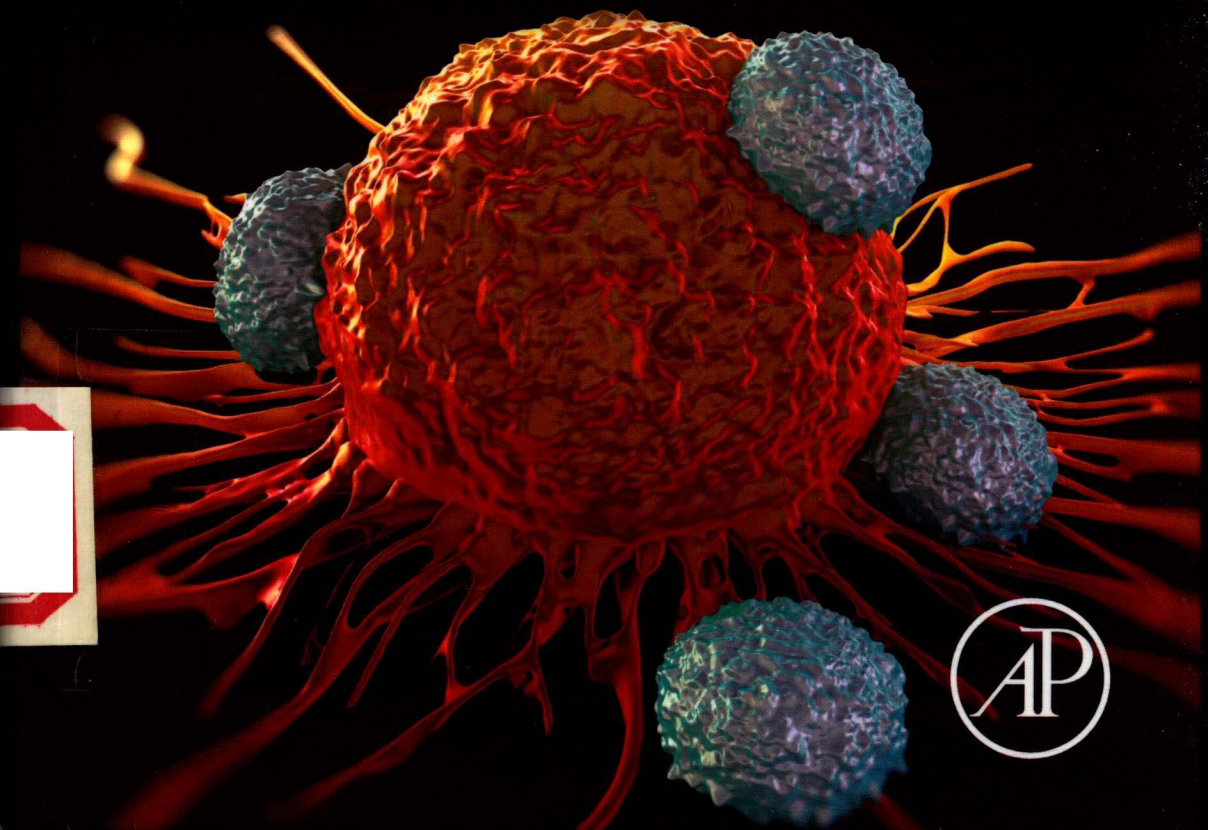
# Translational Immunotherapy of Brain Tumors

Edited by John H. Sampson



# TRANSLATIONAL IMMUNOTHERAPY OF BRAIN TUMORS

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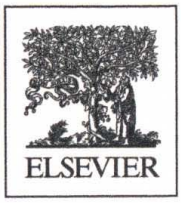


**ACADEMIC PRESS**
**An imprint of Elsevier**
**elsevier.com**

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom



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**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

**British Library Cataloguing-in-Publication Data**
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-802420-1



*Publisher:* Mara Conner
*Acquisition Editor:* Melanie Tucker
*Editorial Project Manager:* Kristi Anderson
*Production Project Manager:* Lucía Pérez
*Designer:* Christian Bilbow

Typeset by TNQ Books and Journals

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# Introduction to Translational Immunotherapy for Brain Tumors

