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Immunology and Immunotherapy in Brain Tumors — Immune Failure and Potential Counteractions

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1. Introduction

The status of the immune system plays a major role tumor progression. Lewis Thomas proposed the association between immune surveillance and tumor progression as early as the 1950's. He suggested that the adaptive immune system has evolved to detect changes in the body's own cell surfaces due to damage or mutation. T cells, which specialize in monitoring cell surfaces, usually in the context of MHC molecular presentation, carry out this role; if a cell is deemed to be abnormal, it is destroyed before a mutant clone has time to proliferate and progress. Thus, the development of cancer could be seen as a failure of the immune system.

This chapter will discuss the ongoing interaction between the tumor development and the immune system, a process that has been called immunoediting. We will focus on tumors involving the central nervous system in both adult and pediatric settings—high grade gliomas (WHO grades III and IV tumors). We will review of the normal mechanisms employed by the immune system in combating tumor cells including cytotoxic T cells, Th1/Th2 cells, Natural Killer (NK) cells, B cells, macrophages, and the complement system.

Furthermore, we will explore the topic of tumor-associated antigens (TAA) in brain tumors, where we will start the review of alternative tactics of brain tumor treatments using immunotherapy. Although there have been relatively few successes in the field of immunotherapy, we will review the recent developments in brain tumor immunotherapy research and the different on-going clinical trials.

2. The immune system

2.1. General defense mechanisms

The immune system is the backbone of the body's defense against foreign invaders including bacteria, fungi, parasites, and viruses. The ability of humans to resist infections is composed of multiple systems working together, the first of which are the physical barriers of innate immunity lining the human body and entry points—skin and mucous membranes. Those physical barriers possess special properties that help fend off unwanted microorganisms including the ability to regenerate and secretion of antibiotics—defensin and cathelicidin families. However, under certain circumstances the physical barriers fail, allowing foreign intruders to venture deep inside the body requiring the activation of the immune system.

The immune system is comprised of two major divisions: the innate and adaptive immune systems. The innate system is not specific to a single pathogen, but is dependent on specific group of proteins and cells to recognize conserved features of pathogens. The main components of the innate system include 1) physical epithelial barriers discussed above, 2) phagocytic leukocytes, 3) dendritic cells, 4) natural killer (NK) cells, and 5) circulating plasma proteins. Using toll-like receptors (TLRs), the innate system is able to recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and respond within minutes. The adaptive immune system, on the other hand, is capable of recognizing new antigens and forming memory cells. The adaptive immune is activated when pathogens overcome the defenses of the innate system. Adaptive responses, however, are slow to respond at initial exposure to a new pathogen since specific clones of B and T cells are not yet activated. There are two types of adaptive immunity: humoral and cell-mediated immunity. Humoral immunity is mediated via antibodies secreted by B-lymphocytes whereas cell-mediated immunity is carried out by T-lymphocytes.

2.2. Induction of immune responses to antigens

Consider a scenario where a pathogen bypasses the physical barriers of the innate immune system. The pathogen is then recognized as foreign, taken up by professional antigen-presenting cells (APCs) and delivered to the nearest lymph node via the lymphatic system where T lymphocytes are activated. Alternatively, antigens can reach the lymph node by passive drainage where they are taken up by macrophages and dendritic cells (DCs). The antigens are then processed and presented to T-lymphocytes in association with MHC molecules. The simultaneous binding of T-cell receptor (TCR) to major histocompatibility complex (MHC) molecules and stimulation via APC's co-stimulatory molecules initiates T-cell activation. The T-lymphocyte may be a "helper T cell" that is now capable of aiding in activating "killer T cells" (cytotoxic T-lymphocytes, CTLs) and B-lymphocytes.

2.3. Immune surveillance

Immune surveillance is the theory that the immune system evolved not only to protect the body against foreign pathogens but also host cells that become tumorigenic. This idea,

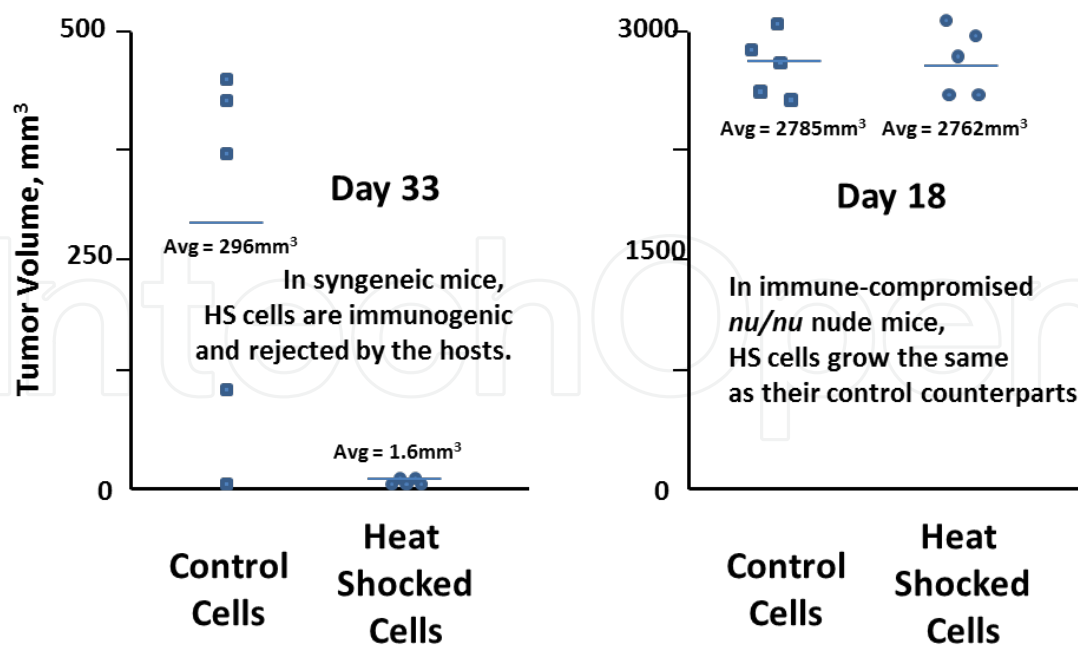


Figure 1. Immune mechanisms in tumor growth control: Tumor cells rendered immunogenic (by heat shock) are rejected by immune competent hosts, but grow unrestricted in immune-compromised mice. In this experiment, heat-shocked murine brain tumor cells became immunogenic (possibly due to the release of stress proteins) compared to non-heat shocked cells and were rejected following injection into syngeneic, immune-competent hosts. However, in immune-compromised mice (athymic nude mice, in this case, lacking T lymphocytes), heat shocked tumor cells grew equally large tumors as the non-heat shocked cells, indicating that the heat stress did not compromise cell viability, and that an intact immune system is required for rejection of the implanted tumors. Note differences in day of tumor measurements and the differences in the tumor volume axes.

