An Innovative Immunotherapy Vaccine with Combination Checkpoint Blockade as a First Line Treatment for Glioblastoma in the Context of Current Treatments

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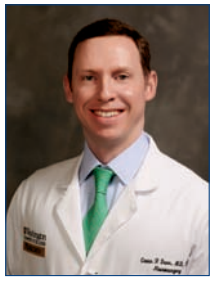
While recent achievements in immuno-oncology have improved the prognoses of many cancer diagnoses, these benefits have not yet translated to glioblastoma multiforme.



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Abstract

Glioblastoma is a devastating disease with a dismal prognosis. While recent advancements in cancer immunotherapy have led to improvements in treating other types of cancer, patients with glioblastoma have not benefited from these new therapies and techniques. Fortunately, neurosurgeons and oncologists at Washington University School of Medicine conducting a cutting edge clinical trial are looking to overcome these persistent challenges in treating glioblastoma through combining a personalized vaccine with new immunotherapy drugs.

Introduction

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor and accounts for approximately 17,000 new cases in the United States annually.[1] GBM unfortunately has the worst prognosis of primary gliomas, with a miserable median survival of 12-15 months despite aggressive treatment.[1,2] The current first line standard of care includes maximal surgical resection, adjuvant radiotherapy with concurrent temozolomide chemotherapy following resection, and six months of maintenance temozolomide after concurrent chemoradiotherapy.[1] Tumors in a large majority of patients will recur, and the lack of effective standard second-line options leads to patients rapidly succumbing to the disease; the current 5 year survival rate is less than 10%.[1] Due to the abysmal prognosis associated with GBM, new safe and effective therapies are desperately needed.

Cancer immunotherapy is a new and active area of cancer research, where various therapies are used to evoke an immune response against a tumor. Contemporary cancer immunotherapies include targeting immune checkpoint signaling pathways with inhibitory antibodies, checkpoint blockade immunotherapy (CBI), or priming the immune response with therapeutic vaccines. Therapeutic vaccinations aim to prime the immune response against tumor antigens, which can include shared tumor antigens and/or personalized tumor-specific antigens, called neoantigens. While other therapies, including cellular therapies such as chimeric antigen receptor (CAR) T cells have shown positive data in other cancer types, this review will focus on past applications of CBI and therapeutic vaccines. We will also present a pioneering clinical trial that combines a personalized therapeutic vaccine with CBI.

Checkpoint Blockade Immunotherapy in Glioblastoma

Landmark discoveries in checkpoint inhibition have revolutionized oncology treatment options for previously devastating diagnoses, resulting in a well-deserved Nobel Prize. Two major immunotherapy targets are the negative immune regulatory checkpoint proteins cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and Programmed cell death protein 1 (PD-1) or its ligand, PD-L1. Both CTLA-4 and PD-1/PD-L1 are coreceptor molecules on the surface of T-cells that inhibit T cell function and play key roles in guarding against autoimmunity.[3] The CTLA-4 pathway regulates T-cell proliferation and priming in the lymph nodes, while the PD-1/PD-L1 pathway regulates T cell response in the tissues later in the immune response.[4] CBIs targeting these pathways can improve the anti-tumor immune response.

CBI has shown efficacy in preclinical orthotopic transplantable GBM mouse models, such as GL261 and SMA-560. Mice with intracranially implanted GL261 tumors show a survival benefit when treated with either anti PD-1, anti PD-L1, or anti CLTA-4 treatment.[5,6] These effects of anti PD-1 or CTLA-4 therapy in GL261 are synergistic when combined with radiotherapy.[7,8] Anti CTLA-4 treatment also confers a survival benefit in mice with intracranially implanted SMA-560 GBM cell line tumors.[9]

Both anti CTLA-4 and anti PD-1/PD-L1 CBI antibodies have been FDA approved or have shown preliminary success in melanoma, squamous and non-squamous non-small cell lung cancer, small cell lung cancer, metastatic renal cell carcinoma, urothelial cancers, head and neck squamous cell carcinoma, and colorectal cancer.[10] The immune system can apparently control tumors in many environments as shown by the success of CBI in multiple organ systems; ongoing clinical trials are investigating the efficacy of CBI in other systems.[10]