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## TRANSLATIONAL IMMUNOTHERAPY FOR BRAIN TUMORS SUMMARY

Malignant gliomas are an especially aggressive group of diseases that are largely recalcitrant to conventional therapies. Even with modern advancements in treatment, the median overall survival for patients afflicted with these diseases is roughly six months.[1] The inability of standard therapies to safely and specifically eliminate all cancerous cells leaves patients vulnerable to tumor relapse and underscores the demand for alternative treatment modalities. In response, researchers across the globe are now attempting to exploit the immune system's natural cytotoxic capacity to mount an aggressive immunological siege upon cancer—a field known as immunotherapy. From this therapeutic paradigm, a variety of novel treatment strategies were born, several of which are showing promise against multiple cancers. In *Translational Immunotherapy for Brain Tumors*, we provide an overview of immunotherapy, with a particular emphasis on its application to malignant gliomas. General topics of discussion will include the immunological landscape of malignant gliomas, the approaches by which researchers and clinicians evaluate immunotherapies, and an in-depth look at the various experimental immunotherapies that are being pursued for malignant gliomas. Although great progress is being made in this area of research, the use of immunotherapy for malignant gliomas is still a relatively new concept. Furthermore, these central nervous system (CNS)-resident neoplasms are proving to be particularly resilient in the face of potent immunological intervention. Despite these obstacles, studies have elucidated the undeniable connection between the immune system and CNS-resident tumors. With each immunotherapeutic attempt that is made, the evasive mechanisms of these tumors becomes more clear,

providing hope that one day immunotherapy will be at least a component of the successful eradication of malignant gliomas.

## PRIMER ON MALIGNANT GLIOMAS

In *Translational Immunotherapy for Brain Tumors*, we pay special focus to immunotherapy for brain tumors. More specifically, most of the discussion is directed toward malignant gliomas, which are among the most difficult tumors to treat. Gliomas arise from glial cells. The three types of glial cells that are known to produce tumors are the astrocytes, oligodendrocytes, and ependymal cells. Astrocytes, which make up 20–40% of all glial cells, are regarded as the supportive cells of the CNS and have a wide range of functions including, but not limited to, metabolic and biochemical regulation, structural support, and maintenance of the blood–brain barrier (BBB). Oligodendrocytes also play a supportive role, albeit not as extensive as astrocytes, by providing electrical insulation by encasing neuronal axons within the CNS with a lipid-rich myelin sheath. Finally, ependymal cells line the ventricular system and central canal and are involved in the production of cerebrospinal fluid. The exact etiological mechanisms that cause these cells to transform into cancerous cells with uncontrolled growth have not been fully clarified, though several intrinsic and environmental factors have been proposed.

Gliomas represent the second most common primary intracranial tumor (~27%), behind only meningiomas (~36%). However, given that most meningiomas are benign, gliomas hold the title of the most common primary malignant CNS-resident tumor by a wide margin (~80%). Although they are not staged, gliomas are classified by a World Health Organization grading system that reflects their organization and structure relative to normal tissue (i.e., differentiation), growth potential, and aggressiveness. Grade I and II gliomas generally have a more differentiated phenotype, are slower-growing, and are less aggressive. These low-grade gliomas make up the majority of brain tumors that afflict children and adolescents. Alternatively, grade III and IV gliomas are poorly differentiated, grow rapidly, and are highly aggressive. These gliomas are designated as malignant or high-grade gliomas, occur more frequently in adults, and make up >90% of all diagnosed gliomas.[2]

GBM is the most common and aggressive primary malignant brain tumor, representing ~55% of all gliomas (Fig. 1), and has an incidence of ~2–3 in 100,000 people in the United States and Europe.[2] Multidimensional genomic analyses have shown that GBM represents a very diverse class of tumors and can be divided into several subtypes, each with their own unique signature. The classical subtype is frequently characterized by aberrations in the EGFR gene. The mesenchymal subtype typically

exhibits deletions in the NF1 gene. The proneural subtype is typified by alterations in the PDGFRA gene, as well as point mutations in isocitrate dehydrogenase 1 (IDH1); this group has been shown to make up a large fraction of secondary GBMs. Finally, the neural subtype is distinguished by the expression of a number of neuronal markers.[3] However, it is important to note that these subtypes are determined by a tumor's bulk expression profile, and studies have shown that an individual GBM tumor can contain cells of varying subtypes.[4]

