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Exploring Novel Neoantigen-Based Treatment Methods for Glioblastoma

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Abstract

Glioblastoma, constituting almost half of all malignant brain tumors, presents a formidable challenge in treatment due to the blood-brain barrier's protective role against certain therapies. These tumors, characterized by rapid growth, tissue invasion, and diverse evolving cells, afflict individuals of all ages and resist conventional cancer treatments despite extensive biomedical research. An innovative approach to glioblastoma treatment involves leveraging neoantigens, specific to cancer cells and targetable by the immune system. Clinical trials suggest that neoantigen-based treatments hold promise, offering more effective and personalized options for patients. Exploring this avenue, including vaccines, immune checkpoint blockers, and adoptive cellular therapies, is crucial for improving outcomes. This paper reviews novel therapeutic options within neoantigen-based treatments, providing insights into potential advancements against glioblastoma.

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1. Introduction

The aim of this paper is to explore and evaluate the efficacy of neoantigen-based vaccines, immune checkpoint blockers, and adoptive cellular therapies as potential treatment methods for Glioblastoma. By referencing scientific literature and clinical trials, this research seeks to accomplish the following objectives:

1.1. Reviewing the History and Background of Glioblastoma Treatment:

- Conduct a comprehensive analysis of the historical context and background of glioblastoma treatment methods utilizing scientific databases such as PubMed, Google Scholar, CINAHL, etcetera.
- Highlight the limitations and challenges associated with conventional treatment approaches, such as surgery, radiation therapy, and chemotherapy.

1.2. Investigating Neoantigens as Potential Treatment Options for Glioblastoma:

- Review scientific literature to examine the role of neoantigens in glioblastoma immunotherapy.
- Explore the mechanisms through which neoantigens can elicit an immune response against glioblastoma tumor cells.
- Evaluate the efficacy and safety of neoantigen-based vaccines in preclinical and clinical settings.

1.3. Assessing the Efficacy of Immune Checkpoint Blockers in Glioblastoma Treatment:

- Examine the role of immune checkpoint blockers, such as PD-1/PD-L1 inhibitors and CTLA-4 inhibitors, in modulating the immune response against glioblastoma.
- Analyze the outcomes of clinical trials and real-world evidence regarding the use of immune checkpoint blockers in glioblastoma patients.
- Assess the potential of combination therapies involving immune checkpoint blockers and other treatment modalities.

1.4. Exploring Adoptive Cellular Therapies for Glioblastoma:

- Investigate the use of adoptive cellular therapies, including chimeric antigen receptor (CAR) T-cell therapy and tumor-infiltrating lymphocyte (TIL) therapy, in glioblastoma treatment.
- Analyze the efficacy and safety profiles of adoptive cellular therapies in glioblastoma patients.
- Evaluate the potential of enhancing the effectiveness of adoptive cellular therapies through genetic engineering and personalized medicine approaches.

By addressing these objectives, this article aims to contribute to the advancement of glioblastoma treatment by shedding light onto the potential of neoantigen-based vaccines, immune checkpoint blockers, and adoptive cellular therapies as innovative and promising therapeutic strategies.

2. Glioblastoma Background

Every year, it's estimated that approximately 10,000 people will be diagnosed with glioblastoma, which yields a 25% one-year survival rate as well as a 6.8% 5-year survival rate.[1] On average, patients survive eight months before succumbing. Glioblastoma is a Grade IV brain tumor[2]—signifying that the cells are actively dividing, and the tumor has dead tissue as well as abnormal blood vessel growth—that stems from malfunctioning astrocytes, glial cells that provide structural support to neurons, modulate synaptic activity, and act as a major component in the blood-brain barrier.[3]

	Primary GBM	Secondary GBM
Origin	De Novo	Grade II/III Astrocytomas
% of Cases	90%	10%
Mean Age Diagnosis	62 years	45 years

Figure 1. *Glioblastoma Statistics*

The age-adjusted incidence of glioblastoma has a positive correlation with age, being 0.15 per 100,000 in children to 15.03 per 100,000 in patients

between 75 and 84 years of age.[4] Glioblastoma develops most commonly in the frontal lobe, but has appeared in the temporal, parietal, and occipital lobes, and could even grow into surrounding brain tissues.[5] Common symptoms of glioblastoma include seizures, coordination issues, paralysis, fatigue, severe headaches, and cognitive impairment. Major risk factors have yet to be identified; however, it was recently discovered that exposure to high ionizing radiation is a major contributor towards glioblastoma development. Moreover, exposure to vinyl chloride, pesticides, smoking, petroleum refining, and synthetic rubber have shown occasional positive association with glioblastoma emergence.[6] Currently, the main treatment methods used include surgical resection, alkylating chemotherapy, radiation therapy, and Tumor Treating Fields.[6]

The majority of gliomas have been found to contain point mutations in isocitrate dehydrogenase 1 and 2. In Glioblastoma, molecular alterations that have been discovered include mutations in genes regulating receptor tyrosine kinase (RTK), rat sarcoma (RAS), phosphoinositide 3-kinase (PI3K), p53, and retinoblastoma protein (RB) signaling.[7] Current research shows mutations in EGFR (57% of GBM patients), HER2, PDGFRA (13%), c-MET (1.6%), FGFR (3.2%), PTEN (41%), and VEGFR genes are therapeutic targets due to having shown amplifications or mutations from dysregulated cell signaling cascades in glioblastoma.[7]

Kinase inhibitors haven't proven to be effective in glioblastoma therapy due to their low efficacy in penetrating the blood-brain barrier; however, a multitude of experimental treatments have emerged and shown consistent progress. AZD3579 (EGFR inhibitor) has shown effective blood-brain barrier penetration in vivo in rats and monkeys, however its safety and efficacy in humans has yet to be tested. Epitinib (EGFR inhibitor) was reported to have optimal BBB penetration, is well-tolerated in patients, and is effective in treating brain metastases as well. WSD0922 (EGFR inhibitor) is reported to have high BBB penetration, reasonable safety, and has shown antitumor properties in Glioblastoma PDX models.[7]

Tucatinib (HER2 inhibitor) can cross the blood-brain barrier and create survival benefits in mice. For patients with breast cancer and brain

metastasis, tucatinib produced better progression-free survival and overall survival. Neratinib (HER2 inhibitor)had limited BBB penetration in mice and was not successful in a phase II trial for patients that had HER2-positive brain metastases.[7]

Additionally, for newly diagnosed Glioblastoma cases, clinical trials involving the drug temozolomide—a monofunctional DNA alkylating agent—have shown significant progress. Adding the temozolomide regimen improved the overall survival and progression free survival in patients with GBM compared with radiotherapy alone. Temozolomide is a lipophilic molecule that crosses the blood-brain barrier and is stable at the acidic pH of the stomach and is therefore administered orally.[7] Temozolomide is currently being tested, in a phase II interventional clinical trial led by *Wen* et al. (NCT02977780) via combination therapy with Neratinib, to study overall survival compared to standard treatment in Glioblastoma patients.[8]

3. Neoantigen Background

Neoantigens are a class of proteins that arise in cancer cells with the mutation of tumor DNA. The first discovery of neoantigens was made in 1988 by De Plaen and his colleagues.[9] By utilizing cDNA library screening on a mouse tumor model, they observed a single nucleotide difference between the normal and tumor gene, resulting in a noticeable amino acid change. This novel finding led to the coining of the term "neoantigen" which was used in further studies on human tumors including melanoma and renal cell carcinoma.[9]

Neoantigens have proven efficient immunogenic targets because of their localization to cancer patients. The mechanism of action by which they form explains the tumor-specificity of neoantigens and their associated potency in developing cancer treatment. First, mutations in tumor DNA cause rise of new and mutated proteins in tumor cells. After completion of function, these proteins are proteolyzed by the proteasome and the degraded peptides are sent to the endoplasmic reticulum via a transporter associated with antigen processing (TAP)protein.[10] The protein-peptide complex is then sent to the Golgi apparatus and then exported to the plasma membrane with chaperone proteins. There, the major histocompatibility

complex (MHC) displays protein fragments (post-proteolysis) to the immune system to fight infection by pathogens.