proposed in the 1950's, was quite prescient given that self monitoring done by T-cells was not discovered until 20 years later [1]. There is some evidence for this attractive notion: 1) Patients with T-cell immunodeficiencies have a higher incidence of tumors, e.g. AIDS patients have a higher incidence Burkitt's lymphoma and Kaposi's sarcoma; 2) Organ transplant patients treated with immunosuppressive drugs had 25 to 100 fold increase in tumor incidence relative to healthy controls; 3) Tumor-Infiltrating Lymphocytes (TILs) have been identified that are capable of recognizing tumor-associated antigens. The most persuasive evidence, however, comes from the chemically-induced sarcomas in RAG2^{-/-} mice. RAG2 knockout mice fail to produce mature B and T cells due to lack of recombinase enzymes that are necessary to generate mature and functional antibodies and T-cell receptors [2]. Tumor cells induced in this knockout strain could be transplanted to new knockout hosts with 100% take rate, whereas wild-type mice of the same strain rejected 60% of the tumor challenges [3]. These experiments suggested that the immune system plays an active role in defending the body against cancerous cells. Additionally, this suggests that certain types of tumors or cells within a tumor may be strongly immunogenic inciting an immune response, while other cells may be weakly immunogenic. For instance, cells that are driven (eg, by heat stress) to be immunogenic are rejected by immune-competent syngeneic hosts, but those cells will grow at the same rates as control (unstressed) cells in immune-compromised (*nu/nu*) mice (Figure 1). We can then conclude that

the immune system of mice plays a role in determining the pro-or anti-immune phenotypes of tumors that arise in mice, and possibly humans, a process termed immunoediting.

2.4. Immunoediting

The interaction between the immune system and tumor development has been termed immunoediting. One can think of the role of the immune system in immunoediting as the selective force on tumors, a process analogous to Darwinian selection. The process of immune editing can result into 3 outcomes: 1) the tumor cells are successfully eliminated as seen in the WT mice (Figure 1); 2) The tumor cells and lymphocytes exist in equilibrium; 3) Tumor cells initiate several responses that result in the suppression of the immune system or avoidance of immune effectors.

2.4.1. Tumor cell elimination

Compared to normal cells, tumor cells display a variety of metabolic abnormalities leading to the expression of damage associated molecular patterns (DAMPs) [4], as well as the stress expression of NK ligands such as MICA/B and ULBP families [5]. The expression of DAMPs and other stress molecules leads to the activation of the innate immune system, which in turn activates the adaptive immune system via APCs and cytokine release. The adaptive response yields stimulated effector lymphocytes. The end result is macrophage and lymphocyte infiltration of the tumor. If the abnormal clone is successfully eliminated, the process of immunoediting ends here. If the abnormal clone persists, however, then it can exist in equilibrium or grow beyond the control of the immune system [6].

2.4.2. Equilibrium

Another outcome to the infiltration of tumors by lymphocytes is incomplete eradication of the tumor. In this scenario, the abnormal clone and lymphocytes exist in equilibrium where the lymphocytes keep the tumor in check, but fail to entirely eradicate it, aided by regulatory T cells (Tregs, a subset of lymphocytes with immune suppressive properties) [7]. This may result in tumor dormancy (sometimes called “occult” cancers), but the mechanisms of this are poorly understood [8, 9].

2.4.3. Escape

The third phase of the immunoediting paradigm is escape of the tumor from immune attacks. This is often regarded as an “immune sculpting” phenomenon [10] that leads to immune pressure selection and outgrowth of unrecognized tumor clones. In combination with the aforementioned Tregs, the tumor may reach sufficient size that it can generate its own immune suppressive microenvironment, leading to growth and metastases in the face of an ineffectual immune response. In light of tumor selection and tumor-induced immune suppression, what factors lead to immune recognition of tumors?

3. Detection of neoplastic tissue

The immune system is generally very successful in eliminating viral infections and certain virus-induced tumors due to the vast difference in structure between viral and self-antigens. So, how are tumors lacking a viral etiology recognized and eliminated?

3.1. Tumor associated antigens

TAAAs are antigens that are expressed by tumor cells and not readily found on corresponding normal cells. TAA can be found on normal cells, but are usually overexpressed or abnormally expressed on tumor cells. There are several types of TAAs: 1) viral gene products; 2) mutant gene products; 3) normal gene products. Viral antigens are expressed in tumors caused by viral infections: e.g. HTLV-1 and HTLV-2 viruses causing mycosis fungoides, human papilloma virus (HPV) in cervical cancer, Epstein-Barr virus (EBV) in Burkitt's lymphoma, and hepatitis B and C viruses (HBV, HCV) in hepatocellular carcinomas [11]. Mutant gene products are often found in transformed cells that may be part of the transformation process, or caused by physical and chemical carcinogens resulting in mutated proteins. Mutant gene products vary from one patient to the other and represent tumor-specific antigens (TSA). It has been estimated that tumors may have scores to hundreds of mutations that lead to recognizable epitopes [12].

Normal gene products are found on corresponding normal cells, but may overexpressed by tumors, such as epidermal growth factor family members (eg, HER2/Neu [13]). Others may be inappropriately expressed outside developmental or differential stages, such as oncofetal antigens and differentiation antigens. Differentiation antigens are lineage specific antigens that are usually overexpressed e.g. prostate-specific antigen (PSA) or the melanoma antigens MART1, TRP1, and tyrosinase. Oncofetal antigens are normally expressed in fetal tissue but not adult tissue. Perhaps the most familiar oncofetal antigen is the carcinoembryonic antigen (CEA) found in patients with colon carcinoma, amongst others. A review describing a comprehensive database for tumor antigens may be found here [14].

3.2. Effectors of the immune system

How does the immune system manage to eliminate tumors once an abnormal clone is recognized? Perhaps the most important cell in the topic of anti-tumor resistance is the CD8+cytotoxic T-lymphocyte (CTL).