The standard of care therapy for GBM is surgical resection, if tumor is operable, followed by external beam radiation therapy (60 Gy delivered in 30 fractions) and concomitant temozolomide (TMZ) chemotherapy (75 mg/m$^2$/day) administered over a period of six weeks. This is followed by adjuvant TMZ chemotherapy (150–200 mg/m$^2$/day) on days one to five of every 28-day cycle for up to six to twelve cycles.[5,6] Despite this aggressive treatment regimen, patient prognosis is poor. Treated GBM patients almost invariably succumb to tumor relapse, surviving approximately 15.6 months (95% CI: 13.3-19.1 months).[1,5] Although there is no established standard of care for alternative (non-GBM) malignant gliomas, treatment is generally similar (i.e., surgical resection, radiation therapy, chemotherapy). As with GBM, therapy only slightly prolongs survival in most cases. Not only are the conventional therapies used to treat malignant gliomas incapacitating and damaging to healthy tissues,

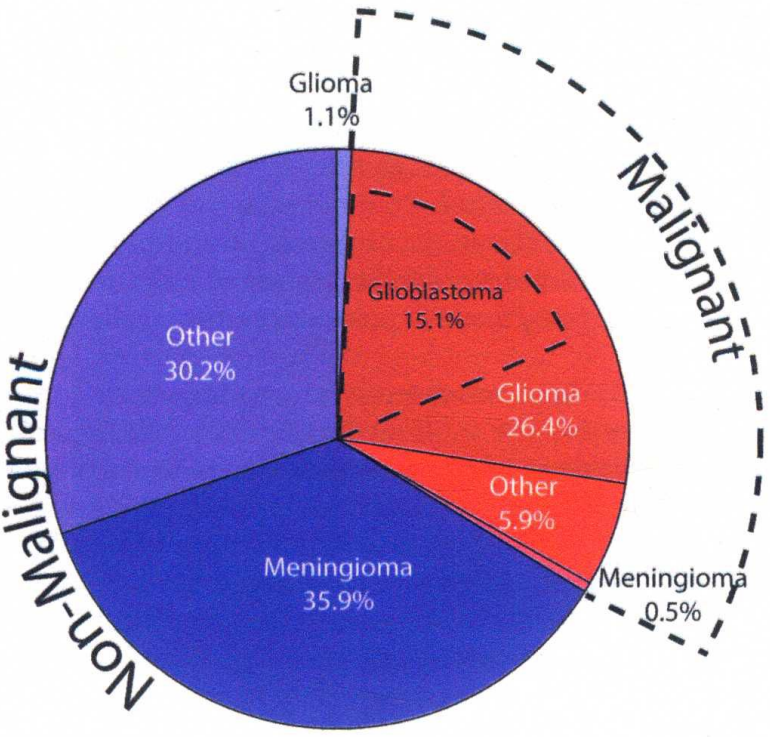


FIGURE 1 Distribution of malignant and nonmalignant brain and CNS-resident tumors.[2]

they ultimately suffer from a lack of tumor selectivity. A hallmark feature of malignant gliomas is their ability to infiltrate and diffuse into normal, healthy CNS tissues. Consequently, any residual tumor cells with stem cell-like attributes that remain following therapy have the potential to repopulate a new tumor. To overcome these limitations, a novel modality that can selectively target malignant glioma cells is likely required.