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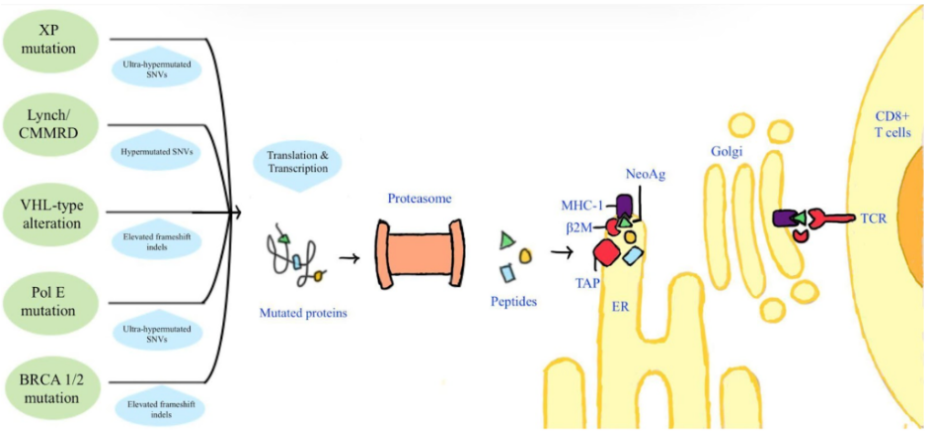

Figure 2. *Mechanism of action of neoantigens*

In humans, the MHC is referred to as human leukocyte antigens (HLA), which are encoded by genes on chromosome 6.[11] HLAs work with T cells to identify 'self' and 'foreign' cells and tissues in the body by displaying short polypeptides to T-cell receptors. These polypeptides can be recognized as 'foreign' by tumor-infiltrating lymphocytes (TILs) which results in the activation of strong tumor-specific immune responses.[12] There are two main groups of MHC molecules, which bind to different peptide sequences and are recognized by separate T-cell types. **MHC-class I** genes consist of HLA-A, HLA-B and HLA-C, and their attached peptides are recognized by $CD8^+$ T-cells.[11] **MHC-class II** genes, on the other hand, consist of HLA-DR, HLA-DQ and HLA-DP, and attached peptides are recognized by $CD4^+$ T-cells.[11]

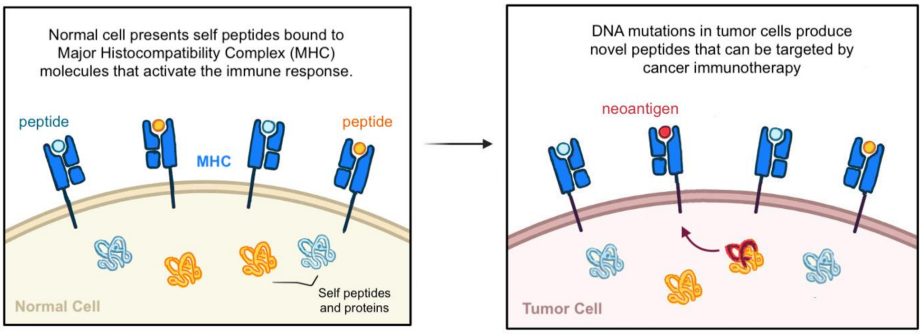

Figure 3. *Role of the Major Histocompatibility Complex (MHC)*

Neoantigens are commonly confused with other tumor antigens that exist, of which there are three different classifications that T-cells can target.[11] **Tumor-associated (TAA) antigens** are a group of proteins characterized by overexpression in cancer cells compared to normal tissue. They consist of *differentiation antigens*, which are normal proteins that are increasingly expressed due to uncontrolled division of cells that have specific functions [eg. prostate-specific antigen (PSA) in prostate cancer.[13] The other class is *overexpressed antigens*, which are proteins that are greatly expressed due to mutated genes characteristic of cancer [eg. Hepsin in prostate cancer].[14] A complete library of tumor-associated antigens can be found in the Cancer Antigenic Peptide Database: https://caped.icp.ucl.ac.be/Peptide/list. **Cancer testis (CT) antigens** are a family of TAA that are strictly found on the testis and placenta. Due to their expression specificity in the germ cells, CT antigens found in other regions of the body indicate oncogenic causes.

Hence, CT antigens are considered promising therapeutic targets. There is also the fact that the testis do not express MHC-class I, therefore CT antigens are recognized as 'non-self.' A comprehensive list of CT antigens can be found in the CTDatabase: http://www.cta.lncc.br/. Finally, **viral antigens** are a family of TAA that are caused by a viral infection. Examples include the human papillomavirus (HPV)which can cause cervical and several other cancers, as well as hepatitis C which may lead to liver cancer and non-Hodgkin's lymphoma. **Neoantigens** are unique to the aforementioned antigen types because they arise from the onset of cancer, thus it is the genomic mutations from the cancer that lead to the rise of new (neo-)antigens in the body.[14]

In exploring neoantigen-based treatment, there is the distinction to be made between public and private neoantigens. Public (shared)neoantigens are common across cancer patients and typically occur in driver oncogenes and tumor suppressor genes. Private (personalized)neoantigens are unique to each patient and therefore require individualized treatment.

4. Potential Treatment Methods

4.1 Neoantigen-based Vaccines

The process of creating a neoantigen vaccine typically involves sequencing the patient's tumor cells to identify the unique neoantigens present. Once these neoantigens are identified, they can be used to create a personalized vaccine that is tailored to the patient's specific cancer. The goal of the vaccine is to prime the immune system to recognize and attack the cancer cells that display these neoantigens. This approach has the potential to be more effective than traditional cancer treatments because it targets the cancer cells directly while sparing normal cells.

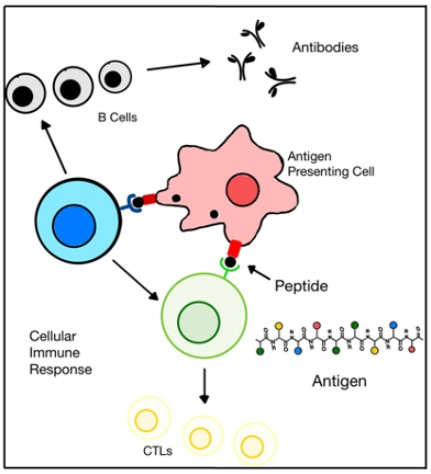

Figure 4. Mechanism of Action for Protein-peptide vaccine

4.2 Protein-Peptide Vaccines

Protein peptide vaccines contain specific antigens which are immunogenic and unique to the patient's tumor, with potential immunomodulating and antineoplastic activities.[15] Upon vaccination, with the neoantigen peptide vaccine, the peptides stimulate the host's immune system to elicit a specific cytotoxic T-lymphocyte (CTL)response against the tumor cells that are expressing the targeted neoantigens, thereby resulting in tumor cell lysis.

According to the FDA, with respect to the mechanism of action, neoantigen-based protein-peptide vaccines use short peptide fragments to induce highly targeted immune responses while simultaneously avoiding allergic responses. Peptide epitopes can bind antibodies in three conformations: alpha-helical, beta-strand/extended, or loop. Since the peptide epitope's spatial conformation among the antigen-antibody complex is significant, vaccines must be engineered in a conformationally correct way to produce optimal results. To constrain peptide epitopes when epitope conformation is important, some methods utilized include covalent side chain-side chain cross linking and integration into a larger scaffold. Emulsions, which act as delivery systems for various peptides, form a depot at the site of injection that attracts immune cells.[16] The presence of antigen depots at organs releasing low-level antigens induces a strong immune response and promotes tolerance. The stability of these emulsions as delivery systems plays a key role in vaccine safety and efficacy.[16]

The process of developing a neoantigen-based peptide vaccine starts with extracting a sample of peripheral blood.[17] Peripheral blood mononuclear cells (PBMCs) are then isolated from the blood sample, and subsequently are differentiated into dendritic cells—the antigen presenting cells. Simultaneously, the peripheral blood sample and a tumor sample are run through Sequencing and Computational Neoantigen Identification: whole-genome sequencing (WGS), whole-exome sequencing (WES), and next-generation sequencing (NGS).[18] The identified neoantigens are validated in vitro via ELISpot, MHC tetramer, and tumor organoids, which have shown in studies to retain the neoantigen features of parental tumors. Then, the selected neoantigens are loaded onto dendritic cells and integrated into peptide vaccines for treatment.[17]

WGS, one of the previous sequencing identification methods, determines the order of the nucleotides in the individual's DNA, however its production of complex datasets that require bioinformatics expertise makes it inefficient timewise. WES utilizes probes and hybridization to analyze the exome, which despite only constituting 1-5% of the genome, contains approximately 85% of disease-related variants. WGS is more time-efficient and allows for greater sequencing capabilities. NGS, reflective of WGS, is used to determine nucleotide sequences in targeted regions of DNA and is capable of sequencing an entire human genome within one day.[19] It's evident that with these three sequencing methods, identifying neoantigens and somatic mutations would be carried out efficiently with regards to time and costs.

