknown etiology: SART2-93 DYSARWNEI—The SART2 protein was first identified in squamous cell carcinoma and has been shown to be expressed in adenocarcinoma, melanoma, renal cell carcinoma, and glioma.[251] This protein has been determined to be absent in normal tissue, including testis and fetal liver.[251] In the phase III ITK-1 multipeptide vaccine trial for 88 patients with recurrent GBM in Japan, receiving vaccination of the SART2-93 DYSARWNEI peptide conferred a negative impact on survival.[192] In this randomized double-blind trial, each patient in the vaccinated group was given a vaccine of four peptides out of twelve possible HLA-A24 restricted peptides based on their pre-vaccination IgG levels for each peptide. The median OS for the 13 patients that received the SART2-93 antigen in their vaccine was 6.6 months compared to eight matched placebo patients with a median survival of 22 months.[192] This difference in survival was not necessarily mediated by receiving the SART2-93 antigen and may instead reflect the immune function of the patients for whom the antigen was selected. Investigators found that prior to vaccination, the patients that were ultimately selected to receive SART2-93 had baseline lower CTL and B cell activity against all possible vaccine peptides, as measured by ex vivo IFNγ assays and antibody assays, respectively.[192] Despite this, investigators still opted to use the same antigen in the TAS0313 vaccine.[193,214] A phase I/II clinical trial for the vaccine in patients with recurrent GBM has not yet published its overall survival (OS), but the median PFS was 1.7 months.[193] The function of SART2 and the reason for its tumor-specific expression remains unknown. While most antigen vaccines have not been harmful and often provided benefit, the mechanism underlying the negative association between the SART2-93 antigen and survival merits further investigation.

Transposable element derived TSAs in glioblastoma—Recent studies have shown that antigens derived from transposable elements (TE) are presented by GBM tumor cells and generate an immune response.[252] Specifically, Bonte et al. identified fifteen TE-derived antigens in GBM that were validated as immunogenic by tetramer-binding assays.[252] While TE-derived antigens have been classified as tumor-specific,[93,253] the study indicated that many TEs are expressed at low levels in normal tissues.[252] Another study identified 19 TE-derived antigens on GBM samples[254]; however, this study did not confirm the immunogenicity of these peptides. One TE that warrants further investigation is human Endogenous Retrovirus K, since it is differentially expressed in GBM and likely contributes to its stem cell niche.[255,256] No clinical trials have used antigens from TEs for GBM, but given the large number of candidate antigens, they hold promise for future therapy.

Viral tumor-specific antigens in glioblastoma—Studies have indicated that 51–100% of GBM tumors, including GSCs, are CMV-positive,[257–260] while surrounding brain tissue is CMV-negative.[257,261] As such, GBM TSAs include peptides from the CMV pp65 protein. One study found that when PBMCs from patients with GBM were exposed to DCs transfected with RNA for CMV pp65 peptides, peptide-specific T cells expanded and could subsequently lyse autologous tumor cells endogenously expressing pp65.[262] However, since many GBM patients are seropositive for CMV,[257] CMV antigens likely are not a bona fide tumor-specific target. In fact, an IMA-950 vaccine trial used a CMV-derived peptide as an internal positive control for ex vivo immunogenicity experiments, citing the high levels of chronic infection with the virus.[173] Nonetheless, both CMV antigens[170,263] and CMV-specific T cells[264–266] have been used safely in clinical trials.

In a clinical trial for 25 patients receiving ACT specific for CMV antigens, at 65 months, ten patients were alive and five were disease free.[264] In addition, in a clinical trial that administered GM-CSF mixed with DCs pulsed with the mRNA of pp65 fused to lysosome-associated membrane protein (LAMP), along with dose intensified TMZ, median PFS was 20, and OS was 33.4 months.[170] The strategy of fusing the antigenic target (in this case, pp65) mRNA to LAMP mRNA has previously demonstrated enhanced activation of the MHC class II pathway and thus, subsequent induction of CD4+ T cells.[267,268] Both in this trial, and another CMV peptide vaccine clinical trial, investigators did not confirm the presence of CMV in patients before enrolling them.[170,263]

Contrastingly, a study that analyzed the immunopeptidome in 19 primary and recurrent GBM samples found no virus-derived antigens.[42] Nonetheless, the increased survival seen in the early clinical trials focused on CMV antigens justify further trials to better explore their therapeutic potential, many of which are already in progress (Table 1).