## THE POTENTIAL OF CANCER IMMUNOTHERAPY

The notion that the immune system can detect and eliminate cancerous cells has been around since 1909, when Paul Ehrlich made this proposition.[7] In the 1950s, Sir Frank MacFarlane Burnet and Lewis Thomas elaborated upon this theory by claiming that malignant cells arise within a host quite regularly but are quickly eliminated by the immune system—a concept known as immunosurveillance.[8] This theory was dealt a significant blow in the 1970s when it was realized that athymic nude mice, which at that time were considered to be devoid of a functioning immune system, did not develop cancer at an increased rate.[9] However, it was later determined that these mice were not so immunodeficient after all,[10,11] and studies with mice lacking immunological effector molecules (e.g., IFN-γ) did exhibit a higher incidence of cancer.[12,13] More recently, in 2004, Gavin Dunn, Lloyd Old, and Robert Schreiber put forth a theory known as immunoediting,[14] positing that tumors are sculpted by the immune system, selecting for tumor cells with a low-immunogenic phenotype. Thus, it would appear that cancer has managed to exploit an evolutionary weak point in the immune system, leaving many to question whether the immune system truly has the capacity to eradicate all malignant cells within a host. Fortunately, however, there seems to be more to the story.

One of the major considerations of cancer immunotherapy regards the sufficiency of the host immune system to target malignant cells; in other words, the immune system must possess immune cells that can detect tumor cells in order for immunological rejection to occur. From an immunotherapeutic standpoint, cancer truly is a wolf in sheep's clothing, insomuch as its components are more or less normal cellular material. Most tumor antigens are identical to normal antigens to such an extent that tumor cells easily evade detection by innate immune cells, which scout for pathogen-associated motifs. Fortunately, evolution has provided us with an adaptive immune system that can be educated de novo to target specific molecules. But again, given the similarity between most tumor and normal antigens, most adaptive immune cells that recognize these homologous antigens are thought to be destroyed or rendered nonresponsive (i.e., anergic) to avoid the consequence of autoimmunity. Nevertheless, several decades worth of preclinical studies have repeatedly demonstrated that

adaptive immune cells do indeed possess the capacity to target tumor cells, proofed by the abrogation of antitumor effects in the context of adaptive immune cell depletion.

Early attempts to target tumor antigens took a brute force approach by using vaccines consisting of autologous, typically irradiated, cancer cells. Despite an exhaustive variety of approaches, these therapies induced only moderate and short-lived antitumor benefits, at best. Then, in the early 1990s, in the wake of cytokine-gene cloning, researchers began evaluating the antitumor effects of tumor cells transfected with genes encoding various immunological signaling molecules. Though results often varied among studies depending on the cancer type and site of tumor challenge, striking antitumor effects were frequently seen in preclinical studies using the immunomodulator granulocyte-macrophage colony stimulating factor.[15,16] As an alternative to the brute force approach, investigators at that time also began identifying specific tumor antigens that could be recognized by adaptive immune cells.[17–19] Notably, several melanocyte-specific proteins (e.g., MART-1) were found to stimulate tumor infiltrating lymphocytes (TILs) isolated from melanoma patients,[18] and vaccines consisting of these melanocytic proteins were able to produce significant antitumor responses in preclinical models of melanoma. This was a rather striking observation considering that clonal deletion of self-reactive lymphocytes was demonstrated a few years earlier.[20,21] We now know these processes are incomplete, and self-reactive immune cells do indeed exist in the periphery.[22] However, activation of these cells does pose the risk of autoimmunity, which is a critical concern in the field of immunotherapy.[23] The readministration of ex vivo-expanded TILs into melanoma patients, while effective in several cases, frequently induced autoimmune effects (e.g., vitiligo)[24,25]—a not too surprising outcome considering the nature of the recognized antigens. Though the treatment of one disease with another is not unprecedented, autoimmunity can have just as debilitating effects as malignant cancer. Consequently, researchers are beginning to focus their attention on safer tumor-specific antigens, which only became possible in recent years.

Upon the advent of next generation sequencing (NGS) in the early 21st century, large-scale tumor sequencing efforts quickly ensued. Using this technology, it became possible to peer inside tumor cells at the molecular level to determine their exact genetic and transcriptomic compositions. Although it was largely known that cancer was a disease caused by genetic mutations, the comprehensive landscape of these alterations was unknown prior to the availability of NGS. Much of the findings of these studies were not all too surprising: (1) genetic mutations were found in coding and noncoding DNA, (2) oncogenic and tumor-suppressor genes were frequently perturbed, and (3) tumors thought to develop from environmental stressors (e.g., melanoma and lung cancer) had a far higher

number of mutations. However, it became evident that it may be possible to target the expressed mutations, or neoantigens, within tumors. The benefit of using neoantigens as immunotherapeutic targets is two-fold: they are inherently tumor-specific and neoantigen-cognate adaptive immune cells are less likely to be subject to deletion or quiescence. Consequently, efforts are now being made to target the repertoire of tumor-specific neoantigens within tumors.