In two clinical trials reported by *Hilf* et al. (30568303) and *Keskin* et al. (30568305) in 2019, neoantigen-peptide vaccines produced somewhat successful therapeutic results for glioblastoma.[20,21] In *Hilf* et al.'s trial, fifteen patients, all of whom were recently diagnosed with glioblastoma, were found to possess the human leukocyte antigen (HLA)-A*-2:01 or HLA-A*24:02. In APVAC1 (actively personalized vaccine 1), patients received a vaccine that targeted unmutated antigens, and in APVAC2, the vaccine targeted neoantigens. Compared to classic treatment, patients who received APVAC1 and APVAC2 had an increase in median overall survival of 29 months, and an increase in median progression-free survival of 14.2

months. In the tumor, the vaccine injection induced T-cell expansion; however, at the vaccine's injection site, nearly all patients experienced some form of disease.[20] In a trial led by *Keskin* et al., ten patients with newly diagnosed glioblastoma were treated with the personalized neoantigen-based vaccine after being treated surgically and with radiotherapy. Though some experienced an increase in CD8+ T cells in the environment of recurrent tumors, as well as a small increase of PD-1+ tumor infiltrating CD8+ T cells, all of the patients ended up dying as a result of disease progression, with an average survival of 16.8 months and progression-free survival of 7.6 months.[21]

Reardon et al. (NCT02287428) is conducting an ongoing phase I interventional clinical trial studying the neoantigen-specific peptide vaccine NeoVax, alongside the monoclonal antibody Pembrolizumab, radiation therapy, and Temozolomide in subjects with glioblastoma.[22] The clinical trial involves five cohorts, each receiving either separate individual or combinations of treatments. The first cohort receives NeoVax and radiation therapy, the second starts pembrolizumab within two weeks of the start of radiation therapy and continues triweekly for two years, the third starts pembrolizumab about three weeks after completion of NeoVax priming and continues triweekly for two years, the fourth receives a single dose of pembrolizumab two weeks after the start of radiation therapy and restarts concurrently three weeks after completing NeoVax priming, and the fifth enrolls patients with tumors for which the MGMT status is (partially) methylated and receives standard temozolomide with radiation and adjuvantly for six cycles after radiation therapy. The primary outcomes are the number of participants with adverse events to determine the tolerability and safety (all cohorts), the number of participants with at least ten actionable peptides to measure the feasibility of the study (cohort 1), and the number of participants able to initiate vaccine therapy after radiation therapy within 12 weeks of the date of surgery (cohort 1). The secondary outcomes are the number of participants who experience IFN-γ T-cell responses at week 16 (all cohorts), the number of participants who are alive and don't demonstrate glioblastoma progression eight months after resective surgery (cohorts 1, 1a, 1b, 1c), and the number of participants who are alive without having glioblastoma progression eleven months after

resective surgery (cohort 1d).[22] As the study is ongoing, final results and data have not been collected yet.

4.3 Nucleic acid (DNA and mRNA vaccines)

Nucleic acid vaccines are another category of treatment methods available for cancer patients, of which there are two types: deoxyribonucleic acid (DNA) and messenger ribonucleic acid (mRNA) vaccines.[23] For DNA vaccines, a bacterial plasmid is used as a vector to introduce a gene encoding antigens. The plasmids then replicate, and by using antibiotics, the vectors exhibiting antibiotic resistance can be separated. DNA vaccines are able to activate both humoral and cellular immune responses, and they can be delivered intramuscularly (IM), intradermally (ID), mucosally, and/or transdermally. The process by which genomic alterations occur include internalization of the DNA vaccine into the cell, transcription of the genomic information at the nucleus, and translation of that info within the cytoplasm.[24] This ultimately leads to expression of proteins in vaccinated hosts, however there are three distinct mechanisms by which these proteins reach the T-cells for recognition and targeting. One way includes the use of MHC I complexes in somatic cells, which presents proteins to CD8+ T cells. Another method uses professional antigen presenting cells (APCs), like dendritic cells (DCs),that are transfected with plasmid DNA and then present antigens to T cells through MHC I or II complexes. A third way involves the phagocytosis of plasmid-transfected somatic cells by APCs, resulting in the cross priming and presentation of antigens to both CD4+ and CD8+ T cells.[23]

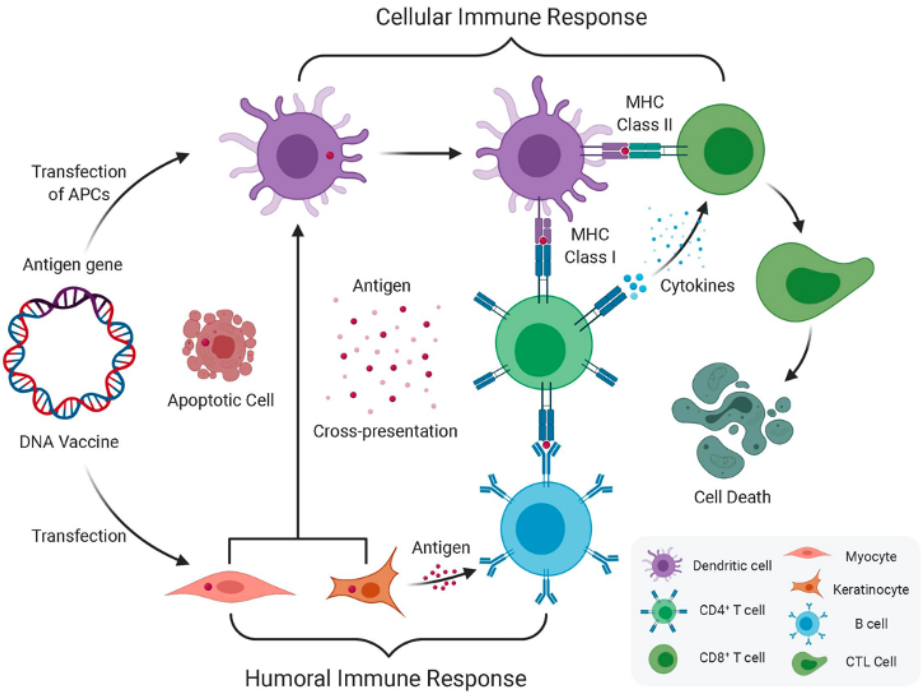

Figure 5. *Molecular mechanism of DNA vaccines*

There are several current clinical trials experimenting with the use of DNA vaccines in patients with glioblastoma. In *Johanns* et al, the initiative is to evaluate the safety, feasibility, and immunogenicity of a personalized neoantigen-based vaccine in subjects with newly diagnosed, unmethylated glioblastoma.[25] In this trial, patients will each receive standard radiation therapy as well as administered neoantigen DNA vaccines using the CELLECTRA®2000 EP Device. The vaccine used is a combination of GNOS-PV01 + INO-9012, DNA vaccines which have previously shown immunogenicity in newly diagnosed GBM patients when administered alongside Libtayo, radiation, and temozolomide. Safety of the vaccine will be determined by dose-limiting toxicities, as per the Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE v5) published by the U.S. Department of Health and Human Services (USDHHS). Alongside safety, feasibility of this DNA vaccine is also being considered, for which there are three necessary conditions: efficiency of vaccine manufacturing, administration, and ability of the vaccine to identify neoantigens in the patient. Additionally, the immunogenicity of the vaccine will be determined by percentage of neoantigens that elicit a neoantigen-specific T cell response, as well as the associated CD8 T-cell response, and overall survival rate.[25]

Some clinical trials are testing the use of DNA vaccines in combination with other immunotherapies to achieve a higher magnitude and breadth of neoantigen-specific T cell responses. In another trial by *Johanns* et al, a personalized neoantigen vaccine is being used concurrently with Retifanlimab PD-1 blockade therapy for patients who are newly diagnosed with glioblastoma.[26] This work extends to other cancer types as well. In *Gillanders* et al, patients with triple negative breast cancer (TNBC) are being actively recruited to conduct a study on the efficacy of adjuvant therapies after administering neoantigen-based DNA vaccines. In this trial, both groups will be given standard therapy as needed (ie. chemotherapy, surgery, radiation therapy, etc.), however one group of patients will be given the DNA vaccine alone. The other group will be given the vaccine along with Durvalumab to test for increased immunogenicity. [27] The purpose of these clinical trials, by both *Johanns* and *Gillanders* et al, is to investigate the effects of using certain DNA vaccines in combination with standard therapies. The findings from these trials will lead to future advancements in the treatments used for diagnosed GBM patients.

