Fire in the Smoke: Battling Brain Tumors

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Editor’s Note: Therapeutic vaccines, drugs, and modified human cells that activate the immune system against cancer have improved outcomes and prolonged lives in some types of cancer in the past few years. For patients with glioblastoma, the most common primary brain tumor in adults, immunotherapy is still struggling to overcome this lethal malignancy.
It was 20 years ago when someone we will call Mr. H set off on a unique path. He was commuting home from work along his usual route on Interstate 95 when he forgot which exit to take. For the next two hours he wandered through the Baltimore suburbs trying to find his way home. Finally, he gave up and called his wife, who called 911.

At the hospital, brain resonance magnetic imaging (MRI) foretold a future that blended the uncertainty of a life changing event with the sobering clarity of now knowing precisely how soon that life will end. Mr. H was in his late 30s and was otherwise healthy, exercising two or three times per week and watching what he ate, while being generally content in his career and the time he spent at home with his wife and two young children. Now, fate had brought him face-to-face with glioblastoma, a deadly form of brain cancer with no cure and a life expectancy of less than two years.

After being rushed into surgery, he awoke to a cacophony of monitors, IV pumps, and conversations full of unfamiliar abbreviations and numbers without units of measure. Pathology had confirmed the diagnosis of glioblastoma multiforme (GBM). He spent the next three days in the hospital recovering and was discharged home to a familiar life that was now anything but familiar. Two weeks later he spiked a fever and noticed redness around the c-shaped incision on his head. Back at the hospital, laboratory results and imaging confirmed that he had a severe wound infection. At the time of surgery, the infection was so extensive that bone had to be removed and could not be replaced. He was started on IV antibiotics and sent home to recover with a helmet to protect his compromised skull.

Once the infection had cleared, the skull defect was repaired and he went back to the planned course of chemotherapy, radiation, and preparing for his family’s future without him. Then the unexpected happened. Months passed, then a year, then two years, with successive MRI scans failing to show any evidence of the tumor returning. Five years later he was in a rare minority: patients who had survived at least five years with GBM. More than two decades later and now in his late 50s, there is still no sign of the tumor that once promised to take his life. His tumor has been studied by the world’s most eminent pathologists and confirmed to be GBM. But if there is nothing
too distinct about this patient or this tumor, what could explain his remarkable clinical course? Could it be the infection?

**A Brief History of Cancer Immune Therapies**

Tumors were first noted in an ancient Egyptian textbook on surgery and medicine. But it wasn't until the 1700s that the dramatic regression of tumors in the presence of an infection was first observed. Scattered reports of this phenomenon were recorded over the next century, and by the mid-1800s such anecdotes led to a few small-scale therapeutic efforts to introduce infection in cancer patients, with limited success.

Purposefully stimulating a patient’s own immune system to fight cancer was first systematically attempted by William B. Coley in the late 1800s. In May 1891, he reviewed the cases that had been reported of patients with infections who had lived longer than expected and concluded that most were sarcoma patients who developed streptococcus infection. Coley injected streptococcal broth cultures in a patient who had a large, recurrent sarcoma of the head and neck. The treatment resulted in a near fatal infection, but the tumor drastically regressed and the patient was once again able to swallow food. According to Coley’s records, the patient would go on to survive for eight years before dying of recurrent disease.¹

More than a century would pass before rigorous study of the immune system yielded clinical therapies capable of reliably generating antitumor responses. This work coalesced into two general lines of research: anticancer therapeutic vaccines that train the immune system to recognize and destroy tumor cells, and immune checkpoint inhibitors that overcome the tumor’s defenses against immune attack.

In 2010, the Food & Drug Administration (FDA) approved the first antitumor therapeutic vaccine for the treatment of castrate-resistant metastatic prostate cancer.² The following year saw FDA approval of the first immune checkpoint inhibitor for the treatment of metastatic or un-resectable melanoma.³ The immunologic strategies exemplified by these agents—stimulating an immune response to a specific cancer antigen or overcoming the tumor’s ability to evade an immune response—have served as the framework for immuno-oncology, forging the way for next
generation immunotherapeutics that have dramatically improved the prognosis for many patients with advanced cancers.

Those with brain tumors, however, have not been among them. Of more than 100 types, the most common malignant brain tumors are gliomas, which arise from the glia, the brain’s supportive cells. Depending on their grade, or degree of differentiation, gliomas can be benign or highly malignant. The most common malignant primary brain tumor among adults is GBM, which is invariably fatal and associated with a median survival of approximately 20 months despite surgery, radiation, and chemotherapy. These and other high-grade gliomas present unique challenges for immunotherapy due to patient, treatment, and tumor intrinsic factors that have thus far limited the effectiveness of immunotherapies.