Due to the success of CBIs in other cancer types, many clinical trials for CBI in newly diagnosed and recurrent GBM are currently underway, but none have reported convincing positive results in large patient cohorts. The Checkmate 498 open label trial for patients with newly diagnosed GBM and an unmethylated MGMT promoter comparing nivolumab (anti PD-1) combined with radiotherapy against standard of care temozolomide with radiotherapy did not meet the primary endpoint of overall survival.[11] The ongoing sister phase III Checkmate 548 (NCT02667587) trial for patients with newly diagnosed GBM and methylated MGMT will compare nivolumab versus placebo combined with standard radiotherapy plus temozolomide. While a phase II trial comparing pembrolizumab (anti PD-1) against concurrent pembrolizumab and bevacizumab appeared safe in both cohorts, it also showed minimal anti-tumor activity in the pembrolizumab only cohort, and combination therapy did not show improved outcome compared to historical bevacizumab controls.[12] A phase Ib trial of pembrolizumab in 25 patients with recurrent PD-1 positive GBM reported 1 partial response and 12 patients with stable disease.[13] In a trial of patients with recurrent GBM, neoadjuvant (pre-reresection) combined with adjuvant pembrolizumab conferred a survival benefit over adjuvant pembrolizumab alone.[14] The results from this neoadjuvant administration study suggest that a strong priming response associated with increased tumor antigen exposure may lead to a better anti-tumor response later. A trial treating 6 recurrent GBM patients with pembrolizumab and bevacizumab reported no dose-limiting toxicities and clinical benefit in 3 of 6 patients.[15,16]

In addition to CBI monotherapy, ongoing clinical trials for both newly diagnosed and recurrent GBM are investigating combination CBI of anti CTLA-4 and anti PD-1. Results from these trials demonstrate that CBI is safe and tolerable in these populations, but do not show a survival benefit. Results from the Checkmate-143 phase I study comparing nivolumab (anti PD-1) alone or in combination with ipilimumab (anti CTLA-4) in patients with recurrent GBM showed no grade 3 or greater SAEs out of 10 patients treated with nivolumab monotherapy (3 mg/kg every 2 weeks).[17] Only 2 of 20 patients in the Checkmate 143 trial treated with nivolumab at 3 mg/kg plus ipilimumab at 1 mg/kg every 3 weeks for 4 doses followed by maintenance nivolumab at 3 mg/kg every 2 weeks experienced grade 3 or greater SAEs.[17] A lower initial 4 doses of nivolumab at 1 mg/kg with higher ipilimumab at 3 mg/kg led to a larger (7/10) proportion of SAEs without a dramatic increase in treatment response.[17] Furthermore, cohort 2 of the Checkmate 143 phase III study, which randomized GBM patients to nivolumab monotherapy or bevacizumab monotherapy, did not show any difference in median overall survival, 12 month overall survival, or progression free survival.[17]

Although the completed trials investigating CBIs in patients with GBM have established a safe treatment protocol, they have not shown a survival benefit compared to standard therapy. The GBM microenvironment and standard of care treatment conditions cause immunosuppression, and the low mutational burden does

not create an immunogenic environment present in other tumors.[18,19] The challenging tumor biology along with past negative trial results suggest that combination therapies are likely necessary to see a clinical benefit.

Therapeutic Neoantigen Vaccination in Glioblastoma

Tumor antigens distinguish tumors from normal tissue and serve as potential targets for immune system neutralization. Shared tumor antigens and neoantigens are both subsets of tumor antigens. Normal tissues or limited subsets of normal tissues express low levels of shared tumor antigens, but tumors overexpress these shared antigens. Examples include MAGE in melanoma[20] and HER2/neu in breast cancer.[21] Tumor-specific antigens, or neoantigens, are the result of somatic mutations only present in the tumor.[22] Therefore, while tumor cells may express neoantigens, normal tissue does not due to a lack of somatic mutations present. The first neoantigen described was mutated cyclin-dependent kinase 4 in melanoma.[23] Other groups have since described neoantigens in multiple other types of cancer.[24,25]