Bacterial TSAs in glioblastoma—Intracellular microbes have been reported in GBM tumor cells.[269] The previously mentioned study that analyzed the antigens present in 19 GBM tumor samples found between 5 and 54 unique HLA class II-restricted bacterial derived antigens per patient,[42] some of which were demonstrated to be recognized by TILs.[42] However, findings from this study challenged the utility of bacterial antigens as therapy for GBM: (1) some bacterial antigens were also found in the brain tissues of control brain tissue (taken from healthy patients or those with multiple sclerosis), and (2) there was minimal overlap of bacteria and bacterial antigens between patients. One clinical trial currently underway targets bacterial antigens from the gut that are designed to induce T cells that are cross-reactive against GBM TAAs and CTAs.[224] However, since only a small number of studies have reported intratumoral bacterial antigens in GBM,[42,269] further preclinical work should be undertaken to validate and further elucidate this antigen type before targeting in therapy.

Translational Considerations

Antigen Selection

Antigens can be harnessed therapeutically in GBM via vaccines or TCR-based therapies, and when selecting target antigens, many factors should be considered (Figure 2). First, the two criteria of being a TA should be confirmed: presentation on tumor cells and immunogenicity. Antigen presentation in the tumor compared to normal cells should be evaluated, weighing the risks of potential autoimmune reactions. Levels of antigen presentation should be evaluated within a tumor, with a preference toward those that are highly expressed. However, relatively low expression of an antigen does not necessarily militate against the success of an antigen as a target. GSCs are a small percentage of total tumor but are drivers of recurrence.[270] Thus, GSC antigens may provide key targets. Levels of antigen presentation should additionally be considered across a tumor spatially, since GBM tumors have been shown to be intratumorally heterogeneous with distinct regions of the tumor expressing different antigens.[140] The function of the antigen's source protein should be considered, too, with the prioritization of those that belong to driver mutations or associate with decreased survival. Finally, HLA restriction of antigens will need to be accounted for, as further discussed below.

Multiple antigens should be targeted because immune response to multiple vaccine antigens has been demonstrated to be associated with prolonged survival in both renal cell carcinoma[271] and in GBM.[172] Polyvalent targeting can counteract antigen heterogeneity, particularly regarding spatial variation in expression; indeed, Johanns et al. demonstrated the feasibility of incorporating multisector sampling of a GBM tumor into antigen vaccine design.[107,140] Directing therapy at multiple antigens can also help mitigate dampened immune response that may arise secondary to "original antigenic sin," the process where immune cells can have weak responses to epitopes that are similar to previously encountered foreign epitopes.[272,273] Lastly, targeting multiple antigens protects against vaccine failure caused by antigen downregulation on recurrent tumors, which has occurred in multiple GBM antigen vaccine clinical trials.[209,243,274,275]

Targeting multiple antigens is feasible. GBM vaccine trials have targeted multiple antigens and had varying degrees of success inducing T cell responses to their peptides.[52,106,173,192,215] A clinical trial for melanoma exhibited that targeting multiple peptides does not decrease the immunogenicity of each peptide, and patients had a greater total immune response to a twelve-peptide compared to a four-peptide vaccine.[276] Lastly, the previously mentioned Latzer et al. study produced personal neoantigen vaccines for GBM patients in around 12 weeks.[172]

Selecting antigens that encompass multiple TA classes likely provides a therapeutic advantage. In two patients who underwent adoptive transfer of tumor-infiltrating lymphocytes (TILs) resulting in successful eradication of their HPV+ cervical cancer, investigators looking into the antigenic targets of the infused TILs discovered the TILs were reactive against HPV antigens, neoantigens, and CTAs.[9] They also demonstrated that these TILs remained functional and elevated in patients' blood during tumor regression and remission.[9]