CTLs are capable of recognizing TAAs presented on MHC class I molecules, which are present on the surface of most cells in humans. Following activation, CD8+CTLs undergo clonal expansion and migrate towards the tumor. CTLs then eliminate abnormal clones by inducing apoptosis through perforin or Fas-mediated pathways. Additionally, CTLs secrete IFN γ upon engagement of their TCR, attracting macrophages. CTLs, however, can be inactivated upon arrival to tumor site as identified in melanoma patients [15, 16]. As a component of normal regulatory mechanisms, CTLs carry a surface marker, PD-1, that when engaged inactivates the

CTL. Many tumor types, however, up regulate PD-1 ligand (PD-L1 or PD-L2) in order to suppress and evade CTL activity.

Another notable cell belongs to a subset of CD4⁺T-lymphocytes, the type 1 T-helper cell (Th1). Th1 cells play a major role in recognition of antigens, production of lymphokines, activation of CD8⁺CTLs, and attracting M1 macrophages. Th1 cells then could play an important role in development of cancer vaccines [17].

Natural Killer (NK) cells are part of the immune system and are also known as large granular lymphocytes (LGLs). Being part of the immune system, NK cells can recognize a range of tumors and stress related markers without prior exposure to antigen (eg, via immunization). NK cells play a role in cancer immunity attacking cells that down regulate MHC class I molecules. Certain types of cancer cells down-regulate MHC class I molecules as an attempt to evade CTLs; the abnormal clone, however, risks detection and elimination by NK cells for reduced expression of surface MHC molecules [18].

Macrophages play a major role in cancer, exhibiting both anti-and pro-cancer behavior. Macrophages are part of the innate immune system and can be divided into the opposing M1 and M2 type macrophages. M1 activity inhibits cell proliferation and causes tissue damage. M2 activity promotes proliferation and repair. Tumors are capable of promoting M2 macrophage phenotypes which then aid in tumor angiogenesis, immune suppression, and tumor progression [19].

Dendritic cells (DC) are professional antigen-presenting cells and are a transitional link between the innate and adaptive immune system, inducing and maintaining T-cell immunity. DCs are outfitted with antigen-processing machinery (APM) allowing them to uptake and process TAAs. Processed TAAs are then loaded on MHC class I and II and presented to the appropriate T-cells. Thus, activated DCs are responsible for antigen specific immune responses by promoting activation and proliferation of T-cells. DCs can be divided into 2 major groups: plasmacytoid and classical DC. Plasmacytoid DCs play a role in antiviral immune response. Classical DCs can be further classified based on surface marker and function e.g. Langerhans cell in human epidermis. DCs are potential vectors for immune priming as vaccines against cancer antigens [20].

Antibodies play an interesting role in cancer immunity. Tumor-reactive serum antibodies from patients have long been viewed as resources for antigen detection, both in terms of vaccine potential and as exploitable biomarkers [21]. In other cases, antibodies isolated from cancer patients were tested for reactivity against cancer cell lines in vitro and in vivo. The antibodies showing specificity for tumors were all germ-line encoded and most belonged to the IgM class, binding to surface carbohydrates on malignant cells [22]. The utility of endogenous anti-tumor antibodies has not been clearly exploited, but may play a role in vaccine scenarios [23].

3.3. The immune response

Immune system activation is classically initiated when foreign antigens are taken up by professional APCs such as DCs, which migrate to the nearest draining lymph node. Figure 2 shows a highly diagrammatic and simplified version of this phenomenon. Protein antigens

are processed during transit and beyond so that peptides may be loaded onto MHC Class I and Class II molecules for surface display by the APC. Once in the lymph node, the APC presents the antigen to the corresponding naïve T-cell to activate it. Activation of the T-cell requires two signals: 1) presentation/display of the processed peptide to the T-cell; and 2) co-stimulation of the T-cell by direct contact with APC surface molecules and by the secretion of activating cytokines (interferons, IL-12, IL-15, granulocyte macrophage-colony stimulating factor [GM-CSF], etc) by the APC. For Signal 1, the peptide displayed must fit into the peptide binding cleft of the host's MHC molecules and bind with sufficient affinity that the peptide-MHC is stably presented at the APC surface. The "T-cell of destiny" will be one whose T-cell receptor (TCR) recognizes the displayed peptide in the context of the MHC molecule. Thus, the T-cell must be antigen-specific. For Signal 2, the APC must be sufficiently stimulated upon and following antigen uptake that it produces co-stimulatory molecules such as CD80 and CD86, whose receptor, CD28, awaits on the T-cells. The APC will also produce the aforementioned stimulatory cytokines, as well, contributing to the activation of T-cells. This cell-cell interaction, with the various presentation of antigens and ligands to receptors requires close contact between the cells, and has been termed "the immunological synapse" [24]; for further review, see [25].

3.3.1. *Tumor evasion*

Given the descriptions of the immune system cited earlier, one would think that attempts by the immune system to eradicate tumors are a rare phenomenon. In fact, TAAs are found in the sera of some cancer patients. Still, however, the development of many human cancers is not blocked by the immune system. A likely explanation considers the antigenic properties of abnormal clones; certain cancers utilize the tolerance of the immune system to self-antigens by only expressing proteins that fly under the immunologic radar. Another possibility is immunoevasion—strategies employed by antigenic tumors after initial insult by the immune system.

3.3.2. *Immunoevasion*

The strategies of immunoevasion enable tumor cells to grow and create clinically relevant tumors. The immune system acts as a selective force on the initial tumor, allowing abnormal clones to escape elimination. Perhaps the most obvious evasive maneuver employed by tumor cells is to hide their identity by ceasing to display specific TAA and TSA. By doing so, abnormal clones evade elimination by cytotoxic T-lymphocytes. Consider a melanoma patient who was vaccinated with tyrosinase protein expressed by his melanoma cells. Initially, his melanoma regressed as a result of the immune response. Soon, however, tyrosinase-negative clones emerge while the tyrosinase-positive clones continue to regress. The tyrosinase-negative clones continue to proliferate rapidly until his death, a process called "immune escape". A possible explanation to the rise of the tyrosinase-negative melanoma cells is the diverse population of abnormal clones created by faulty DNA replication in cancerous cells. The tyrosinase-negative clone was then selected for by the immune system. This scenario is evident