The other type of nucleic acid vaccines, mRNA vaccines, currently holds two widely acknowledged forms: non-amplifying mRNA and self-amplifying mRNA, and each bears its mechanistic differences.[23] The main difference between the two is in the additional length of self-amplifying mRNA, which is attributed to the nonstructural proteins that extend the duration and amplitude of gene of interest (GOI) expression.[23] In contrast to DNA vaccines, mRNA vaccines do not have to be transcribed by the host. Instead, the antigen-encoding genetic information is directly delivered to antigen-presenting cells (APCs). After reaching the host cell, the mRNA can be released and translated into proteins which are then proteolyzed into peptide epitopes. Then, the epitopes are sent to the Golgi apparatus, after which they are transported to the plasma membrane and combined with MHC class I complexes via a cross-presentation pathway.[23] An immune response arises when CD8+ T cells are activated as a result of the peptides reaching the cell surface of APCS.

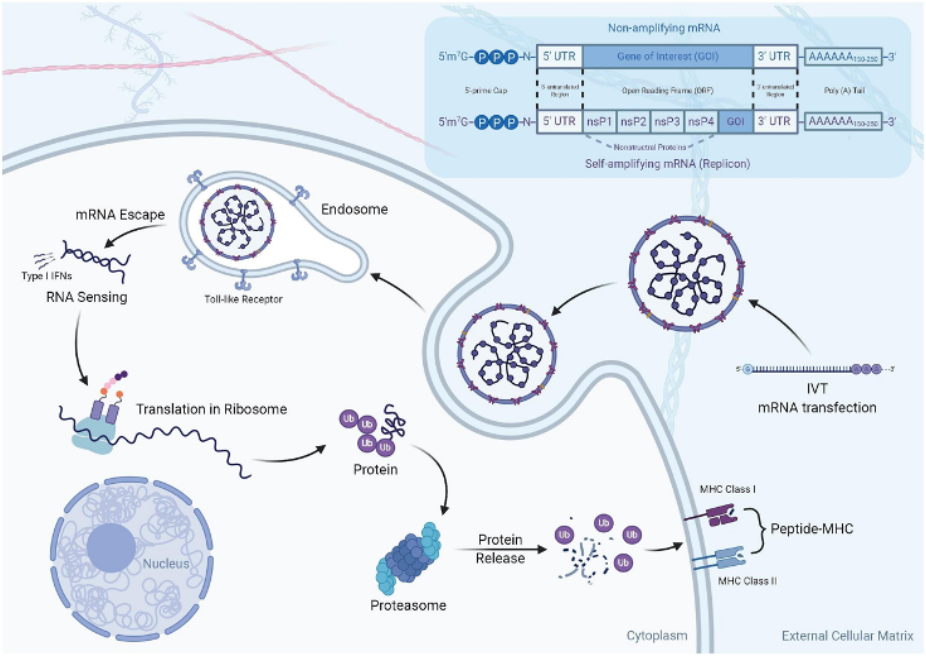

Figure 6. *Molecular mechanism of mRNA vaccines*

Currently, there are studies being done assessing the potency of mRNA vaccines in patients with glioblastoma (GBM). In *Sayour* et al, the objective is to first assess the manufacturing feasibility and safety of a RNA-lipid particle (RNA-LP) vaccine and then evaluate the maximum tolerated dose in GBM patients.[28] Participants will take part in a dose-escalation study using the Bayesian Optimal Interval (BOIN) design with an initial embedded accelerated titration design (ATD).[28] In other studies, such as *Desjardins* et al, mRNA is currently being combined with dendritic cell vaccines and then evaluated for feasibility, potential adverse effects, and survival rates.[29] Similar to clinical trials using DNA vaccines, the purpose of the experiments led by *Sayour* and *Desjardins* et al. is to advance the therapies available for GBM patients. As their approach uses less-studied mRNA vaccines, the current initiative is to evaluate the potency of these vaccines in patients.

4.4 Autologous Dendritic Cells (DC) Vaccines

Autologous DC vaccines are composed of autologous, immature dendritic cells (DCs) with potential immunostimulating and antineoplastic abilities. Upon leukapheresis, immature dendritic cells are isolated and re-administered intratumorally. The immature DCs internalize and process

the neoantigens or tumor-associated antigens (TAAs), migrate to the lymphatic system, and expose the immune system to the TAAs, thereby inducing a specific cytotoxic T-lymphocyte (CTL) response against the cancer cells, leading to tumor cell lysis. The process of vaccination has the potential to either reinforce the reaction against TAAs or induce a new reaction. The mechanism of action for DC vaccines involves the MHC-II molecules on the surface of the DCs, which make them professional antigen-presenting cells (APCs). The DCs move between lymphoid and nonlymphoid tissues to regulate chemokine gradients and cytokine gradients and active T-killers. To create a DC vaccine, immature dendritic cells are isolated from human blood, then utilize a cytokine cocktail and autologous tumor antigens to promote maturation, then readminister the autologous DCs back into the human body via the DC vaccine.[30]

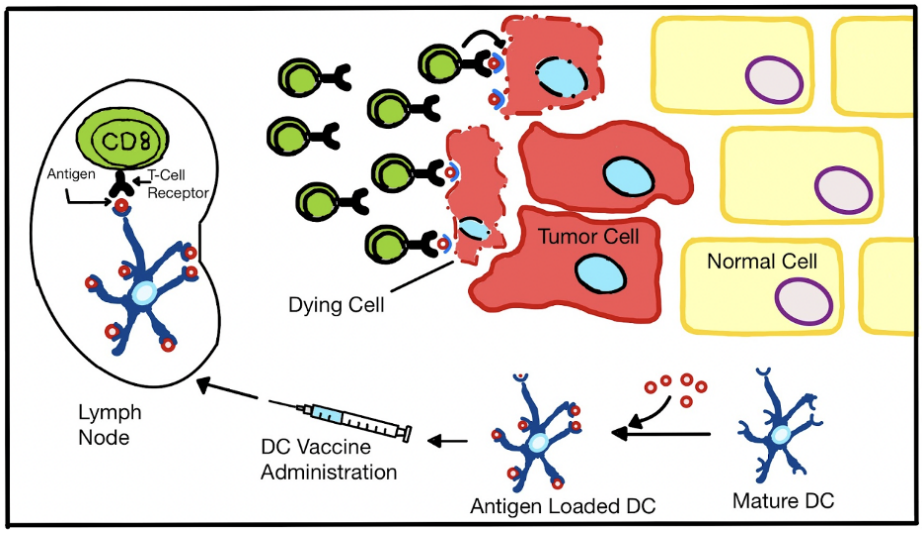

Figure 7. *An overview of the mechanism of action of autologous DC vaccines*

The efficacy of the DC vaccine is dependent on the quantity of neoantigens present in the tumor. The tumor mutational burden (TMB), representing the frequency of neoantigen-associated mutations per megabase, is about 10 mutations/1.4 Mb.[31] For patients undergoing TMZ chemotherapy, the TMB typically experiences an increase, thus making neoantigen discovery more feasible.

The autologous neoantigen-based DC vaccine Neo-MoDC was tested in a patient with advanced metastatic gastric cancer (35661819). The patient had initially undergone laparoscopic-assisted D2 radical distal gastrectomy. Multiplex immunohistochemistry (IHC) revealed low expression of PD-1 and high expression of PD-L1. Once the patient chose to alter their treatment to receive the aforementioned trial's treatment methods, Neo-MoDC vaccines were created using autologous dendritic cells generated from monocytes in peripheral blood mononuclear cells.[14]

Neo-MoDC was administered on its own initially, however after two months, due to continued progression of the tumor, it was administered alongside combination therapy with nivolumab—a monoclonal antibody used to treat different forms of cancer. The Neo-MoDC vaccine elicited a healthy immunogenic response via an increase in neoantigen-specific CD4+ and CD8+ T cell activation, as well as an increase in neoantigen-specific T cell clones in peripheral blood. Upon the onset of combination therapy, the tumor volume experienced a rapid decrease, and continued application of the Neo-MoDC vaccine resulted in complete regression for 25 months until the present. To study T cell clone activation due to the Neo-MoDC vaccine, primary tumor tissue and blood samples at different intervals of vaccination were collected. Activated peripheral blood lymphocytes (PBLs) containing mutant peptides, TCRB clonotypes, and CDR3-regions of the TCRB chain were also analyzed. Approximately 35.3% to 86.5% of TCRB clones in PBLs were found in the tumor tissues, and the frequency of tumor-enriched TCRB clones in PBLs increased from 0% to 16.8% after four doses of Neo-MoDC.[14] Evidently, Neo-MoDC induced immunogenicity, however it required concurrent administration of nivolumab to produce significant results in tumor regression.