Targeting both MHC class I and II peptides should be prioritized. While the majority of studies have looked into MHC class I epitopes, in part due to the limitation of prediction algorithms, MHC class II epitopes are important for anti-tumor immunity.[8,277–279] Class II expression by tumor cells is associated with improved survival in many cancers.[280] Plus, CD4+T cells reactive to class II-restricted neoantigens have been observed in glioma.[232,281,282] One multivalent neoantigen vaccine for GBM primarily induced CD4+T cell responses, despite being designed to induce CD8+T cell responses.[52] Other neoantigen vaccines for GBM have similarly demonstrated the ability to provoke CD4+T cell responses.[247,283] Lastly, targeting both classes may be necessary to counteract tumor immune evasion via tumor downregulation of either MHC class.[89]

Moreover, consideration should be paid to the changing antigenic landscape that occurs temporally as GBM tumors evolve, especially in response to standard therapy,[175,284] antigen-based therapy,[247] and immune pressures.[285] While personalized antigen vaccines can lead to antigenic loss,[106] treatments have also been shown to induce new antigenic targets.[175,286–288] A potential approach to antigen targeting might thus involve vaccinating patients against antigens known to be induced by a treatment in conjunction with administration of the treatment.[289] Lastly, recurrent tumors are distinct from primary tumors[290] with presumably discrete antigenic targets and thus may require different treatments. For example, tumors expressing the EGFRvIII mutation at diagnosis have been observed to lose its expression at recurrence.[238,291]

All these factors should bear upon the design of antigen-directed therapy, as should other factors, like the immunological response state of each patient's tumor, which can be evaluated with techniques like CIBERSORT,[292,293] as well as the vaccine delivery platform, adjuvant therapies, and timing and route of administration. These topics merit their own review.