Recent negative results of large clinical trials have placed researchers at a crossroads: can immunotherapy in fact generate robust, durable responses in brain tumors? The discussion below aims to provide a framework for understanding cancer immunotherapy, highlight how deviations from this framework might explain the resistance of gliomas, and suggest a path forward.

**Initiating an Immune Response: Lessons from Vaccines**

Tumor vaccine development is predicated on many of the same principles that govern vaccine development against infectious pathogens. An antigen (a foreign molecule that induces an immune response) and adjuvant (a substance that enhances that immune response) are introduced. They stimulate immune T cells that recognize that specific antigen undergo clonal expansion. Unlike foreign pathogens, tumors are derived from host tissues and typically express antigens that the immune system recognizes as self. This triggers processes that have evolved to protect the immune system from targeting the body’s own cells (to prevent an “autoimmune” response), resulting in immune tolerance to the tumor rather than immune activation.

In addition, vaccines targeting antigens that are not only on the tumor but are also expressed on normal tissues may generate unacceptable autoimmune side effects. An anticancer vaccine, therefore, must target antigens expressed only on tumor cells (neo-antigens) or on tumor cells as well as expendable normal tissues. The latter strategy, for instance, enables use of a therapeutic
vaccine for treating prostate cancer. This vaccine targets prostatic acid phosphatase (PAP), which is exclusively on prostate tissue. Since this tissue does not serve a vital function, damage by the immune system is well-tolerated.  

Another example of a successful vaccine-type approach is the use of genetically engineered T cells targeting a B cell antigen known as CD19 in the treatment of lymphomas. CD19 is expressed exclusively on B cells and is often over-expressed on lymphoma cells. The treatment eliminates normal as well as cancerous B cells, but the normal cells recover.

Since gliomas are derived from non-expendable cells of the brain (glia), vaccination strategies have primarily targeted neo-antigens and are produced by tumor-specific mutations that are not shared by healthy tissues. The best studied of these, EGFRvIII, is a mutated form of a normal protein known as epithelial growth factor receptor. This mutated protein is expressed on approximately 40 percent of GBMs. The relative lack of expression on normal tissues makes this a promising target for immunotherapy. One EGFRvIII peptide vaccine, rindopepimut, showed promise in phase II clinical trials that included patients who had undergone complete resection of all tissue identified on a preoperative MRI and demonstrated an absence of tumor progression after radiation and chemotherapy. These trials were the basis for a randomized, placebo-controlled trial of the vaccine in patients with newly diagnosed GBM. It was stopped, however, in 2016 when an interim analysis concluded that the primary endpoint of improved overall survival was unlikely to be met. A randomized trial of rindopepimut in combination with bevacizumab (a drug that inhibits development of blood vessels to feed a tumor) for recurrent GBM also failed to meet its primary endpoint of progression-free survival at six months.

A consistent finding in these studies was the absence of EGFRvIII antigen in up to 80 percent of recurrent tumors. While it is possible that recurrent tumors down-regulate EGFRvIII expression, independent of immunologic pressure, it is more likely that the tumor escapes by down-regulating EGFRvIII or expanding tumor cell clones that do not express it. This process is known as immune-editing, a sort of cellular Darwinian process whereby an external pressure (immune response) selectively destroys subtypes of cells within the tumor while allowing resistant cells to continue.
growing unimpeded, resulting in a change in the molecular composition of the tumor so that it is no longer susceptible to destruction by the immune system.

Two additional findings from this work are important lessons moving forward. First, antibodies against EGFRvIII were consistently detected in patients undergoing treatment, but their presence did not predict clinical response. This underscores that not all immune responses are created equal when it comes to fighting cancer. Specifically, even though the immune system recognizes the antigen and produces an antibody, the presence of antibodies does not guarantee tumor regression. Rather, the immune response must be of a specific type directed toward cell lysis, similar to the immune responses to viruses or intracellular bacteria. Accordingly, while a humoral (antibody) response may coincide with a cytotoxic response, antibody titers alone are not a reliable biomarker of antitumor activity.

Second, radiographic tumor responses were observed in patients with recurrent tumors or when a larger volume of residual tumor tissue remained following surgical debulking. There has been an assumption in immune-oncology that if a tumor is immunosuppressive, eliminating the bulk of the tumor prior to initiating immunotherapy will result in a more vigorous immune response. This finding appears to undermine this assumption and may suggest that having more available antigens at the initiation of immunotherapy may be advantageous even in the setting of a higher tumor burden. Although this remains to be proven, we believe that this phenomenon may be mediated by a process known as epitope spreading. Epitope spreading occurs when antigens other than the targeted antigen (in this case EGFRvIII) are recognized by the immune system and an immune response is generated. An immune response is then generated against these “bystander” tumor antigens even if EGFRvIII is no longer present in the tumor.