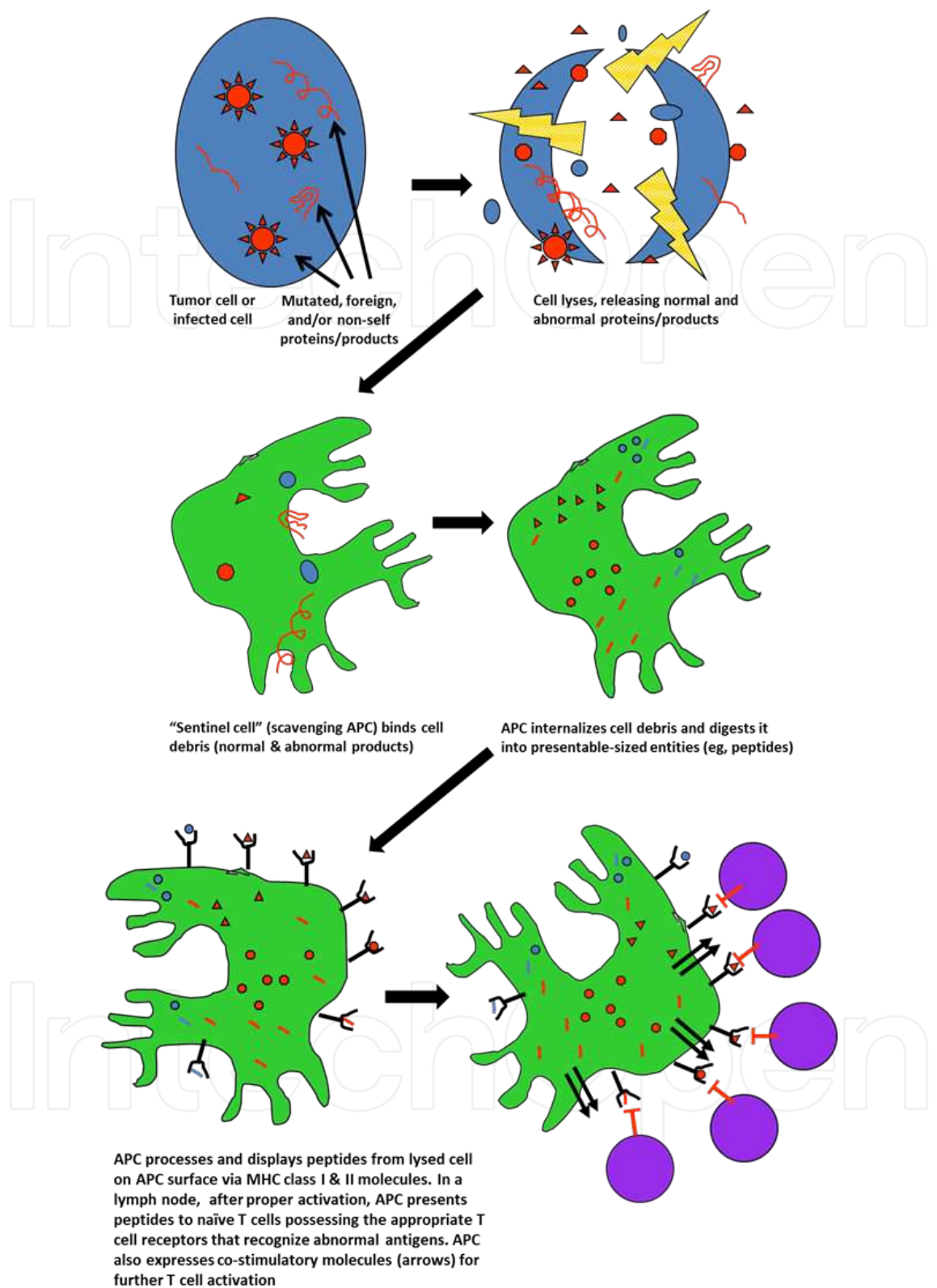


Figure 2. Antigen uptake, processing, and presentation. Pathogenic cells may lyse for a number of reasons, releasing normal and abnormal (viral, mutated, mis-expressed) proteins or other products. Scavenging Antigen Presenting Cells (APCs) may encounter the debris, internalize, and process it into peptides that are loaded onto MHC molecules and presented with co-stimulation to T cells in the lymph node, activating the T cell.

both in murine [26] and human studies [27], and is a concern where a particular antigen may be inconsequential to tumor physiology.

Some antigens, however, are essential for the function of tumor cells and neoplastic growth. In cancer cells that cannot down regulate TAAs, an alternative immuno-evasive strategy is employed—downregulation of MHC class I molecules by repressing MHC gene transcription, instability of MHC at the cell surface by reduction of $\beta 2$ microglobulin, or with proteasomal or transporter associated with antigen processing (TAP) deficiencies leading to poor peptide processing/loading [27]. Such strategy can be seen in various forms of human cancers (e.g. lung, breast, colon, head & neck squamous cell carcinoma). Such downregulation or loss of MHC class I expression in human cancer represents a poor prognosis. Nevertheless, downregulation of cell surface MHC class I molecules attracts the attention of Natural Killer (NK) cells. NK cells patrol the body looking for cells that have reduced their cell surface display of MHC molecules. Decreased level of MHC class I molecules or total loss prompts destruction of cells by NK cells. This phenomenon explains why certain cancers block a minute fraction of surface MHC class I molecules. Perhaps this small fraction of surface MHC molecules prevents NK cell attack, but strategies to upregulate MHC I expression would be a consideration [28].

Another way by which abnormal clones evade NK cells is by repressing NKG2D ligands—stress-signaling proteins displayed by cells in stressful situations such as viral infections and neoplastic transformations. NK cells display a receptor on its surface (NKG2D) capable of recognizing various stress signals such as MICA and MICB. Binding of the NKG2D receptor to stress signals results in activation of NK cell's cytotoxic response and rapid killing of cells expressing the stress signals. Abnormal clones then can repress expression of NKG2D ligands in various ways, such as by secretory release or shedding of NKG2DLs [29] or regulation of expression at several levels [30]. The shedding strategy employed by human melanoma cells allows for continued expression of stress signals such as MICA, but as decoy ligands—the stress signals are released in surrounding medium instead of being displayed on cell surface. This diverts the attention of NK cells and CTLs from the ligands displayed on the cells surface.

Macrophages also play a role in tumor elimination responding to several signals on the surface of tumor cells. One protein expressed on the surface of various normal cells throughout the body, CD47, is used to protect the cells from random attacks by macrophages. Consider the downregulation of CD47 in erythrocytes as they progress through their life cycle. Such downregulation ensures that older cells are discarded by macrophages. Circulating malignant mammary cells have used CD47 to their advantage; by over-expressing CD47, they are able to evade the innate immune system acting via macrophages [31].