4.5 Immune Checkpoint Blockade Therapy

Many signaling pathways associated with the development of cancer. But there are also key regulatory checkpoints termed immune checkpoints that can negatively regulate these same signaling pathways. Immune checkpoint blockade therapy takes advantage of these checkpoints and suppresses them, thereby limiting tumor progression. Some known signaling molecules

associated with pathways that lead to cancer development include PD-1, PD-L1, and CTLA-4.[32,33]

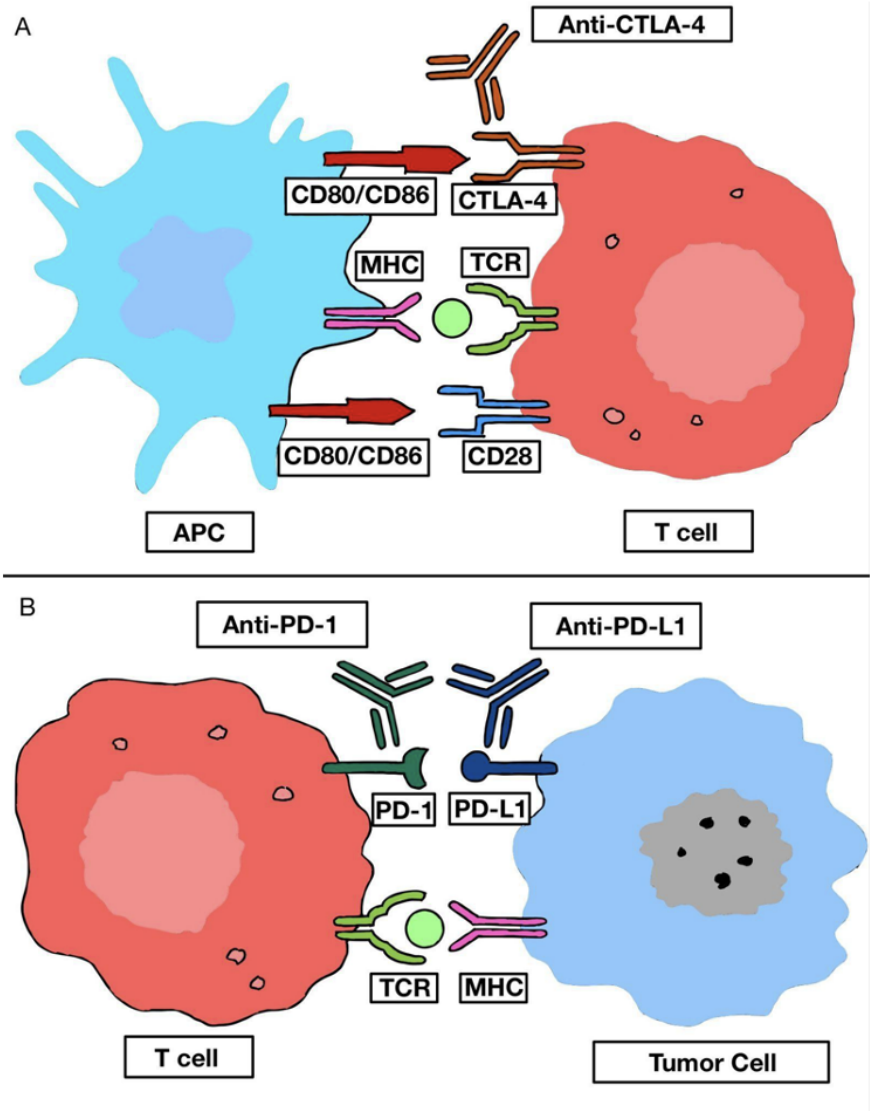

***Figure 8*. Mechanisms of Immune Checkpoint Inhibitors (ICIs).** ICIs act on certain key proteins involved in the signaling pathways that activate T cells. (A) T cells, via their CD28 protein, interact with antigen-presenting cells (ACPs) and their CD80 or CD86 proteins. This interaction can lead to subsequent activation of the T-cell. Another signaling protein on the same T-cell, CTLA-4, has more affinity for the CD80 and CD86 proteins and can lead to subsequent inactivation of the T-cell. Anti-CTLA-4 inhibitors prevent the inactivation of the respective T-cell by disrupting the function of the CTLA-4 protein. (B) Cytotoxic T cells $CD8^+$ interact via their PD-1 protein with tumor cells via their

PD-L1 protein. The interaction between these signaling proteins can lead to negative regulation of the anti-tumor T cell response. Anti-PD-1 and Anti-PD-L1 inhibitors prevent the binding of PD-1 and PD-L1 and thus prevents inactivation of T cell anti-tumor responses.

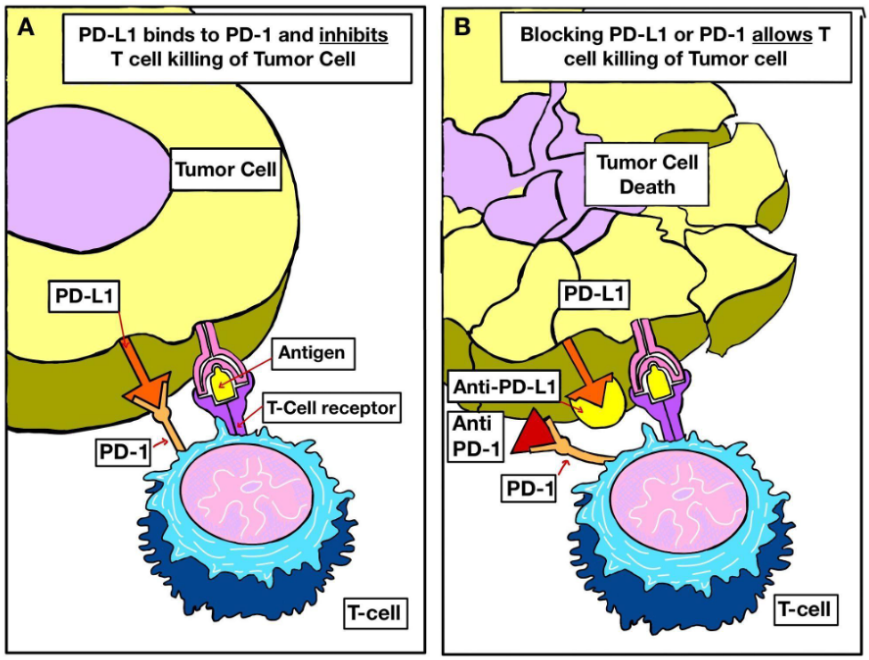

Figure 9. *A comparison of normal T-cell receptor binding vs. T-cell receptor binding after the addition of Anti-PD-L1 ligand and PD-1 blockade(A) T-cell binds to PD-L1 on tumor cells via PD-1 receptor; anti-tumor killing response is inhibited. (B) T-cell's PD-1 receptor binds to Anti PD-1 and PD-L1 binds to Anti-PD-L1; subsequent activation of the anti-tumor killing response is observed.*

More specifically, monoclonal antibodies are being used to target these signaling molecules. When understanding the mechanisms by which monoclonal antibodies function, we focus on a key immune cell, the B cell. B cells are a very important part of the immune system since they have antibodies on their surface membrane. Moreover, each B cell has antibodies that are specific to one antigen and are useless against other antigens. This can be advantageous or disadvantageous depending on the type of antibody and respective antigen. When a B cell and its antibody interact with a

specific antigen, the B cell becomes activated and differentiates into either a plasma cell or memory B cell. Plasma cells produce a vast amount of their specific antibody and memory B cells remain in the host and serve as a part of immune memory so that if they are needed again, they can be activated.[32,34] Looking further into antibodies or immunoglobulins, they are made up of two heavy chains and two light chains and are arranged in a Y-shape. The lower portion of the antibody contains an FC portion, which remains the same amongst all antibodies and is then used to bind to cells of the immune system. Cells of the immune system have an FC receptor that binds to the FC portion of the antibody. The upper portion of the antibody contains the variable region, which has many configurations and is designed to attach only a single type of antigen. There are many ways by which antibodies can stimulate the immune response which include by activating the classical complement system, by attaching themselves to antigens in order to neutralize toxins, by attaching themselves to certain receptors to disrupt function of that receptor, by attaching themselves to a certain pathogen or disease causing agent and agglutinate, or by acting as opsonins, which involves antibodies attaching themselves to pathogens or disease causing agents and making it convenient for the phagocytes to recognize and destroy those pathogens that would have otherwise not been able to recognize them because of primitive recognition structures associated with certain immune cells.[32,33,35] Another very important role they can play is simulating antibody-dependent cell mediated cytotoxicity, in which antibodies attach themselves to pathogens or abnormal cells and then they help immune cells to recognize the pathogen and destroy it.[32,35]

Utilizing the information that antibodies provide an effective way of targeting certain disease-causing agents and are very specific as to what antigen they target, this can be translated over to mechanisms by which monoclonal antibodies function. When treating a patient, it is possible to translate this key process of recognition and subsequent action to stopping a disease-causing pathway. Certain proteins or antigens that are associated with harmful processes can be identified and targeted by monoclonal antibodies. Monoclonal antibodies are a single type of antibody [MP1] that target a specific protein. There are many types of monoclonal antibodies on the market today that have their own specific target protein.[33,34] Many

monoclonal antibodies have been designed to target previously mentioned key signaling molecules to inhibit immunosuppression and restore antitumor responses of the immune system. These antibodies include nivolumab, ipilimumab, avelumab, atezolizumab, retifanlimab, and many more that have proven to be efficacious against many cancer types, including Glioblastoma.[32] Multiple clinical studies have been undertaken to understand the efficacy of monoclonal antibodies against cancer.