Numerous studies have demonstrated the immunogenicity of tumor-specific and tumor-associated antigens. How, then, are endogenous immune responses to these antigens prevented? The answer lies, in part, in the developmental process of a tumor. From incipience, cancers evolve an immunosuppressive phenotype that develops gradually, balancing on the cusp of immunological rejection with constant signals relayed to the immune system that it is simply a normal, healthy cluster of cells. Paracrine-acting suppressive molecules secreted by the tumor subdue local effector functions, whereas endocrine signaling molecules diminish activation of new antitumor immune cells in the secondary lymphoid organs, as well as promote systemic immunosuppression. In those unlucky few, the balance between immunological rejection and immunosuppression skews in the favor of tumor development. By the time of diagnosis, the arsenal of immunosuppressive mechanisms exhibited by the tumor is frequently evidenced by the infiltration of immunosuppressive immune cells (e.g., regulatory T-cells [Tregs]), the reeducation of the stroma into an immunosuppressive phenotype, and the elaboration of immunosuppressive signaling molecules from the tumor cells themselves.

Overturning the effects of tumor-mediated immunosuppression is now a primary focus of immunotherapy. The history of this strategy dates back to the late 1800s, when a bone surgeon by the name of William B. Coley noticed that a patient suffering from a recurrent neck sarcoma experienced tumor regression following a case of erysipelas, or *Streptococcus pyogenes*, infection.[26] In response to this observation, Dr. Coley crafted his own treatment cocktail consisting of killed *S. pyogenes* and *Serretia marcescens*—eponymously termed Coley's toxin. Although the antitumor effects of Coley's toxin are mixed, several cases of tumor regression following treatment have been documented. Several explanations of the antitumor effects of these bacterial components have been proposed, including the activation of innate toll-like receptors; however, the exact mechanisms are unknown. Suffice it to say that the stimulation of the host's immune system by Coley's toxin seems to be sufficient to reverse immunological suppression, thereby promoting antitumor responses, in some instances. As the field of immunology progressed, the suppressive pathways of the immune system began to develop understanding. In the late 1980s, a molecule known as cytotoxic T lymphocyte associated antigen 4 (CTLA-4) was discovered,[27] and shortly thereafter, the receptor programmed

death 1 (PD-1)[28] and its ligands (PD-L1[29] and PD-L2[30]) were identified. These molecules are collectively known as checkpoints due to their role in keeping the immune system in check by promoting immunosuppression. Checkpoint inhibition via antibody-mediated blockade is gaining considerable attention in the field of immunotherapy in light of the potent antitumor responses it engenders against several tumors (e.g., melanoma and lung cancer).[31,32] These effects highlight the role of immunological suppression in subduing endogenous antitumor responses. Unfortunately, these therapies do not seem to be equally efficacious in all cases or against all tumors. Further optimization of clinical protocols using checkpoint inhibitors is required before their true effectiveness can be confirmed. It is unlikely, however, that checkpoint blockade alone will be the "magic bullet" envisioned by Dr. Ehrlich in the 1900s. The list of immunosuppressive mechanisms utilized by cancer is an extensive one, several of which will be discussed throughout this book, and therapeutic remediation of these pathways is showing great promise against certain cancers.