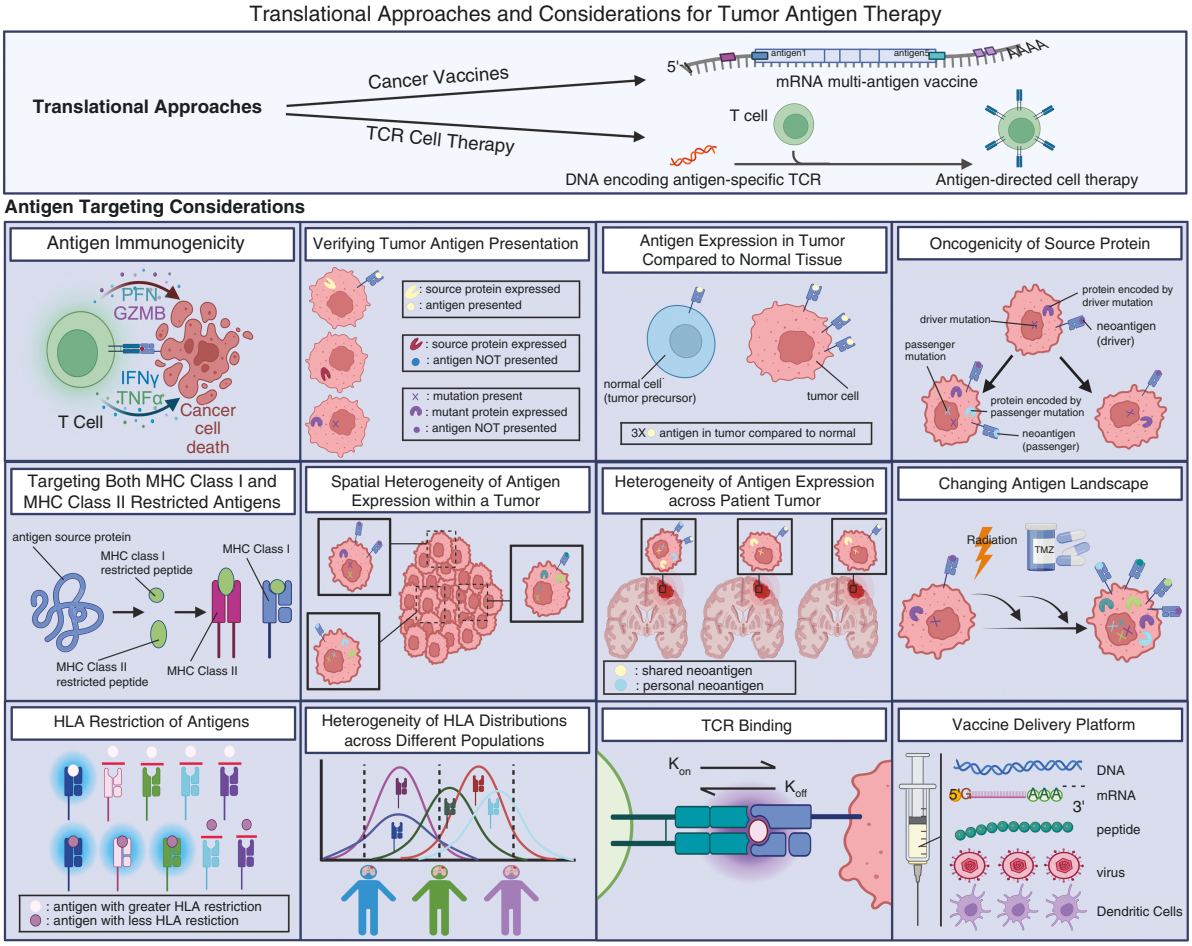


Figure 2. Tumor antigens can be harnessed for therapy against GBM either via vaccines or TCR-based therapies. Considerations for the design of the therapy include: focusing on antigens confirmed to be immunogenic; prioritizing antigens that are confirmed to be present in the immunopeptidome; focusing on antigens that have little to no expression in normal tissue to minimize the risk of autoimmune reactions; whether the protein source of the antigen contributes to the tumor's oncogenicity, with a preference toward antigens sourced from driver mutations to target the most deleterious tumor cells; ensuring both MHC I and MHC II restricted antigens are targeted to activate both CD8+ and CD4+ T cells, respectively; the intratumoral spatial heterogeneity caused by clonal and subclonal tumor populations with discrete antigen targets; intertumoral heterogeneity of antigen expression; temporal evolution of antigen expression that occurs secondary to immune pressures and standard treatment like temozolomide and radiotherapy; HLA restriction of antigens; accounting for the fact that HLA molecules are differentially expressed across populations and ensuring that antigens of different HLA restrictions are studied to avoid excluding certain populations from benefiting from therapy; antigen binding to TCRs, specifically the affinity, avidity and cross-specificity; method of delivery for antigen-based vaccines, which could include DNA, mRNA, peptide, virus, or pulsed dendritic cell delivery platforms.

TCR Engineering

Recent preclinical advances in targeting GBM antigens via ACT have been promising. One study demonstrated that ACT specific for an endogenous neoantigen in the murine GBM model GL261 resulted in intratumoral infiltration by the T cells and long-term cures in the majority of the mice.[294]

When developing TCR-based therapies, including ACT, three important TCR qualities must be considered: (1) affinity, (2) avidity, and (3) cross-specificity. Affinity describes the strength of interaction between a TCR and cognate peptide-MHC molecule. While some studies suggest that TCR affinity relates to T cell activation,[295] others show that TCR affinity does not relate to T cell response.[124,125,296] One group found that changing the catch bond duration between a TCR and cognate pMHC, while keeping the affinity the same, correlated with TCR activity.[297] Structural avidity measures the number of interactions between all the TCRs of a T cell and MHC molecules on the target cell. Functional avidity measures the capacity of a T cell to respond to a given concentration of peptide. Higher avidity indicates that at lower concentrations of a peptide, a T cell will be activated. Generally, higher functional avidity is associated with increased T cell function[298–300]; however, TCR-independent factors like T cell differentiation states[301] and epitope density[302] can affect functional avidity levels.

