Immune Landscape of the CNS and Glioblastoma

Kristine Dziurzynski, M.D.

Penn Medicine Lancaster General Health Physicians
NeuroScience & Spine Associates

Second in a series

Editor's Note: *The first article in this series, Advancement of Therapy for Glioblastoma, appeared in the Spring 2020 issue of this Journal. The third article will focus on advances in surgical technology and approaches. The final article will be on the application of these advances at Lancaster General Hospital.*

Though this article is focused on immune therapy for glioblastoma, the mechanisms discussed are important to understand for treatment of many other malignancies.

The central nervous system (CNS) has historically been perceived as immunologically privileged. This perception originated from transplant studies conducted in the 1950s, in which grafted cells and tissues were accepted when implanted in the brains of rodents, but rejected outside the CNS. [1,2]

Recent studies reveal that CNS immunity is more robust. By the 1980s, antigens and T cells were reported to travel to deep cervical lymph nodes.[3] This finding was further elucidated in 2012. when a glymphatic system was described in the CNS. CSF was identified to flow along arterial perivascular spaces, [4] then translocate into the interstitium through a water channel, and exit along venous perivascular spaces. By 2015, functional lymphatic vessels were described in the meninges that drained into cervical lymph nodes.[5]

In addition to the work uncovering the cerebral lymphatic system, immune cells within the CNS have been further characterized. The principle resident immune cell in the brain is microglia, whose phenotype is subjugated to the type of inflammatory stimulus. Consequently, they will either promote or inhibit an immune response. Elucidating the precise mechanisms by which this occurs remains an active area of research.[6] Finally, the concept that the blood-brain barrier prohibits entry of immune cells has been discredited, as effector T cells have been reported traveling in the CNS.[7]

Pertaining to glioblastoma, however, immune suppression is the hallmark of its microenvironment.[8] Mechanisms that prevent an anti-tumor immune response to glioblastoma include:

- secretion of immunosuppressive cytokines such as IL-10 and TGF-β;
- infiltration of immune suppressive T cells known as Tregs, whose function ordinarily is to modulate the immune response to prevent autoimmunity;
- fewer tumor infiltrating T cells (TILs) compared with other cancers, therefore reducing the cytotoxic response against the tumor;
- T cell exhaustion of those in the tumor;
- immune suppressive macrophages and microglia, whose phenotype had been co-opted by the immune suppressive tumor environment to inhibit an anti-tumor response;
- a paucity of unique tumor proteins that can serve as antigens.

It is against this backdrop that immune therapy for glioblastoma is being investigated. Multiple directions are being explored, from agents that inhibit immune regulators to viral and vaccine strategies.

CURRENT IMMUNOTHERAPIES UNDER INVESTIGATION

Immune checkpoint inhibitors

Immune checkpoint inhibition is a class of immune therapy that is now included as part of the standard of care for treating many types of cancer. The most success has been seen in melanoma, but this modality has also been used to treat lung, breast, and even kidney cancer.

These agents work by direct inhibition of the receptors and ligands comprising physiologic regulatory check points that stop or inhibit an immune response, or prevent autoimmunity. They include inhibitors to cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1), and programmed

cell death ligand 1 (PD-L1), which are all expressed on immune cells. Once these receptors are engaged, the T cell is no longer able to carry out a cytotoxic response. These agents are all monoclonal antibodies that prevent binding of their respective ligands or to their respective receptor, thus enabling the T cell to become or remain activated.

Nivolumab, the anti PD-1 antibody drug, has been studied the most in glioblastoma, frequently in the setting of recurrent disease. This series of clinical trials is collectively referred to as the "Checkmate" studies. The first study to report results was Checkmate 143, which showed no improvement in overall survival.[9] A principle challenge to the success of these agents is the fact that many patients with glioblastomas require corticosteroids for symptom control throughout the course of their illness. Corticosteroids are a potent immune suppressive agent, and those patients who received more than a certain dose of steroids during the Checkmate 143 study performed worse than those who received a low dose or none.