Not only can tumor cells evade the attacks of the immune system, but also they can launch counterattacks on lymphocytes. Cytotoxic lymphocytes utilize the Fas Ligand (FasL) molecule on its surface to bind and activate Fas receptors on target cells. Activation of Fas receptors leads to activation of the extrinsic apoptotic pathway. Cancer cells acquire resistance to the FasL through mechanisms that are not well understood. This leads to abnormal clones that are resistant to destruction by cytotoxic T-lymphocytes. Additionally, they also acquire the ability to synthesize and secrete soluble forms of FasL. The secretion of FasL does not affect the already resistant strain; however, some studies have shown that they do affect some lymphocytes

leading to activation of extrinsic apoptotic pathway and lymphocyte death. This strategy ensures a safe microenvironment for the growing neoplasm [32].

In addition to FasL, some human cancers have been shown to secrete TGF- β or Interleukin-10 (IL-10). Both IL-10 and TGF- β are immunosuppressive agents secreted by normal cells of the immune system with TGF- β being the most potent immunosuppressive cytokine. TGF- β has many biological effects including the inhibition of 1) APC antigen presentation; 2) APC maturation; 3) T-cell activation and differentiation. Studies have shown that TGF- β is upregulated in glioma clones that are resistant to CTLs [33]. IL-10 on the other hand is capable of downregulating the expression of MHC class II antigens and Type 1 T-Helper (Th1) cytokines. The expression of IL-10 in glioma tissue has been correlated with tumor grade [34, 35].

In addition to CTLs and macrophages, tumor cells have been shown to recruit regulatory T-lymphocytes (Treg) to essentially fend off attacks by other lymphocytes. Treg cells are capable of suppressing T-helper lymphocytes and CTLs. These may be immature T cells committed to the regulatory lineage, or antigen-driven induced Tregs [36]. Research has shown that the percentage of Treg cells increases by 3-5 fold in cancer patients especially in tumor infiltrating lymphocytes (TILs). Furthermore, the degree of Treg infiltration correlates with tumor grade [37]. The ability of tumor cells to attract Treg cells depends on the secretion of chemokine CCL22. CCL22 acts on CCR4, a receptor on the surface of Treg cells to attract Treg cells towards the tumor [38, 39]. While present in the tumor, Treg cells are capable of indirectly suppressing both the humoral and cellular branch of the immune system through inactivation of T-helper cells. The existence of Treg cells in the tumor mass questions the association between the total number of TILs and tumor prognosis. TILs were assumed to be cytotoxic T-cells, but if a substantial fraction of TILs are Treg cells then this notion casts a shadow of doubt over the significance of TILs in tumors [40].

Myeloid-derived suppressor cells (MDSCs) are another cell type recruited by tumors to evade host immune defenses. MDSCs comprise a heterogeneous group of immature myeloid cells that play a major role in the immune suppressive tumor microenvironment (TME) [41]. As a part of their normal physiologic role, immature myeloid cells play a role in replenishing DCs and macrophages in early phases of trauma and stress and avoiding immune pathology in later stages [42]. The TME, however, influences local myeloid cells to become immunosuppressive [43]. Additionally, tumors initiate myelopoiesis, thus recruiting more immature myeloid cells [44]. MDSCs play a role in tumor progression by inhibiting T-cell response and their elimination has been shown to improve anti-tumor immunity [45]. Although treatment aimed at MDSCs could potentially be effective, efforts at characterizing MDSCs have been fruitless providing inadequate information to understand their phenotypical and functional heterogeneity.

3.4. Immunology of the CNS

The ability to restrict collateral damage caused by the immune response is essential, especially in the CNS. As a result, a status of immunological privilege is maintained in the brain limiting the magnitude of the immune response and inflammation.

Due to the delicate nature of the cells composing the central nervous system (CNS), the blood brain barrier (BBB) tightly controls molecular passage and cellular migration in and out of the CNS. The BBB is composed of both capillary and post capillary vessels. The ability of the BBB to tightly regulate passive diffusion of hydrophilic molecules results from the selectivity of the tight junctions (TJ) between endothelial cells in the CNS vasculature [46]. Consequently, the BBB has been implicated in the regulating the immune response in the CNS by restricting molecular access to cerebral interstitial fluid (CIF).

As discussed above, the activation of the immune response is maintained throughout the body: APCs uptake antigen, migrate to lymphatics, appropriate T-helper cells and CTLs are activated. However, professional antigen presenting cells (APCs) such as dendritic cells in the systemic circulation have not been described in CNS parenchyma, but DCs are present in vascular-rich regions of the CNS [47]. Instead, microglia are the primary resident APCs in the CNS [48]. Microglia express MHC class II antigens and T-cell co-stimulatory molecules giving them the ability to present antigens to T-helper cells. Once antigens are taken up by APCs in the CNS, presentation of the antigen seems to take place in the cervical lymph nodes (Dunn et al., 2007). T-cells are not normally found in the brain unless activated [49], but T-cells and antibodies do have access to the brain [50].

Migration of leukocytes towards the CNS starts with the interaction between leukocytes attracted to chemokines and adhesion molecules on endothelial cells. The chemokines secreted by the site of inflammation activate G protein-signaling, thus activating the leukocyte and up-regulating integrins. Through a tight interaction involving adhesion molecules on lymphocytes and endothelial cells (VCAMs, ICAMs, and LFAs), the cells transmigrate into the parenchyma [51].

4. Neoplasia in the CNS

4.1. Glioblastoma multiforme (GBM)

Brain tumors exist as two distinct types, malignant and benign. This chapter will focus malignant tumors originating in the brain, primarily glioblastoma multiforme (GBM). Tumors originating from astrocytes/glia cells are named gliomas with (GBM) being the most common and aggressive primary adult brain tumor. GBM is a grade IV astrocytoma arising from astrocytes and is characterized by central areas of necrosis surrounded by anaplastic cells. Median survival time is less than 15 month and significantly less for patients with recurrent tumors [52]. GBM can present as primary or secondary tumor. Primary GBM is generally seen in older patients as a result of EGFR overexpression, PTEN mutations, and mdm2 gene amplification [53]. Primary GBM is thought to be a single step transformation with no clinical background. Secondary GBM is seen in younger patients as a slow multi-step transformation process. Secondary GBM results from p53 inactivation or overexpression of PDGF ligand, receptor, or both [54].