Breaking Immune Tolerance

Although cancers are derived from healthy tissues, the mutations that drive malignancy result in a molecular signature that distinguishes them from their normal counterparts. These tumor-specific neo-antigens can be recognized by the immune system, resulting in elimination of cancer cells before they organize into a solid tumor. For malignant cells to progress to a tumor, they must usurp the mechanisms that protect healthy tissues against an autoimmune attack. These immunologic
brakes that protect against autoimmunity, known as “checkpoints,” are non-redundant signaling pathways that reduce the degree and duration of immune responses. Clinical development of agents that block these pathways has revolutionized oncology, but an understanding of which patients and cancers will respond to this approach remains elusive.

Two signals are required for an immune T cell to kill a cell with which it comes into contact. The first signal is the T cell recognizing the antigen presented on the surface of the tumor (or healthy) cell. Each T cell recognizes a single cognate antigen. In essence, this is the key that turns on the immune cell’s engine. The second signal is a co-stimulatory molecule that puts the immune cell in drive. Without the second signal the immune cell determines that the cell it has come into contact with is part of normal tissue and should not be destroyed. The first immune checkpoint discovered, CTLA-4, was initially identified based on its similarity to the co-stimulatory molecule CD28. Research demonstrated that CTLA-4 prevents activation of the second signal. This work led to the understanding of immune checkpoints as negative feedback mechanisms that mitigate collateral damage from overly vigorous and/or non-specific inflammatory responses.

With the discovery of several additional immune checkpoints, we now know that these pathways are much more nuanced than simple immunologic on/off switches. Each immune checkpoint has a distinct function and can signal alone or in combination with others. For example, the two most extensively studied immune checkpoints, CTLA-4 and Programmed Death 1 (PD-1), have specific effects on systemic and local immune responses. CTLA-4 is up-regulated upon initial T cell activation in lymph nodes, while PD-1 signaling occurs primarily within peripheral tissues.

In this way, CTLA-4 is roughly analogous to the master switch in a circuit breaker box, while PD-1 turns off the activity in individual circuits. Adding further complexity, PD-1 binds at least two biologically relevant molecules (PD-L1 and PD-L2), and both the location and expression patterns of these molecules may further modulate immune function. Most other immune checkpoints currently under clinical investigation fine-tune the immune response in more subtle ways, by amplifying or dampening the functionality of activated immune cells.
For immune checkpoint blockade to be effective, a baseline immune response must be present. It is no surprise, therefore, that most of the cancers that respond well to these therapies are highly immunogenic (they elicit a strong immune response). PD-1 and CTLA-4 blocking antibodies, for example, are approved for a growing list of solid malignancies, including melanoma, renal cell carcinoma, and non-small cell lung cancer; they can generate objective responses and significantly improve survival in more than 20 percent of patients with advanced cases of these cancers, which carry a grave prognosis and previously had few treatment options.

Other malignancies, however, including GBM, show little or no response to PD-1 or CTLA-4 inhibitors. The reason is unclear and a topic of intense study. PD-L1 expression, mutational burden (a high number of mutations), and DNA repair deficiencies are some characteristics that correlate with responses to checkpoint blockade. Mutational burden and DNA repair deficiency reflect back on the first strategy of immunotherapy illustrated by vaccines—recognition of foreign antigens and initiation of an immune response. Each mutation in a tumor further differentiates tumor cells from their normal counterparts. Therefore, a tumor with a high burden of mutations provides more targets for the immune system, increasing the probability that an immune response will be specific to the tumor and fueling epitope spreading as the immune response evolves.

Unique Challenges

Despite encouraging laboratory data, clinical results with immunotherapy for patients with GBM have generally been disappointing. The largest trial of PD-1 blockade was stopped early when the PD-1 blocker nivolumab failed to show a survival benefit over the angiogenesis drug bevacizumab, which is standard of care for recurrent GBM. Despite the overall negative results, however, in a small subgroup of patients (eight percent) the response was significantly more durable than that observed for bevacizumab. In addition, there have been anecdotal reports of GBM patients, particularly those with tumors that have unusually high mutational burdens, whose response to PD-1 blockade was remarkable. Ultimately, the question is whether the dismal prognosis for GBM patients can be reliably and meaningfully improved with immunotherapy.

These findings indicate that GBM may play by some of the same rules as other tumors that respond favorably to immunotherapy, but if this is the case, why do so few patients benefit? The situation
for GBM patients is dire. They are traveling through one of the remotest regions in medical science, night is falling, and the temperature is rapidly dropping. There is little time for indecision and we, the medical professionals specializing in this disease, are their guides. In this oncologic wilderness, the rare durable responses are like smoke on the horizon of neuro-oncology that keeps us moving forward. But where’s the fire?