Other studies are under way that utilize these agents in combination with other drugs, and in newly diagnosed patients versus those with recurrent disease. One of these trials employing a combination of immune checkpoint inhibitors is currently enrolling newly diagnosed glioblastoma patients at Lancaster General Hospital's Ann B Barshinger Cancer Center. The current hypothesis being tested is that these drugs will prove useful as part of combination therapy.

Vaccine Therapies

The concept behind vaccine therapy for glioblastoma involves exposing a patient's immune system to a tumor protein that serves as an antigen to trigger a cytotoxic T cell anti-tumor response. Creative strategies have been adopted to develop this approach. Thus far, no ubiquitous glioblastoma antigen has been identified, due to the glioblastoma's heterogeneous genomic and polyclonal make up.

Dendritic Cell Vaccines

Dendritic cells (DCs) are recognized as the principle immune cell to initiate an immune response. Two clinical trials using dendritic cells primed with glioblastoma antigens are presented here

1. DCVax is being evaluated in a Phase III clinical trial, in which each patient with glioblastoma undergoes surgery for tumor removal, and the remaining excised tissue is preserved for trial use after the pathologist confirms the diagnosis. The patient's own dendritic cells are then harvested and isolated by leukapheresis. These dendritic cells are then pulsed with tumor lysate prepared from the remaining tumor tissue, comprising the vaccine. Now the dendritic cells are considered primed, with a mix of tumor proteins serving as antigens. In theory, the cells will migrate to the lymph nodes draining the vaccine injection site. They will induce an anti-tumor immune response by contact with other antigen-presenting cells, and initiate maturation of effector T cells. The vaccine is administered by intradermal injection into the upper arm after patients undergo standard of care chemotherapy and radiation following their surgery.

Interim results from this trial reported a median overall survival (OS) of 23.1 months,[10] compared with a historically reported range of 14-18 months. Median progression free survival (PFS), the interval from diagnosis to recurrence of disease, was not reported.

Despite the reported improvement in OS for the study's patient population, there were many criticisms of this report. The PFS should have been included; of all patients screened for eligibility, only 21% were enrolled; there was a high crossover rate into the treatment group; and patient characteristics were not reported for each arm of the study (those receiving vaccine vs. placebo).[11] This trial is now closed, and final analysis remains pending at the time of this publication.

2. A second dendritic cell vaccine trial in earlier phases of investigation uses DCs pulsed with the RNA of the cytomegalovirus (CMV) pp65 antigen. It is based on the controversial premise that CMV antigens are identified in over 90% of glioblastomas, without involvement of surrounding normal brain tissue.[12,13] However, once early Phase I data were reported in 2015, this controversy diminished. The focus is now on the trial results presented below.

To improve vaccine efficacy, the investigators postulated that if the draining lymph node at the planned vaccine injection site is conditioned with a strong recall antigen (one the body has seen previously and already possesses memory cells), it will improve homing of those dendritic cells primed with tumor antigens to those lymph nodes, and chances of initiating an anti-tumor immune response would be improved.[14]

Twelve patients were enrolled and randomized into two groups. After receiving the standard of care, they all received an intradermal injection of

the vaccine into the groin. In one of the randomized groups, the groin was primed with tetanus/diptheria (Td) toxoid, while the other group was primed with a placebo injection in the groin. The group of six patients that received vaccine with placebo priming demonstrated a median PFS of 10.8 and OS of 18.5 months, consistent with matched historical controls of patients treated with only standard of care. Among the group primed with Td toxoid, three of the six were still alive 36.6 months later. Thus, median PFS and OS for this group still remain to be determined. A Phase II trial is ongoing.

Another Phase I trial using the DC pp65 vaccine admixed with granulocyte-monocyte colony stimulating factor (GM- CSF) also reported significantly improved median PFS and OS compared with historical matched controls: 25.3 vs. 8 month PFS, and 41.1 vs. 19.2 month OS.[15] This trial has also moved to Phase II.

Peptide Vaccines

A peptide vaccine for glioblastoma consists of synthetic tumor proteins designed from genetic analysis of an individual patient's tumor. Two trials underway in the United States and Europe include detailing individual immune response to help tailor this vaccine design for efficacy in future trials. Again, timing of vaccine administration was after standard-of-care surgical resection, chemotherapy, and radiation.