4.2. Glioma immunity vs immune suppression

A number of potential TSA and TAA have been identified in gliomas (a listing may be found here [55]) including a number of antigens previously found in melanoma (eg, gp100, MAGE-1 and-3, MART-1, NY-ESO-1, tyrosinase and related proteins 1 and 2). Others include tenascin-C, IL13R α 2, EphA2, and EGFRvIII. Whole proteins or peptides derived from these antigens could be used in vaccine scenarios with a goal of providing antigen to APCs (presumably DCs), usually in the context of an adjuvant or immune stimulant to promote antigen uptake and activation of the DCs. Alternatively, the DCs may be harvested as progenitors from patients, differentiated and bulk-proliferated ex vivo, supplied with antigen, and then returned to the patient as a cellular vaccine. In other scenarios, lysates or particular proteins that may “sample” the antigenic peptide repertoire of the tumor (eg, heat shock proteins or chaperone proteins) [56, 57] may be employed to provide “blanket” immunogenicity by theoretically supplying all antigens rather than selected ones.

Unfortunately, gliomas are capable of employing some or all of the immunoevasive strategies discussed above. Patients with malignant gliomas often have weak adaptive immune systems due to the increased percentage of Tregs [58, 59]. In addition, malignant gliomas have been shown to secrete TGF- β and VEGF capable of direct immune suppression as well as inducing myeloid-derived suppressor cells [60]. VEGF has been shown to inhibit NF- κ B signaling in hematopoietic progenitor cells, thus inhibiting dendritic cell maturation.

4.3. Current therapies

Available therapies for GBM and other brain tumors include chemotherapy, fractionated radiotherapy, and image-guided tumor resection. Current chemotherapeutic options for glioblastoma include Gliadel wafers (carmustine/BCNU), cisplatin, and temozolomide (the drug that is part of the standard of care regimen concurrent with radiation, and then in the adjuvant setting [52]).

Carmustine is an alkylating agent and was the first drug approved for the treatment of GBM. Carmustine inhibits cancer growth via alkylation of O6-guanine position on DNA and thus crosslinking the helix. Carmustine has shown modest improvement in patient survival in early trials (reviewed here [61]) and has been the cornerstone of GBM adjuvant therapy. Although carmustine can cross the blood brain barrier (BBB), delivery to target site is difficult. Additionally, carmustine effectiveness hindered by its short half-life, systemic toxicity and tendency for chemo-resistance.

For better delivery of carmustine to action site, Gliadel wafers are used. Gliadel wafers are made of carmustine-loaded biodegradable polymers placed in the resection cavity formerly occupied by the tumor post-surgical excision. As the polymer is degraded, carmustine is slowly released. A study conducted by Westphal et al. in 2003 [62] showed that Gliadel-treated patients had a median survival rate of 13.9 month compared to 11.6 month in placebo controls. The complications of Gliadel wafers are serious and life threatening--seizures, edema, and hydrocephalus [63, 64].

The current standard of care for GBMs is maximal surgical resection followed by concurrent fractionated radiation and temozolomide (TMZ), with TMZ then given in the adjuvant setting

[52]. There are variations on this theme, including the addition of the anti-VEGFA antibody bevacizumab (as a form of anti-angiogenesis), but surgery, radiation, and TMZ are the staples [65]. TMZ is an oral DNA alkylating agent capable of crossing the BBB and inducing apoptosis [66, 67]. Attempts at combination treatments using TMZ and other chemotherapy drugs have shown little or no benefit when compared to TMZ alone [68]. TMZ efficacy varies, however, in patients depending on the action of the enzyme repairing the lesion produced by the drug, O6 methyl guanine DNA methyl transferase (MGMT) [69].

Cisplatin is a cis platinum complex containing 2 chloride and 2 amine groups. Once in the body, cisplatin triggers apoptosis by crosslinking DNA. Cisplatin's efficacy on brain tumors has shown few benefits. A phase 3 trial showed no significant outcome improvement in patients administered carmustine and radiation therapy versus cisplatin, carmustine, and radiation therapy [70].

4.3.1. Understanding Glioma-Associated Antigens (GAAs)

Considering the low median survival time, current therapies are inadequate and there is a strong need for novel therapies with superior safety and efficacy. In order to develop new therapies, it's important to be familiar with potential glioma-specific molecular targets. In light of the potential utility of immunotherapy as a novel therapeutic strategy for patients with GBM, this section will discuss Glioma-Associated Antigens (GAA), some of which were briefly mentioned above.

IL13R α 2

IL13R α 2 is a glycoprotein overexpressed on the surface of many glioma cells. The only other normal tissue where IL13R α 2 can be found is in the testes; therefore it represents a great potential target for glioma therapy [71].

EphA2

A receptor tyrosine kinase overexpressed on the plasma membrane of gliomas and tumor-associated vasculature. EphA2 is thought to play a role in developmental processes and carcinogenesis. EphA2 has been shown to provoke a response from cytotoxic T-lymphocytes against glioma clones [72].

EGFRvIII

Type III variant mutation of EGFR (EGFRvIII) is seen with patients with primary and recurrent GBM and is currently the most prevalent TSA found on glioma. EGFRvIII promotes and enhances carcinogenesis [73]; EGFRvIII encodes a constitutively active tyrosine kinase that does not need to dimerize nor bind ligand for activity [74, 75].

Survivin

Although not specific for gliomas, survivin belongs to the inhibitor-of-apoptosis protein family that is overexpressed in the majority of human cancers [76]. It is considered a marker of poorer prognosis in patients with GBM [77].

WT1

Wilm's Tumor 1 (WT1) is a transcription factor that is important in the developmental biology of many organs; its mis-expression may drive epithelial-to-mesenchymal transitions prevalent in many cancers [78]. WT1 is overexpressed in solid tumors, leukemias, and gliomas [79]. WT1 has both oncogenic and tumor suppressor capacities depending on mutational status, over-expression, and tissue source [80]. It is considered to be a viable tumor antigen with multiple applications [81].

SOX

Sry-related high mobility group box (SOX) is a family of transcription factors involved in directing the development of various tumors and cell types [82]. SOX2, SOX5, SOX6, and SOX11 are preferentially overexpressed in tumors of the CNS [83] and in glioma stem cell lines [84].

5. Immunotherapy

Understanding how cancer escapes the immune system provides clues for researchers and clinicians to intervene at critical points and empower the immune system. Although the field of cancer immunotherapy has much to prove, it has the potential to treat various forms of cancer with high specificity and relatively low toxicities. The superior therapeutic specificity in particular makes immunotherapy an attractive, tolerable alternative or possible adjuvant to chemotherapy for patients. Available cancer immunotherapeutic options include vaccines, monoclonal antibodies, adoptive cell therapy (ACT), and cytokine therapy, and the so-called "checkpoint blockades".