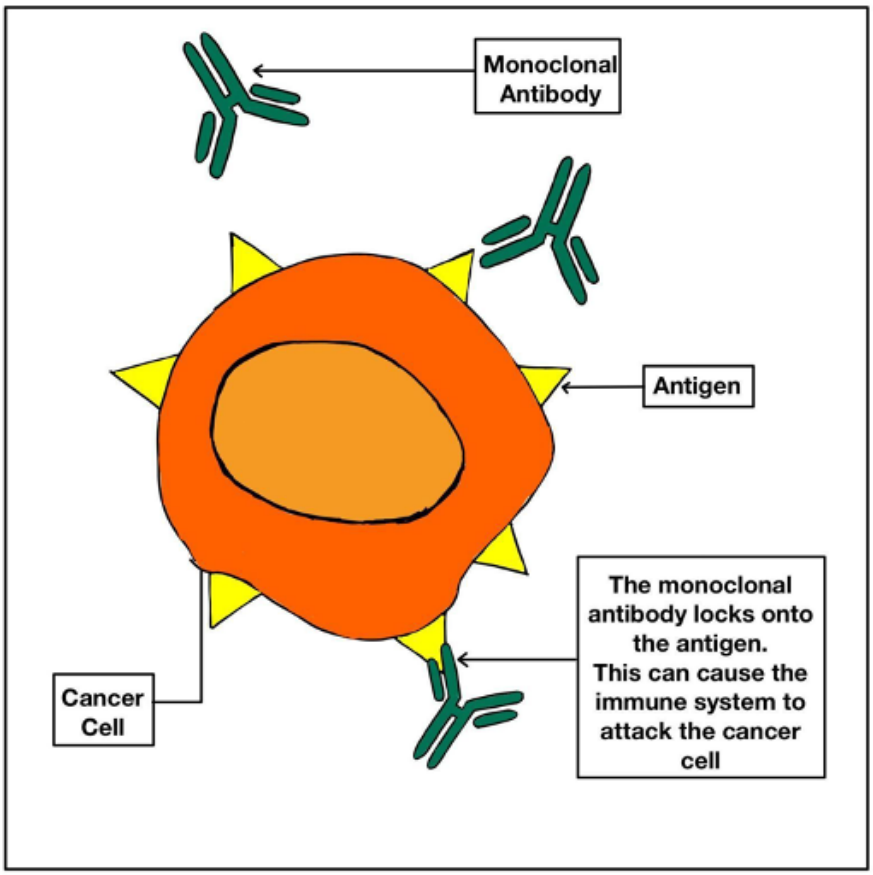

Figure 10. *An overview of the mechanism of action for a monoclonal antibody*

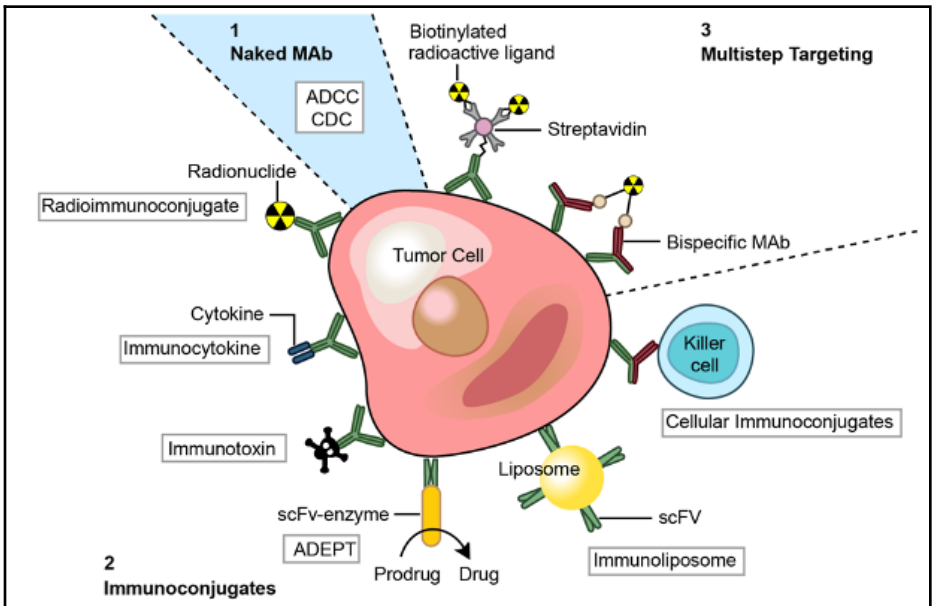

Figure 11. Monoclonal Antibodies (MAb) against Tumor Cells. *Monoclonal antibodies can be found in many different forms and thus based in many different ways. MAbs can be organized into three categories:*
(1) Naked-MAb, (2) Immunoconjugates, (3) Multistep targeting. All these categories represent recombinant or "chimeric" MAbs that are capable of mediating antibody dependent cellular toxicity.

One such Phase I clinical trial, observed treatment with Neo-MoDC (vaccine) and Nivolumab, which is a monoclonal antibody currently being studied for glioblastoma therapy (FDA). PD-L1 and PD-L2 are ligands that typically bind to PD-1 receptors in T-cells, which subsequently inhibit T-cell proliferation and cytokine production (FDA).[14] Nivolumab competitively inhibits PD-1 by binding to the PD-1 receptor and preventing PD-L1 and PD-L2 from binding, thus allowing enhanced T-cell function as well as a resulting anti-tumor response. The recommended dosage for nivolumab, as tested in unresectable and metastatic melanoma, metastatic non-small cell lung cancer, advanced renal cell carcinoma, and classic Hodgkin Lymphoma is typically 240 mg per 2 weeks or 480 mg 4 weeks, as well as 3 mg/kg every 2 weeks for pediatric patients under 40kg. Some possible adverse reactions include fatigue, rashes, musculoskeletal pain, pruritus, diarrhea, nausea, asthenia, dyspnea, arthralgia, urinary tract infection, and upper respiratory tract infection. Although its safety has not been assessed in humans yet, there were no notable effects caused by

nivolumab in the male and female reproductive organs in monkeys, however in animal models inhibition of PD-1 signaling led to an increase in the severity of some diseases, such as tuberculosis in mice.[14]

Phase II Clinical Trial NCT03718767 was an interventional study of neoadjuvant nivolumab in patients with glioblastoma. The patients were due to receive 3mg of nivolumab every 2 weeks (anti-PD-L1 and PD-L2). Its primary outcome measures were changes in the level of expression of PD-L1 by tumor cells and lymphocytes, measured from the baseline until the end of nivolumab treatment. The secondary outcome measures were efficacy, with the response rate assessed by Response Assessment in Neuro-Oncology (RANO) criteria, and safety, with toxicity being assessed by Common Toxicity Criteria (CTC). The study revealed that neoadjuvant nivolumab resulted in enhanced expression of chemokine transcripts, higher immune cell infiltration and augmented TCR clonal diversity among tumor-infiltrating T lymphocytes, supporting a local immunomodulatory effect of treatment.[36] As such, the use of neoadjuvant nivolumab can be used a method of early termination for developing tumor. Some key things to mention are that this study involved the use of patients who have IDH1 or IDH2 mutated gliomas with hyper mutator phenotypes (HMP). These patients had a high number of mutations in their tumors and the study revolved around understanding if neoadjuvant nivolumab can stop or reduce tumor size, showing characteristics of controlled tumor growth. One of the drawbacks of this study was their secondary outcome measure of improvements in quality of life, which was self-reported alongside symptom severity and interference with daily activities. Self-reporting may lead to biases or false speculation in the reports from the patients' end, which in turn would produce skewed secondary results.