The U.S. trial is targeted specifically to patients with a known limited response to temozolomide, the only chemotherapy used to treat glioblastoma up-front. These patients express activity of a DNA repair enzyme that counteracts the chemotherapy-induced DNA damage. They are identified at the time of diagnosis, as testing for this enzyme's activity is a component of the tumor's molecular profile. In this trial, patient-specific tumor protein coding mutations are made, to be used as the antigen to provoke an immune response.

The median PFS and OS were reported as 7.6 and 16.8 months for 10 patients.[16] Importantly, this study objectively demonstrated the deleterious effects of steroids on the robustness of the immune response. Data were provided showing these patients were unable to generate interferon γ, an essential cytokine to propagate an immune response. The use of steroids to manage brain edema not only from the tumor, but from treatment with radiation and chemotherapy, is ubiquitous throughout the course of care

for glioblastoma patients. It represents a significant barrier to be overcome prior to seeing greater success with immunotherapy. (We described this issue earlier as confounding success with check point inhibitors.)

The Glioma Actively Personalized Vaccine Consortium (GAPVAC) is a large multi-center trial being conducted throughout Europe to vaccinate patients with a combination of non-mutated and mutated tumor antigens. A two-phase vaccine strategy was tested where patients would initially receive vaccination with the unmutated tumor antigen followed by a second vaccination with mutated tumor antigen. Immune priming with polyriboinosinic-polyribocytidylic acid poly L-lysine carboxymethylcellulose (Poly-ICLC) and GM-CSF was utilized. Among the 15 patients vaccinated, a median PFS of 14.2 and OS of 29 months were reported.[17] Based upon these results, a Phase II trial is underway.

Overall, these vaccines are considered safe in comparison with other treatments. Of the studies presented here, one patient developed a severe reaction to administration of GM-CSF, three developed brain edema requiring steroids, and two experienced anaphylaxis after receiving APVAC, believed related to the GM-CSF. Otherwise no other significant toxicities were reported.

Viral Therapies

Initial studies using viruses to treat tumors focused on a strategy to infect the tumor cell with a virus engineered so it cannot replicate. Tumor cell death results either from viral infection, or delivery of a lethal gene. Success was limited, but since anti-tumor immune responses were observed in these studies, they were redesigned to include stimulation of a downstream immune response.

The most widely recognized of this next generation of brain tumor viral therapies is the polio-rhinovirus chimera, PVSRIPO, which is a re-engineered form of the live-attenuated Sabin type 1 oral poliovirus. The re-engineering prevents viral spread throughout healthy neural tissues. The receptor entry site for polio virus is CD155, which is expressed at extremely low levels or not at all in most cells of the central nervous system, but is overexpressed in malignant gliomas.[18]

Pre-clinical studies have shown this viral therapy has a two-fold effect. It is taken up by tumor cells expressing CD155, resulting in cell death. Since CD155 is also expressed in dendritic cells and macrophages, which will be traveling through a malignant

glioma, once PVSRIPO is taken up by this population of cells they incur a chronic sub-lethal infection with the re-engineered virus. Consequently, anti-viral interferon responses are activated, generating an immune response consisting of cytotoxic T cell activation, believed to also target the tumor.[19]

In 2018, results from the Phase I clinical trial conducted among patients with recurrent glioblastoma were reported in the *New England Journal of Medicine*.[20] PVSRIPO was delivered by convection enhanced delivery, in which the recombinant virus is directly infused into the tumor cavity through a specialized catheter, while patients are in the Neuro Intensive Care Unit. The catheter was placed at the time a biopsy was done to verify recurrent disease. Varying and escalating doses were used for each patient enrolled, to determine the maximum tolerable dose and safety profile.

A total of 61 patients were enrolled. Overall survival among the patients who received PVSRIPO was 21% at 36 months. Two patients remained alive 5 years after administration. 19% of the patients had a PVSRIPO-related adverse event of grade 3 or higher. (Adverse events are graded 1-5, with 1 representing a mild side effect and 5 subsequent death; Grade 3 is considered severe.) A Phase II trial, designed to assess efficacy, is underway. Recruitment is currently closed; follow up and data analysis are ongoing.