5.1. Glioma vaccines

Vaccines harness the immune system and allow it to target glioma-associated antigens via the processes of antigen provision/uptake to APCs, followed by the stimulation of specific lymphocytes by the APCs. The field of antitumor vaccines is one of the most studied and established modalities of immunotherapy. There are 3 general types of glioma vaccines: whole cell/tumor lysate vaccines, peptide-based vaccines, and dendritic cell vaccines. These vaccines may be used with various adjuvants or immune stimulants; they may be used amidst the standard of care; they may involve additional immune stimulation or regulatory suppression strategies.

Whole cell glioma vaccines involve administration of irradiated allogeneic or autologous glioma cells providing multiple GAA for the immune system to target. Obviously, autologous whole cell vaccines would provide the most personalized treatment using GAAs specific to each patient. On the other hand, such tumor cells may also provide tumor suppressive entities [85]. Manufacturing whole cell vaccines, however, is a very demanding task especially when handling large-scale glioma cell cultures, and when time is of the essence for patients with short times to progression [86]. Additionally, this approach puts patients at a theoretical risk

for autoimmune encephalomyelitis [87]. However, several autologous cell vaccine trials by Schneider et al among others have reported no major adverse effects [88].

An alternative to whole cell glioma vaccine and their associated autoimmunity risks are peptide-based vaccines. Peptide-based vaccines use synthetic peptides based on shared GAA epitopes. Unlike whole cell glioma vaccines, peptide-based vaccines target one or few GAAs specific to each patient's tumor. This may be beneficial in generating higher levels of specific responses to a particular antigen; it may also provide an easier outlet for immune escape by antigen loss. However, this strategy does present a lower risk for development of autoimmunity, and peptide manufacturing is relatively simple compared to handling large-scale glioma cell cultures. Peptide-based vaccines targeting EGFRvIII have been shown to be safe and potentially beneficial in the ACTIVATE (A Complementary Trial of an Immuno-therapy Vaccine Against Tumor-Specific EGFRvIII) series of trials (including ACT II, ACT III, ACT IV, ReACT [89, 90]). The ACTIVATE phase II trial recruited 18 patients with EGFRvIII expressing tumors that received CDX-110 (14-amino acid EGFRvIII epitope conjugated to a keyhole limpet hemocyanin as a hapten carrier with GM-CSF as an adjuvant) along with standard radio- and chemotherapy. The median time to progression was 14.2 month and median survival was 26 month; no adverse events were recorded. ACT IV is a phase III trial comparing vaccine/GM-CSF+TMZ vs TMZ and placebo alone (see Table 1).

A somewhat different version of peptide-based therapy is that of chaperone protein or “heat shock protein” (HSP)-based vaccines [56, 57]. The concept here is that chaperone/HSPs bind intracellular peptides as part of their chaperone duties. Thus, purification of particular chaperone/HSPs from tumor cells results in a population of peptides that are specific to that particular tumor, even though the chaperone/HSPs may appear identical. Upon vaccination, this would provide APCs with a “peptide fingerprint” of the tumor, making the vaccine tumor — and patient — specific, even if the chaperone proteins were ubiquitous in different cell types (see Figure 3). The current chaperone protein vaccine in use for GBMs is variously called Prophage, Oncophase, and HSPPC-96; the protein purified from tumors for vaccine generation is glucose-regulated protein (GRP) 94, also called glycoprotein (gp) 96.

A key common feature of most peptide-based vaccines is the inclusion of immune stimulatory factors as adjuvants (eg, DNA constructs such as polyICLC [91], GM-CSF [92], Freund's adjuvant [93], TLR agonists [94], and cytokines [95]). This is necessary because the lack of APC stimulation will result in poor activation of T cells, with possible conversion to an anergic state. The chaperone/HSP vaccines utilize the “danger signal” effects of extracellular HSPs as innate immune stimulators to essentially provide their own adjuvanticity [96]. An advantage of peptide antigens of known sequence is that provides investigators with a means of immune monitoring by measuring and tracking the immune response, by T cell readouts [97] and sometimes by antibody responses [23]. One question that remains is whether results from immune monitoring yield true prognostic information, as often there is a positive correlation with immune measures of vaccination, but these do not necessarily appear related to clinical benefit [98].

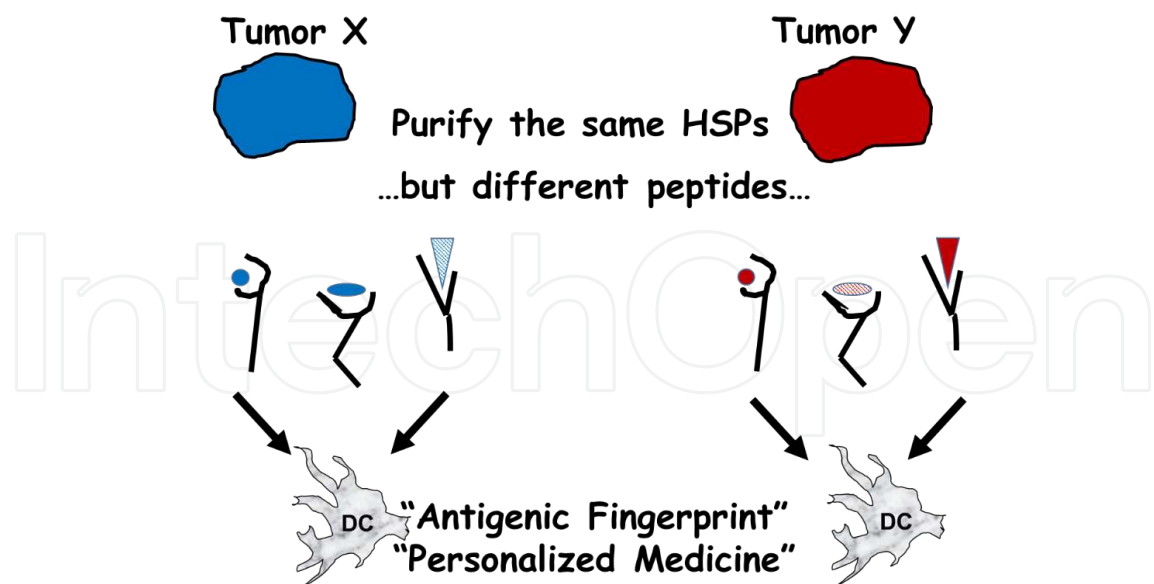


Figure 3. Chaperone/Heat Shock Protein (HSP) vaccines from tumors. Various HSPs have been shown to be immuno-genic when purified from tumors. However, purification of the same HSPs from different tumors does not generate cross-protection (eg, if HSPs are purified from Tumor X and used as a vaccine, but the host is challenged with Tumor Y, there will be no anti-tumor protection, because the antigenic peptide repertoire differs between the two tumors, even if the HSPs appear the same).