Because they are targets for immune cells, vaccinating a patient against their own tumor antigens could stimulate an anti-tumor immune response. The obvious benefit to using neoantigens over shared tumor antigens is that they are unique to the tumor, therefore mitigating autoimmune effects. Phase II clinical trials in patients with GBM using rindopepimut, a vaccine against the neoantigen EGFRvIII, combined with standard temozolomide and radiation showed benefits in overall survival and progression free survival over historical controls.[26–28] However, a phase III trial of rindopepimut with temozolomide failed to show a survival benefit.[29] Additionally, EGFRvIII is only expressed in 20-30% of GBMs, so most patients would not be candidates for this therapy.[30] While disappointing, these results warrant investigation into combining neoantigen vaccines with additional immunotherapy such as CBI. Within the past year, several publications show that personalized neoantigen vaccine therapies for glioblastoma are feasible, generate neoantigen specific T cells that infiltrate the tumor, and have favorable safety profiles.[31–33] A predictive approach to identify immunogenic neoantigens is necessary to streamline any potential therapy that would benefit more than a small subset of patients.

The Future of Brain Tumor Immunotherapy

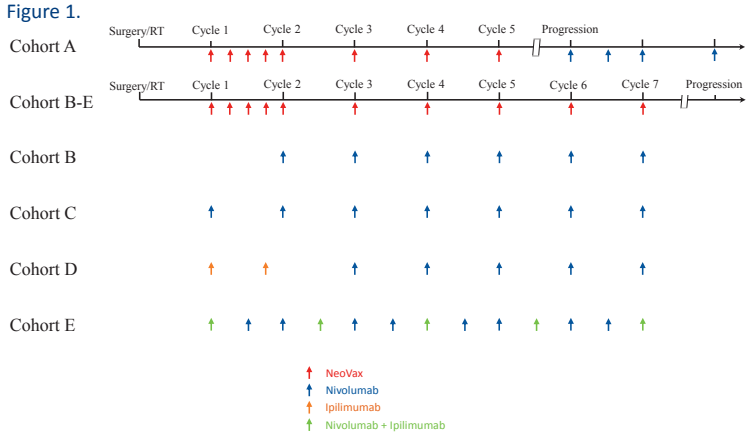
Given the favorable safety profile and different mechanisms of immune activation, combining CBI with neoantigen vaccines makes therapeutic sense. These two approaches could synergistically increase the anti-tumor immune response, using CBI to overcome the problem of immunosuppression and using neoantigen vaccination to counteract the poor immunogenicity of the tumor. Blocking CTLA-4 and PD-1 with CBIs potentially enhances anti-tumor neoantigen-specific T cell responses in melanoma[34] and NSCLC respectively.[35]

An innovative clinical trial (NCT03422094) currently recruiting patients at Washington University School of Medicine (WUSM) combines a state-of-the-art personalized neoantigen vaccine (NeoVax) with different CBIs, specifically nivolumab (anti PD-1) and ipilimumab (anti CTLA-4). Eligible patients must have newly diagnosed GBM with an unmethylated MGMT promoter. Patients who enroll in the trial receive NeoVax and their designated nivolumab or ipilimumab infusions at Siteman Cancer Center in St. Louis. This trial seeks to investigate how the timing of CBI administration combined with a personalized neoantigen vaccine affects the clinical and immunological response. Because CTLA-4 has a role in early priming and PD-1 in later local tissue response, sequentially administering different CBIs which target these separate pathways could synergistically boost the anti-tumor immune response.

Preclinical data has shown a synergistic effect of combining a vaccine with either anti PD-1[36] or anti CTLA-4[37] in mice with intracranially implanted GL261 tumors. Multiple clinical trials in various cancer types have investigated concurrent tumor antigen vaccination with checkpoint blockade monotherapy without an increase in adverse events over CBI alone.[38] These results indicate that combining a vaccine with CBIs should be safe for patients with GBM.