An additional combined monoclonal antibody treatment for glioblastoma (NCT03367715) that is currently being studied involves nivolumab and ipilimumab (FDA). For the mechanism of action, ipilimumab binds to CTLA-4 to block its interaction with the ligands CD80 and CD86—which negatively regulate T-cells, therefore resulting in tumor penetrating T-effector cell activation and proliferation (FDA). Inhibiting CTLA-4 signaling reduces T-regulatory cell function, contributing to an increase in

T cell responsiveness. When ipilimumab and nivolumab are used concurrently, PD-1 and CTLA-4 dual inhibition results in enhanced T-cell function that is more effective than either antibody on its own, and they've shown to produce more significant antitumor responses.[38]

Currently, there are many trials being conducted to test the efficacy of single-monoclonal antibody treatments as well as combined monoclonal antibody treatments. The aforementioned trials are just some of those few.

4.6 Adoptive Cell Therapies (ACT)

Adoptive Cell Therapies (ACT) involve the use of *in vitro* amplification of key immune cells, which include dendritic cells (DC), tumor-infiltrating lymphocytes (TIL) and cytokine-induced killer cells. These cells are extracted from a patient, are amplified, and then injected back into the patient to enhance the tumor-killing response. More specifically TIL based cell therapies have proven to be efficacious in recognizing antigenic epitopes on the surface of tumor cells and killing them.[39,40,41] ACT with TILs has been proven to be efficacious against metastatic melanoma.[42] Neoantigen specific T cells were detected in the TILs of patients with metastatic melanoma and neoantigens were identified that increased the response of T lymphocytes in the peripheral blood post-TIL infusion.[33,43] However, the process that goes into amplifying T cells is complex and it is difficult to obtain high affinity TCR^+ T cells and T cells produced *in vitro* do not last for a prolonged period of time following infusion.[33,40,42] In addition, the tissue from which these T cells are extracted and then amplified can lead to variation amongst the types of antigens produced, making it less likely for a shared/public treatment method.[41,42]

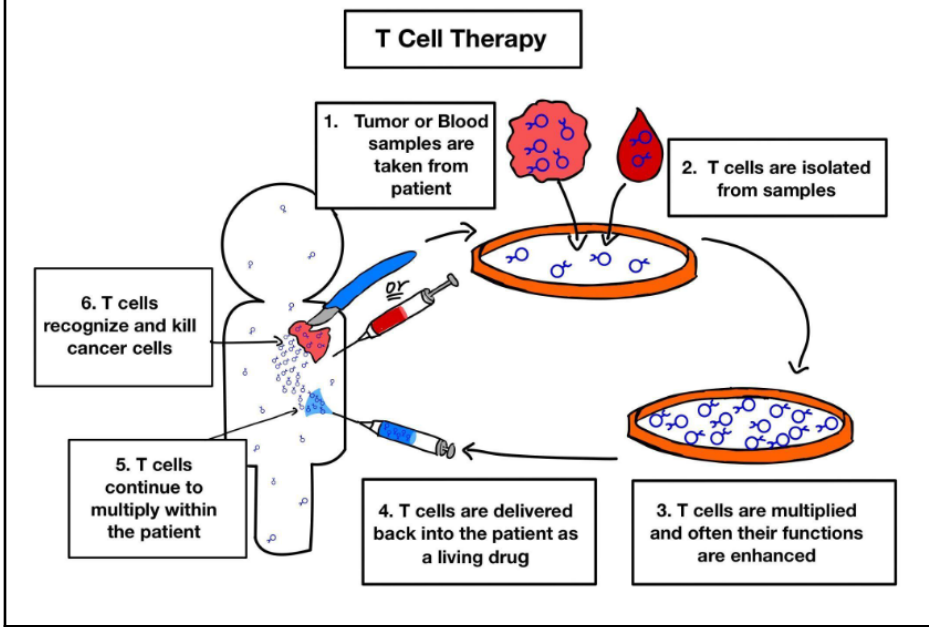

Figure 12. *A sample workflow of T-cell Therapy*

In a Phase III clinical trial NCT05685004 a combination of TVI-Brain-1 immunotherapy and standard therapy compared to standard therapy alone as a treatment for newly diagnosed MGMT unmethylated glioblastoma patients. The general procedures include the collection and testing of cancer tissue samples after surgery and chemoradiation therapy (radiation and temozolomide). For the patients randomized into the investigational study treatment group, they will also receive two vaccinations created from their own cancer cells, undergo leukapheresis to collect immune T-cells from their blood, and transfer of those activated effector T-cells after chemoradiation therapy. All patients are followed with MRIs at follow-up visits. This study acknowledged MGMT promoter methylation changes and investigated whether these changes should be considered in the treatment decision.[44,45]

There is a related therapy called the Chimeric Antigen Receptor (CAR) T cell therapy which utilizes an antibody and T cell receptor complex. CAR can be used to modulate host T cells in order to enhance the cancer killing response of the immune system.[41] CAR T-cell therapy is a type of immunotherapy that uses a patient's own immune cells, called T-cells, to fight cancer. The therapy involves genetically modifying a patient's T-cells in the laboratory to produce chimeric antigen receptors (CARs) on their

surface.[40,41,46] These receptors can then recognize and bind to specific proteins on cancer cells, which helps the T-cells to identify and attack the cancer cells.

However, the use of CAR T-cell therapy for the treatment of solid tumors is still being studied in the early stages of clinical testing. One of the challenges in treating solid tumors with CAR T-cell therapy is that the CAR T-cells need to be able to penetrate the tumor microenvironment and effectively target the cancer cells, which can be difficult to achieve.[41,46]

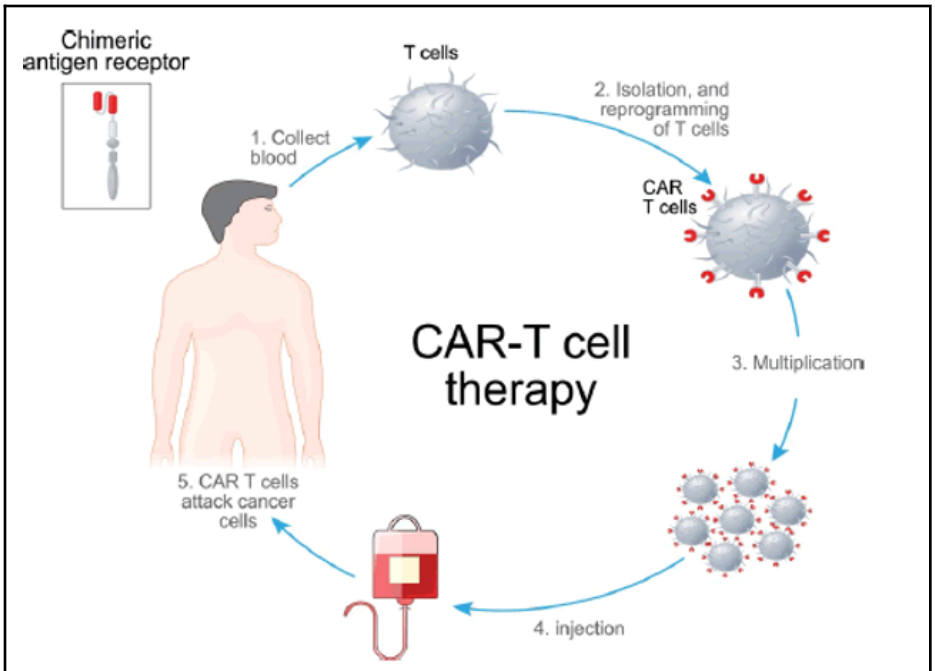

Figure 13. *A simple workflow of CAR-T cell therapy*

In a Phase I Clinical trial NCT04003649, the efficacy of IL13R alpha 2-CAR T cells when given alone or together with nivolumab and ipilimumab and their effects on treating patients with glioblastoma that has come back (recurrent) or does not respond to treatment (refractory). Biological therapies, such as IL13R alpha 2-CAR T cells, use substances made from living organisms that may attack specific glioma cells and stop them from growing or kill them. Immunotherapy with monoclonal antibodies, such as nivolumab and ipilimumab, may help the body's immune system attack the cancer, and may interfere with the ability of tumor cells to grow and spread.[47] It was found that IL13R alpha 2-CAR T

cells enhance anti-tumor activity and T-cell persistence and showed evidence of bioactivity in patients. In addition, it was found that local intracranial delivery of CAR T cells increased anti-tumor efficacy as compared to intravenous administration.[40,47] Overall, this study defined parameters for the clinical translation of CAR T cell therapy for the treatment of brain tumors.