Delta virus is another viral therapy being explored that utilizes the ubiquitous adenovirus, a common cause of respiratory illnesses that is easily modified genetically. 5-Delta 24RGD (DNX-2401) is an adenovirus that has been developed to selectively enter tumor cells through integrins expressed at higher levels in tumor cells but not healthy brain tissue. Upon cellular entry, it replicates using the abnormal function of cell cycle proteins in cancer cells, whereas non-malignant cells have normal function of these proteins (the Rb pathway).

A Phase I clinical trial reported in 2018, conducted among recurrent glioblastoma patients, reported 20% (five of 25) survival beyond 3 years after a single administration of DNX-2401.[21] Three of these five patients had greater than 95% reduction in tumor volume that occurred over several months after treatment. A total of 37 patients were enrolled; 25 in a dose-determining response group, and 12 in a group to determine the biologic effects of the viral injection on the tumor and microenvironment

All patients underwent stereotactic biopsy to verify recurrent disease, and to implant a specialized catheter for virus delivery. 72% of patients in the dose determining response group demonstrated reduction in tumor size, with median survival of 9.5 months. The patients in the other group had surgery two weeks after injection, to resect the tumor for study. Those patients in the group that underwent surgery cannot be adequately compared with the former group which did not have their recurrent tumor removed. Persistent immune responses were identified in both pre-clinical and post-mortem studies. Only two patients developed an adverse event, none higher than grade 2.

Adoptive T cell therapy

A chimeric antigen receptor (CAR) is a re-engineered protein containing the antigen receptor domain of an antibody, combined with the T cell activation domain from a T cell receptor. CAR T cell therapy has shown the most success in treating B cell lymphoma, targeting CD19, the biomarker for B cells. Here, survival rates at 76% were seen at one year and even some patients surviving up to 4 years without additional treatment.[22]

Two antigens are being studied as targets for CAR T therapy: IL13 Receptor alpha 2 (IL13Rα2) and Epidermal Growth Factor Receptor variant three (EGFRvlll). The former is a normal cellular protein overexpressed in glioblastoma as well as in other cancers; the latter is a mutated protein uniquely expressed only in glioblastoma, and constitutively active to help drive tumor cell growth. Both agents are in early stages of clinical trials. Case reports and early publication have demonstrated clear clinical and radiographic responses for a portion of this small number of patients,[23] in addition to proof of concept.[24]

Although sound in principle and demonstrating solid early results, this type of therapy faces many challenges. Glioblastoma does not possess a universal biomarker antigen, nor does it consist of a monoclonal population of cells as in lymphoma. Neither of the two antigens under investigation are universally expressed in a patient's tumor, rendering subsets of patients ineligible from the onset. IL13Ra2 has been reported to be expressed in up to 75% of glioblastomas,[25] and EGVRvIII in approximately 33%.[26]

Furthermore, there is concern that the immune suppressive microenvironment can overcome activation and effectiveness of the CAR T cell. To address this concern, an immune check point inhibitor has been added to ongoing clinical trials, but no results have been reported to date.

Finally, the generation of CAR T cells involves sophisticated, costly technology and is labor intensive. After diagnosis and surgery, a patient first undergoes leukapheresis, whereby their T cells are removed from their body. Next, these harvested T cells are genetically re-engineered to express the CAR, expanded in culture, then re-infused into the patient. Some cost estimates are around one million dollars per patient.[22] Despite these challenges, interest persists in this therapy owing to the significant impact on survival seen in patients with lymphoma.

CONCLUSION

Therapy for glioblastoma is limited. Although immune therapy has yet to demonstrate meaningful prolongation of survival across the entire glioblastoma population, the dramatic responses seen in a select cohort provide an important opportunity. Studies to elucidate the mechanisms that render an individual a responder will result in knowledge to guide the design of future therapies and their trials. Glioblastoma is a complex, horrible disease where much remains to be learned to understand it, and even more to overcome it.

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Kristine Dziurzynski, M.D.
LG Health Physicians NeuroScience & Spine Associates
1671 Crooked Oak DriveLancaster, PA 17601
(717) 569-5331
Kristine.Dziurzynski@pennmedicine.upenn.edu