The immune system truly is a remarkably complex, and at times seemingly inextricable but equally astonishing, network of cells and cellular processes that staggers the imagination when its ontogeny is contemplated. From a therapeutic perspective, the immune system boasts potent cytotoxic potential with the ability to resolve structures at the nanometer level, giving it a distinct advantage over conventional cancer treatment strategies. Leveraging these capabilities to produce safe, selective, and durable antitumor responses is the foremost goal of immunotherapy, and, while this field has been mired with hurdles, great progress has been made and intriguing discoveries unearthed along the way. Studies are continuously showing that the body does indeed have a defense network in place that can eradicate malignant cells, but cancer—masquerading as a normal cell—is a formidable expert at attrition warfare and has decisively co-opted the immune system for its own benefit. However, there have been several strong indications that cancer's immunological evasive mechanisms are reversible, as will be illustrated throughout this book. We truly are in the eve of the "Golden Age" of cancer immunotherapy, and these next few years will be critical in establishing immunotherapy as a respected modality in the armamentarium of cancer therapeutics.

## CHALLENGES OF BRAIN TUMOR IMMUNOTHERAPY

From the serendipitous discovery of bacteria-mediated tumor suppression in the 19th century to the striking response rates promoted by modern molecular-guided immunotherapies, immunotherapy is proving to be a powerful modality for the treatment of cancer. Nevertheless, hopefulness is met with reasonable skepticism, particularly with regard

to brain tumor immunotherapy. Although some types of cancer do seem to be amenable to immunotherapeutic intervention, there are several aspects of brain tumors that make the use of immunotherapy a controversial proposition.

CNS tissues represent a very unique immunological environment compared to peripheral tissues (Fig. 2). Seminal studies in the early 20th century demonstrated that grafts implanted within the CNS are rejected much slower than grafts placed in the periphery,[33] leading to the notion that the CNS was, in essence, an immunologically privileged site. This concept was further supported by the apparent lack of draining lymphatics[34] in the brain and CNS tissues and the presence of a highly restrictive blood–brain barrier BBB.[35] From an evolutionary standpoint, it is reasonable to assume that the CNS has developed means to safeguard itself from the destructive effects of inflammation, given the indispensability of these tissues; however, we now know that the immune privilege of these tissues is not absolute. Studies have shown that immune cells do indeed have the potential to access these compartments, as evidenced by disease