4.7 Combination Therapies with Neoantigen-based Vaccines

The use of tumor vaccines, monoclonal antibodies, and adoptive cell therapies are potential and efficacious treatment methods for Glioblastoma. Specifically looking at tumor vaccines, they have extreme potential when developing personalized treatment methods for patients. Anti-tumor vaccines, such as the proposed neoantigen vaccines, activate certain pathways in the host immune system that can create a susceptible environment for concurrent or subsequent treatment methods.[48,49] This is an important aspect to note when considering neoantigen-based vaccines as a treatment method for Glioblastoma. As shown in previous clinical trials and studies, cancer vaccines are a very innovative way to approach cancer treatment but they have very low efficacy, with an objective clinical response rate of only >7% and an overall rate of clinical benefit of only ~20%.[49,50] There is no question that cancer vaccines stimulate and modulate the immune system, and this can be taken advantage of when treating patients. Utilizing neoantigen-based vaccines can prime the host immune system to be more responsive to subsequent therapies that can enhance the tumor-killing response of the host immune system.[48]

In a Phase II clinical trial NCT03047928, the programmed death 1 (PD-1) regulatory antibody Nivolumab and a peptide vaccine consisting of programmed death ligand 1 (PD-L1) and Indoleamine 2,3-dioxygenase (IDO) peptides were tested in patients with metastatic melanoma.[51] The primary endpoints for this study was feasibility and safety of the combined treatment. The secondary endpoints were efficacy and immunogenicity. Overall, the study revealed that the vaccine-reactive T cells comprised CD4+ and CD8+ T cells with activity against IDO- and PD-L1-expressing cancer and immune cells, thus increasing the immunomodulatory effect of the combination therapy.[51,52]

There are many other clinical trials currently being conducted to understand the use of combination therapies as a treatment method for different types of cancers including Glioblastoma. Overall, while further research is needed to fully understand the potential of neoantigen vaccine combination therapies in glioblastoma, early results suggest that these approaches may hold promise as a way to improve outcomes for patients with GBM.

5. Practical Considerations

Glioblastoma is a type of brain cancer that is difficult to treat. Novel neoantigen-based treatment methods are being explored as a potential solution to this problem. Neoantigens are unique proteins that are expressed on the surface of cancer cells, but not on healthy cells, making them a promising target for cancer treatment.[53] Here are some practical considerations when exploring novel neoantigen-based treatment methods for glioblastoma:

Identification of neoantigens: The first step is to identify the neoantigens that are present in the glioblastoma cells. This can be done through genomic analysis, transcriptomic analysis, and/or proteomic analysis. The identification of neoantigens is critical for developing a personalized treatment plan.

Personalized treatment plans: Because each patient's glioblastoma is unique, a personalized treatment plan is necessary. This involves identifying the patient's specific neoantigens and developing a treatment plan that targets those specific neoantigens.

Delivery methods: One of the challenges of neoantigen-based treatment methods is delivering the treatment to the brain. Traditional delivery methods, such as intravenous injections, are not effective for brain tumors. Therefore, new delivery methods, such as direct injection into the brain, are being explored.

Immunogenicity: It is important to ensure that the neoantigen-based treatment method is immunogenic, meaning that

it stimulates an immune response. This is critical for the treatment to be effective.

Combination therapies: Neoantigen-based treatment methods may be more effective when used in combination with other therapies, such as chemotherapy or radiation therapy. Therefore, the potential for combination therapies should be considered.

Clinical trials: Clinical trials are necessary to test the safety and efficacy of neoantigen-based treatment methods. These trials should be designed to address the specific challenges associated with treating glioblastoma, such as delivery to the brain and the development of personalized treatment plans.

Overall, exploring novel neoantigen-based treatment methods for glioblastoma requires careful consideration of several practical factors, including identification of neoantigens, personalized treatment plans, delivery methods, immunogenicity, combination therapies, and clinical trials.

6. Conclusion

Glioblastoma remains a formidable challenge in the field of oncology due to its aggressive nature and limited treatment options. However, advancements in immunotherapeutic approaches, such as neoantigen-based vaccines, immune checkpoint blockers, and adoptive cellular therapies, have demonstrated promising efficacy in repressing glioblastoma tumor cells. Our comprehensive review of scientific literature and clinical trials has shed light on the potential of these treatment methods and identified areas for improvement to further enhance their effectiveness.

Neoantigen-based vaccines have shown promise in priming the immune system to recognize and attack cancer cells displaying unique neoantigens. Protein-peptide vaccines have been utilized to stimulate a specific cytotoxic T-lymphocyte (CTL) response against tumor cells expressing targeted neoantigens. The clinical trials conducted by Hilf et al. and Keskin et al. demonstrated increased overall survival and progression-free survival in

patients who received neoantigen-based vaccines, albeit with some side effects. Ongoing trials, such as the Reardon et al. trial, are investigating the combination of neoantigen-based vaccines with other therapies to assess their synergistic effects.

Nucleic acid vaccines, including DNA and mRNA vaccines, have also emerged as potential treatment methods. DNA vaccines introduce a gene encoding antigens using bacterial plasmids as vectors, while mRNA vaccines deliver the antigen-encoding genetic information directly to antigen-presenting cells. Clinical trials led by Johanns et al. and Sayour et al. are evaluating the safety, feasibility, and immunogenicity of personalized neoantigen-based DNA and mRNA vaccines in glioblastoma patients, respectively. Combination therapies with DNA vaccines, such as the trial by Johanns et al., show promise in enhancing immunogenicity and improving patient outcomes.

Autologous dendritic cell (DC) vaccines aim to stimulate a specific CTL response against cancer cells by presenting neoantigens or tumor-associated antigens to the immune system. The Neo-MoDC vaccine demonstrated immunogenicity and induced tumor regression in a patient with advanced metastatic gastric cancer when administered alongside nivolumab. The findings suggest the potential of autologous DC vaccines in treating glioblastoma, but further research is needed to optimize their efficacy and explore combination strategies.

In addition to vaccine methods, immune checkpoint blockade therapy, particularly through the use of monoclonal antibodies, has shown promising results in the treatment of glioblastoma and other cancers. By targeting key signaling molecules such as PD-1, PD-L1, and CTLA-4, immune checkpoint inhibitors can suppress immunosuppression and restore anti-tumor responses, leading to improved outcomes for patients. Monoclonal antibodies provide a highly specific and effective way to target disease-causing proteins or antigens. By utilizing their ability to recognize and bind to specific targets, monoclonal antibodies can disrupt harmful pathways and stimulate the immune response against cancer cells. Various monoclonal antibodies, including nivolumab, ipilimumab, avelumab, and

atezolizumab, have shown efficacy in glioblastoma and other cancer types. Combination therapies, such as the concurrent use of nivolumab and ipilimumab, have demonstrated enhanced T-cell function and significant antitumor responses. These combinations target multiple checkpoints simultaneously, leading to more effective immune responses against cancer cells.

Adoptive cell therapies, such as TIL-based cell therapies and CAR T-cell therapy, offer another avenue for glioblastoma treatment. TIL-based cell therapies can recognize and kill tumor cells, while CAR T-cell therapy genetically modifies a patient's T-cells to enhance their ability to target and attack cancer cells. However, further research is needed to optimize these therapies for solid tumors like glioblastoma.

To further advance glioblastoma treatment, several areas of improvement should be considered. First, optimization of sequencing methods, such as whole-genome sequencing (WGS), whole-exome sequencing (WES), and next-generation sequencing (NGS), can enhance the identification of neoantigens and somatic mutations efficiently in terms of time and cost. Additionally, strategies to overcome immune evasion mechanisms employed by glioblastoma, such as upregulation of immune checkpoint molecules, should be explored. Combination therapies involving neoantigen-based vaccines, immune checkpoint blockers, and adoptive cellular therapies hold promise in overcoming resistance and enhancing treatment responses.

In conclusion, the development and utilization of neoantigen-based vaccines, immune checkpoint blockers, and adoptive cellular therapies have opened new avenues for glioblastoma treatment. While these treatment methods have demonstrated efficacy, ongoing research and improvements in sequencing techniques, combination therapies, and immunomodulatory strategies are crucial for achieving further breakthroughs. The integration of personalized medicine approaches and a deeper understanding of the complex interplay between the tumor microenvironment and the immune system will contribute to the development of more effective and targeted therapies for glioblastoma patients, ultimately improving their prognosis and quality of life.

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