Arguably, the most important variable to consider is TCR cross-specificity. TCRs that have been synthetically affinity matured have been powerful in clinical trials,[303–306] but have also led to significant adverse events due to cross-reactivity. Although in vitro the affinity-enhanced MAGE-

A3-specific TCR did not bind off-target,[307] in clinical trials two patients died due to cardiogenic shock,[308] since the TCR was cross-reactive against a titin-derived peptide. Overall, these studies underscore the importance of thorough preclinical testing of TCR-based therapies.

TCR Therapies Compared to CAR-T

A full exploration of CAR-based therapies for GBM is not within the scope of this review, since the "antigens" that CARs target are portions of whole proteins, not T cell antigens. However, it is worth briefly delineating TCR-based versus CAR-based therapies, as they are two arms of cell-based immunotherapy. Unlike TCR therapies that can use endogenous or engineered TCRs to recognize HLA-bound antigen displayed on the cells surface, CAR-based therapies use engineered receptors to recognize cell-surface targets that are not bound by MHC.[309] Thus, CAR-T cells are limited to surface-expressed proteins as targets but are not limited by MHC restriction. ACT is constrained by MHC but can target antigens derived from both intra- and extra- cellular proteins. Plus, in the case of ACT therapy using TILs, like lifileucel, the FDA-approved TIL therapy for use in melanoma,[310] a patient's T cells are removed from their tumor and expanded ex vivo without any genetic engineering, since the TCRs of the TILs presumably already have tumor specificity. CAR-T cells on the other hand, are often manufactured by taking T cells from a patient's blood prior to engineering them to recognize a defined target.[311]

TCR and CAR therapy design should borrow principles from each other, as strategies have been developed for CAR-T cells to mitigate tumor heterogeneity, antigen escape, and off-target effects for non-tumor-specific antigens.[312] For example, SynNotch, which can be engineered to require the presence of a tumor-specific antigen in order to deploy a CAR against a non tumor-specific antigen that is homogenously expressed in the tumor, showed promise against targets in a GBM model in mice.[313] Plus, it may be beneficial to administer CAR and TCR therapies together, as using both modalities likely increases the number of possible therapy targets and decreases the possible mechanisms of immune escape by the tumor.

Developing Equitable Antigen Therapy

Antigens are often HLA-restricted: certain antigens only bind particular HLA molecules.[313,314] Since HLA distributions vary across ethnic populations,[314,315] in the case of designing "off-the-shelf" vaccine peptides or TCR therapies, the choice of which peptides to target and their concomitant HLA restriction has significant implications for who benefits. For example, Tebentafusp, the therapy for advanced uveal melanoma is FDA-approved only for patients that are HLA-A*02:01 positive.[29,316] A recent cross-sectional study of all U.S. clinical trials that required a certain HLA for enrollment found that due to HLA enrollment criteria, people of European descent were 46% more likely to be eligible for a clinical trial with HLA restriction than those of Asian or Pacific Islander descent and 60% more likely than those of African descent.[316] Notably, in the United States, minorities are already underrepresented in both oncology clinical trials[317] and brain tumor clinical trials.[318]

It is imperative that GBM antigen therapies do not inadvertently lead to structural racism[319] or exacerbate the already present disparities[320–322] in brain tumor care. The SurVaxM vaccine presents a proof of concept for the design of equitable therapy, since it includes a peptide that encompasses antigen binding motifs for multiple class I HLAs and successfully induced immune responses to a variety of HLA-restricted peptides.[183] Designing equitable antigen therapy in the future hinges on how research is conducted in the present: investigating antigens that bind to HLAs of different classes. Plus, prioritizing equity in research design will strengthen insights and translate to improved patient outcomes.

Conclusion

Targeting TAs represents an exciting therapy for GBM. Treatment design needs to account for the unique properties of GBM and overcome the limited neoantigens by targeting multiple classes of TAs, and both MHC I and II restricted antigens. Additionally, further research must be done to confirm target antigens are presented on tumor cells and that immune responses to peptides in vaccines translate to survival benefit. More broadly, understanding the immunogenic landscape of GBM is crucial to knowing how the immune system discriminates GBM from normal and, unquestionably will lead to translational insights that will change the lives of patients with this disease.

Supplementary Material

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

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