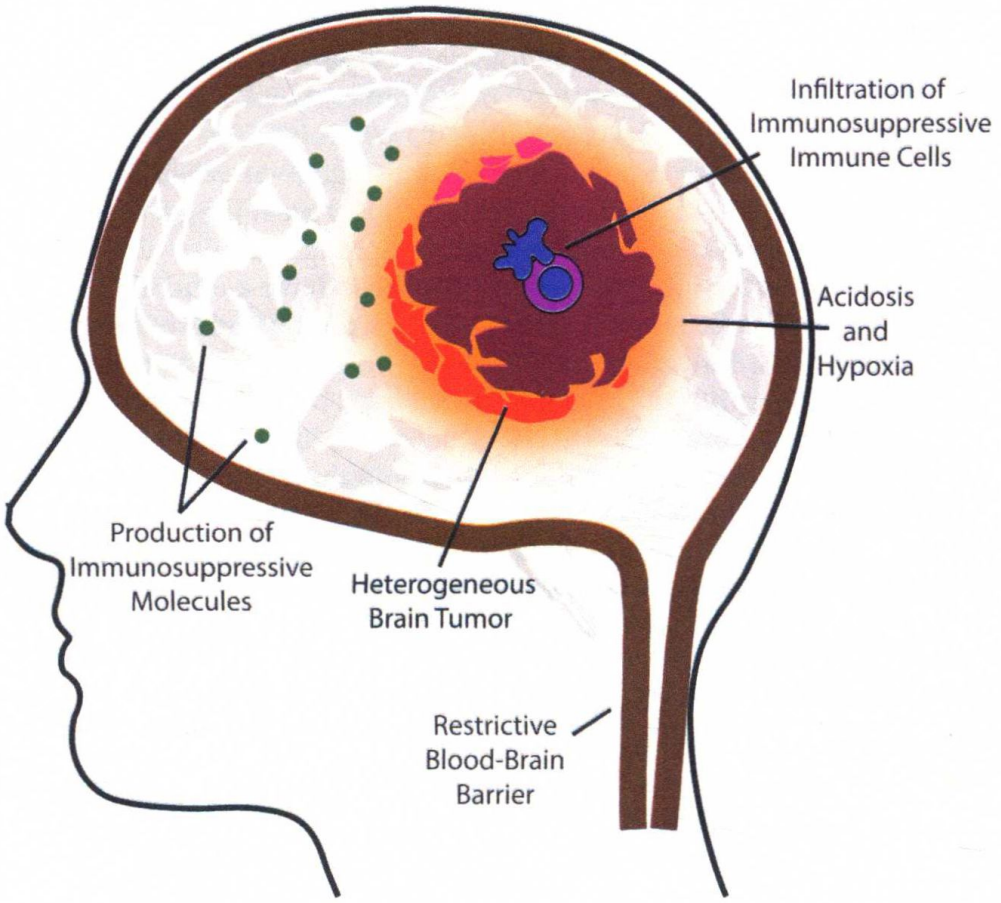


FIGURE 2 The immunological barriers to CNS-resident tumors.

states such as multiple sclerosis and cancer. The ability of immune cells to access the CNS-resident tumors can further be aided by the disruption of the BBB by invading glioma cells.[36] Additionally, a lymphatic system was recently elucidated in the dural sinuses of mice,[37] which carry immune cells and antigen to the deep cervical lymph nodes.[38] Thus, it is currently well-established that there exists an appreciable degree of immunological surveillance of CNS tissues and CNS-derived antigen, albeit not as extensive as that which occurs in peripheral tissues. Nevertheless, it is also clear that these mechanisms are not sufficient to promote complete endogenous rejection of CNS-resident tumors.

A second aspect that can limit the effectiveness of brain tumor immunotherapy has less to do with the anatomy of the CNS, but rather the architecture and behavior of gliomas (Fig. 2). Immunological evasion is one of the hallmarks of cancer,[39] and malignant gliomas are true experts. Much like viruses that frequently mutate to avoid immunological detection, malignant gliomas exhibit profound heterogeneity[40–42] that enables them to escape monovalent immunotherapeutic intervention.[43] Even upon the activation of high numbers of diverse tumor-reactive immune cells, malignant gliomas may still be protected by additional lines of defense. For example, the malignant glioma milieu is often associated with acidosis and hypoxia. This environment is extremely detrimental to effector immune cell function but is able to sustain immunosuppressive immune cells (e.g., Tregs[44]), as well as stimulate angiogenesis.[45] Additionally, malignant gliomas are known to produce a host of immunosuppressive molecules, such as transforming growth factor (TGF)-β, interleukin (IL)-10, and indoleamine-2,3-dioxygenase (IDO), that dramatically inhibit the immune response. These factors are also thought to contribute to the profound immunosuppression identified in malignant glioma patients, characterized by lymphopenia and T-cell dysfunction.

The aforementioned characteristics of brain tumors arguably represent the worst case scenario for immunotherapy. Few other cancers are situated behind such a vital organ that also maintains partial immunological seclusion. Nevertheless, the need for safer and more selective therapies for brain tumors is undeniable, and the evaluation of brain tumor immunotherapy has, thus far, not been a fruitless endeavor. The studies carried out in this pursuit have provided great knowledge into the immunobiology of the CNS and CNS-resident tumors. It is now clear that immunotherapy has the potential to stimulate immunological changes within patients afflicted with brain tumors and, in several cases, impart molecular changes within the tumor itself.[43] The next hurdles will be to overcome the issues of tumor-mediated immunosuppression and tumor heterogeneity, and there are several strategies in the therapeutic pipeline that are being tested to fulfill this demand.

## CHAPTER OVERVIEW

*Translational Immunotherapy for Brain Tumors* is divided into several sections to help guide the reader through the rapidly expanding field of brain tumor immunotherapy. In Section I, we look at the immunological features of brain tumors, including many of the immunosuppressive aspects associated with these cancers. Section II details the methods that researchers and clinicians use to evaluate brain tumor immunotherapies. Finally, in Section III, we review several experimental immunotherapies that are currently being evaluated for the treatment of brain tumors.