Creating a neoantigen vaccine specific for each tumor requires a robust, accurate, and cost-effective pipeline. Fortunately, investigators at the Siteman Cancer Center and the McDonnell Genome Institute at WUSM have developed an immunogenomics approach to identify patient neoantigens for a vaccine. Compared to cancer genome sequencing, identifying neoantigens with tumor versus normal tissue exome sequencing is robust, accurate, and conserves resources.[39] To ensure that the tumor expresses enough of the identified mutant neoantigens, RNA sequencing will measure tumor mRNA mutant allele expression levels of neoantigens identified by tumor/normal exome sequencing. An in silico prioritization algorithm then filters the mutant neoantigens to identify the top candidates that have high HLA class I or class II allele epitope binding affinity for presentation to the patient T

Figure 1.



Study schema of "A Pilot Study to Assess the Safety, Feasibility, and Immunogenicity of a Neoantigen-based Personalized Vaccine Combined with Immune Checkpoint Blockade Therapy in Patients with Newly Diagnosed, Unmethylated Glioblastoma" (NCT03422094). Note that patients in cohorts B-E will all receive the same NeoVax schedule in addition to CBI.

cells. Scientists then manufacture a vaccine against the prioritized neoantigens with a synthetic long peptide vaccine combined with a poly-ICLC adjuvant. In preclinical studies, our group successfully predicted and screened neoantigens from the GL261 and SMA-560 murine GBM models using this screening strategy.[40]

Patients who have a potential GBM and want to participate in the trial can receive surgery and treatment from experts at Barnes Jewish Hospital and Siteman Cancer Center. After surgery, patients receive the standard course radiotherapy (60 Gy over 30 treatments, Monday through Friday for 6 weeks) without temozolomide. Temozolomide is excluded due to the immunosuppressive effects and lack of efficacy in MGMT unmethylated GBM, a similar strategy adopted in Checkmate 498 to avoid the immunosuppressive effects of chemotherapy. During radiotherapy, scientists and physicians at WUSM analyze the tumor and generate a personalized neoantigen vaccine as described above. After radiotherapy, patients enter the vaccine priming phase where they receive their unique NeoVax treatment weekly for 4 weeks. After priming, patients then move to the boosting phase, where they receive NeoVax once every 4 weeks. While the NeoVax schedule is the same for all patients, the schedule for CBIs differs among the cohorts. Patients in cohort A will receive only NeoVax until progression, when they begin nivolumab infusions every other week. Patients in cohort B receive only NeoVax during the priming phase, and then receive nivolumab and NeoVax during the boosting phase. Patients in cohort C receive nivolumab and NeoVax during the priming and boosting phases. Patients in cohort D receive ipilimumab and NeoVax during the priming phase, and then switch to nivolumab and NeoVax during the boosting phase. Patients in cohort E receive all 3: nivolumab, ipilimumab, and NeoVax during both the priming and boosting phases. Figure 1 depicts the administration schedule for the NeoVax trial.

To date, only 8 other trials across various cancer types are recruiting patients for clinical trials involving a therapeutic vaccine and combination CBI. Some of the ongoing trials will begin reporting results next year. The NeoVax trial at WUSM is the only enrolling trial administering a therapeutic vaccine with combination checkpoint blockade immunotherapy for patients with glioblastoma. Our group will soon open a new clinical trial (NCT04015700) investigating a therapeutic DNA vaccine with combination CBI.

Conclusion

GBM is a uniformly lethal disease. While recent achievements in immuno-oncology have improved the prognoses of many cancer diagnoses, these benefits have not yet translated to GBM. These setbacks are likely due to the unique and immunosuppressive nature of GBM, which will require ingenuity to overcome. Despite these roadblocks, trailblazing new treatments like NeoVax and combination checkpoint blockade at Washington University are giving hope to patients with GBM and shaping the future of cancer treatment.

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Disclosure

GPD is a co-founder and Chief Scientific Officer of Immunovalent Therapeutics, Inc. TMJ has served on scientific advisory boards for Geneos Therapeutics and NovoCure. **MM**