Combination immunotherapy is being explored as a means of improving responses in tumors that do not respond well to single immunotherapeutic agents. This “get a bigger hammer” approach may work well in tumors that employ multiple common immunosuppressive pathways. We believe, however, that not all “cold” tumors are the same and that GBM, in particular, should be considered a distinct immunologic entity. Not only does GBM activate multiple immune checkpoint pathways and secrete immunosuppressive cytokines, but its location in the immunologic milieu of the central nervous system (CNS) presents unique challenges for immunotherapy. Furthermore, GBM induces a profound state of systemic immunosuppression infrequently encountered with other tumors. Failure to understand how the immune system interacts with gliomas locally, regionally, and systemically is the most significant impediment to successful implementation of immunotherapy.

Although it has long been known that patients with GBM exhibit signs of immunologic dysfunction, recent work has begun to delve into the underlying mechanisms of immunosuppression and its effect on patient outcomes. A study in 2011 by Stuart Grossman and colleagues showed that GBM patients receiving chemotherapy and radiation experienced profound and prolonged reductions in immune CD4 counts that negatively correlated with survival. One of the unanswered questions from this study is the relative contribution of the disease process vs. side effects of treatment. Nevertheless, the correlation of poor immune function with decreased survival from a tumor that is thought to have little or no immunogenicity is provocative. If there is no immune response to the tumor, why would immune suppression matter? If there is an immune response to GBM, how can we fan the flame? Intrigued by these possibilities, we and others are attacking immunosuppression in GBM on multiple fronts.

Any successful immunotherapy for GBM is likely to be administered in combination with chemotherapy and radiation, both of which are immunosuppressive. We have shown that focal,
single fraction radiation therapy can work synergistically with PD-1 blockade,\textsuperscript{22, 23, 24} and hypothesize that a single-dose regimen may be immunologically superior to standard, fractionated radiation therapy by minimizing exposure to normal tissues and circulating immune cells. Similarly, orally administered temozolamide, a chemotherapy drug that is standard-of-care for newly diagnosed GBM, is profoundly immunosuppressive; when delivered locally however, it mitigates unwanted effects on memory T cell populations and potentiates the efficacy of PD-1 blockade.\textsuperscript{25} We envision a paradigm shift from standard oral chemotherapy and radiation to local chemotherapy and intense, abbreviated radiation therapy, which will minimize immune dysfunction and may prime an antitumor response by increasing the availability of tumor-associated antigens.

In parallel with our efforts to optimize conventional therapies, we are exploring the relative contributions of tumor and host factors to immunosuppression. While experimental models of GBM are intrinsically immunosuppressive,\textsuperscript{26} we have shown in a non-glioma model that CNS location induces more profound immune dysfunction than equivalently progressed tumors at other sites.\textsuperscript{27} Interestingly, our data suggest that CNS tumors induce a state of systemic tumor antigen-specific tolerance. In other words, having a brain tumor suppresses not just local immune activity, but the entire immune system in a way that has not been described in other tumors. In these experiments, vaccination, adoptive transfer of high-affinity T-cells, and radiation can mediate tumor regression; however, a measurable degree of immune dysfunction persists in brain tumors compared with tumors outside the CNS. Our data indicate that a circulating factor is responsible, possibly in relation to the TGF (transforming growth factor)-beta pathway.

Others have corroborated and expanded on the principle of systemic immune dysfunction in GBM patients. For example, it has been shown that immune cells of these patients are sequestered in the bone marrow and, therefore, are unable to access the brain tumor.\textsuperscript{28} Investigations into the mechanisms of brain tumor-mediated tolerance are ongoing, and we think this will be a critical step in developing glioma-directed immunotherapies.

The tragically rare, but undeniably compelling stories of patients like Mr. H offer hope that the immune system can conquer this devastating disease. Ultimately, we believe that immunotherapy will play a pivotal role in significantly prolonging survival for patients with GBM, and other brain
tumors. An effective approach will need to generate and maintain a robust response against multiple tumor antigens in the CNS, while minimizing collateral damage. Patients must have a normally functioning baseline immune system to generate such a response; therefore, reversing the profound systemic immune suppression associated with CNS malignancies is of paramount importance. The negative results of clinical trials to date represent a call to action for a more intense focus on the unique aspects of brain tumor immunology.

Bios

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**Christopher Jackson**, M.D., is a senior resident in neurosurgery at The Johns Hopkins Hospital. His research interests focus on how malignant brain tumors alter local, regional, and systemic immune function as well as developing therapeutic approaches to reverse brain tumor-mediated immune suppression. Jackson graduated *Summa Cum Laude* from Wake Forest University with a BA in English and obtained his M.D. from the Johns Hopkins University School of Medicine.
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