Section I–Immunological Features of Brain Tumors focuses on the salient immunological aspects associated with malignant gliomas. We begin with a basic immunology overview in Chapter 1, including a brief discussion of the innate and adaptive arms of the immune system. This chapter also includes a detailed look at the physiological components that give the CNS partial seclusion from the immune system, including such elements as a restrictive BBB, limited antigen presentation, and the once thought absent CNS-resident lymphatic system that was only recently discovered in the murine brain.

Chapters 3, 4, and 5 focus on an issue that is proving to be a formidable obstacle to brain tumor immunotherapy: profound immunosuppression. Chapter 2 begins with a brief history of the seminal studies that first observed immunological dysfunction in patients with malignant glioma. These early studies were at times contradictory but most demonstrated compromised T-cell functionality and lymphopenia. Although the finer details of these deficiencies remain to be elucidated, it appears as though malignant gliomas have managed to co-opt the immunological signaling networks and immunosuppressive pathways to protect themselves from immunological rejection. Two well-known players in malignant glioma immunosuppression—regulatory T-cells (Tregs) and IDO—are further described in Chapter 3. Tregs, demarcated by the expression of the transcription factor Foxp3, are an immunosuppressive subset of CD4 T-cells that are selectively recruited to malignant gliomas through the production of chemo-attractants by tumor-resident cells. Tregs, which can persist within the hypoxic tumor microenvironment, have been shown to diminish effector T-cell function by several mechanisms, including contact-mediated cytolysis, IL-2 growth factor consumption, elaboration of immunosuppressive cytokines (e.g., TGF-β and IL-10), as well as several alternative mechanisms. Another mediator of immunosuppression that is frequently upregulated in the glioma microenvironment and known to inhibit effector cell function is IDO, the rate-limiting enzyme involved in the catabolism of the essential amino acid tryptophan to kynurenine. Together, Tregs and IDO represent two promising targets for immunotherapeutic intervention.

In Chapter 4 we discuss the role of myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature myeloid cells with impaired ability to differentiate into macrophages, granulocytes, and dendritic cells, in immunosuppression. MDSCs are found in GBM where they suppress innate and adaptive antitumor immunity by subduing T-cell functions and other mechanisms that have not yet been fully understood. This chapter describes the origins, activation, expansion, and known mechanisms of MDSCs.

Finally, this section concludes in Chapter 5, with an overview of the conserved tumor-specific mutations, or neoantigens, that are found in gliomas. Large-scale sequencing efforts have demonstrated that most genetic mutations are unique to an individual tumor; however, several mutations occur in gliomas at disproportionate frequencies, including the R132H isocitrate dehydrogenase mutation in low-grade and secondary glioblastomas, EGFRvIII in malignant gliomas, and histone mutations found in pediatric gliomas. The incidence of these mutations suggests that they may play a role in oncogenesis, and, given their conserved nature and tumor specificity, they may serve as ideal immunotherapeutic targets.

In Section II–Studying Brain Tumor Immunotherapy, the approaches that make it possible to study brain tumor immunotherapy are reviewed. Chapter 6 takes a look at the various preclinical models that are available to researchers for the study of brain tumor immunotherapy. These include a wide range of mostly murine spontaneous, chemically induced, virally induced, or genetically altered glioma models. Additionally, severely immunocompromised mice are available that enable the engraftment of human cells, generating reliable xenograft and humanized models. Although murine glioma models have been extremely useful for preliminary immunotherapeutic studies, limitations arise from their dissimilarities with human gliomas (e.g., lack of heterogeneity and spontaneity). To bridge the gap between murine and human gliomas, several researchers have recently turned their preclinical focus toward spontaneous canine gliomas, which occur at a relatively high frequency and afford the benefits of heterogeneity and spontaneity. Preclinical studies in these nonhuman models are essential for determining the safety and therapeutic potential of various immunotherapies. However, these factors are often determined using highly invasive methods that are not typically translatable to human studies.

Given the vital nature of the brain, noninvasive methods of determining an immunotherapeutic response at the early stages of treatment are needed to limit unnecessary outcomes. Although this requirement can be fulfilled by advanced imaging techniques, assessment of CNS-resident responses is frequently complicated by pseudoprogressive inflammation, which is a sign of an active immune response and may not be associated with clinical decline. Conventional assessment criteria (e.g., Response Evaluation