Trial Name	Phase	n	Therapy	Primary Outcome	Trial Identifier
Vaccine therapy + sargramostim	I	9	ISA-51/survivin peptide vaccine + sargramostim	Safety	NCT01250470
HSPPC-96 vaccine + Temozolamide	II	55	HSPPC-96 + Temozolamide	Safety	NCT00905060
ACT III	II	82	CDX-110 1 TMZ 1 RT	Progression-free survival	NCT00458601
ACT IV	III	440	CDX-110 1 TMZ	Overall survival	NCT01480479

Table 1. Summary of ongoing peptide-based vaccine trials for malignant glioma. Data obtained from National Institutes of Health. Information is available by Trial Identifier at <http://www.clinicaltrials.gov>.

5.2. Antibodies

Antibodies are utilized in cancer immunotherapy usually as human, humanized, or mouse-human chimeric monoclonal antibodies (mAb) that target tumor-associated antigens (TAA) and tumor-specific antigens (TSA), generally on the surfaces of cancer cells, or targeted against secretory molecules. There are 2 categories of mAbs most frequently used: naked and conjugated antibodies. Naked antibodies work independently without an attached radiolabel or toxin. Once bound to the target cell, naked antibodies mark cells to be eliminated by the immune system, usually neutrophils and macrophage. If such mAbs target surface receptors, they may interfere with ligand binding or downstream function. Conjugated or “armed”

antibodies, on the other hand, are mAbs joined to a toxin, chemotherapy drug or a radioactive particle. Conjugated antibodies are essentially vehicles specific for targeting abnormal clones, sparing normal cells from toxic therapy. Regardless of the type, it is crucial that the mAb bind with high affinity and specificity to target [99]. Monoclonal antibody therapy has been successful in treating lymphomas (rituximab), breast cancer (trastuzumab), and more recently, recurrent glioblastoma (bevacizumab). Malignant gliomas are vascular tumors producing vascular endothelial growth factor (VEGF), which promotes angiogenesis and tumor progression. Bevacizumab is a humanized monoclonal antibody against VEGFA administered in combination with chemotherapy [100]. Inhibition of VEGF via bevacizumab followed by cytotoxic chemotherapy and radiation therapy has generated encouraging results in several studies [100].

The use of mAbs to treat GBM has been investigated in preclinical systems. Y10, anti-EGFRvIII naked murine IgG2a, significantly increased survival time in mice bearing EGFRvIII expressing tumors by an average of 286% [101]. Additionally, Y10 was shown to inhibit cell proliferation and DNA synthesis in vitro by complement activation and antibody dependent cell-mediated cytotoxicity (ADCC). Another type of anti-EGFRvIII (or “delta 2-7”) is mAb 806, which has also shown pre-clinical efficacy against EGFR-amplified tumors [102] and has shown good targeting capability and pharmacokinetics in a phase I study [103]. Anti-EGFRvIII mAbs such as L8A4 (murine IgG1) have also been radiolabeled and utilized in pre-clinical testing [104], including boronation [105].

Given the role of EGFR in the progression of malignant glioma, efforts to inhibit EGFR have culminated in phase I study conducted by Faillot et al which demonstrated the ability of murine anti-EGFR mAb EMD55900 to bind the tumor in vivo and is well tolerated in patients [106]. Repeated infusions only resulted in one patient developing human anti-mouse antibodies (HAMA response). Despite the substantial binding of EMD55900 to targets, phase I/II clinical trials using EMD55900 administered intravenously showed no significant tumor regression [107]. The use of another anti-EGFR antibody (chimeric humanized), cetuximab, showed good tolerability but limited efficacy in patients with recurrent GBMs [108]. A recently-developed chimeric humanized anti-EGFR antibody, nimotuzumab, is in clinical trials for malignant gliomas, but the studies are not mature enough to provide much information [109].

5.3. DC Immunotherapy

The superior antigen presenting ability of DCs has been harnessed in novel cancer vaccination strategies. DC vaccines take advantage of the antigen presenting machinery of these cells to activate CD4⁺ and CD8⁺ T cells that are specific for TAAs/TSAs. DCs are generated using autologous peripheral blood leukocytes that are incubated with specific cytokines to induce differentiation of monocytes into DCs; the type of cytokine used dictates the quality of DCs generated e.g. GM-CSF and IFN α generate DCs highly potent in T-cell activation. DCs are then pulsed with 1) synthetic tumor antigens (TAAs) or 2) autologous tumor lysate, or 3) tumor RNAs to promote activation and antigen presentation, and are injected back into the patient [110].

Phase I trials assessing safety have been carried out by Yu et al. in 7 patients (+2 with anaplastic astrocytomas) who received injections of autologous glioma peptide pulsed-DCs; these peptides were acid-eluted from tumor cell surfaces (presumably from MHC molecules). Four out of 7 patients developed tumor specific T cell cytotoxicity, and others had T cell infiltrates into their tumors at reoperation [111]. The Cedar Sinai experience in DC vaccine clinical trials is discussed here [112]. In another phase I trial done by Liao et al., 12 patients were administered DCs also pulsed with autologous glioma-eluted peptides from surface of resected tumors. Fifty percent of the patients showed increased systemic and intracranial immunologic responses while reporting no adverse effects from vaccination [113]. More recent studies by that group have utilized autologous tumor lysate as the antigen source due to its availability from surgical resection, whereas the use of specific GAAs requires patients to express suitable MHC molecules able to present the GAA [114]. Prins et al have also conducted a phase I trial using TLR7 agonists (imiquimod or polyICLC) combined with autologous tumor lysate-pulsed DC vaccination. Twenty-three GBM patients were enrolled showing a mean overall survival of 31 months and 47% 3-year survival [115]. The use of DC vaccines pulsed with tumor-derived RNAs (total polyA+RNAs or specific mRNAs) was demonstrated almost 20 years ago [116], and this strategy has made it to clinical trials for patients with GBMs [117]. An advantage of RNA as a source of antigen is that it can be prepared by standard conditions, or synthesized cheaply.

DC-based vaccine trials have been prevalent in neuro-oncology, and many of those are reviewed here [110], along with a listing of current DC vaccine trials in glioma (Table 2).

Trial Name	Phase	n	Therapy	Primary Outcome	Identifier
ATTAC	I	16	CMV pp65-LAMP mRNA-loaded DCs	Safety	NCT00639639
NY-ESO-1 intranodal vaccine	I	30	DEC-205-NY-ESO-1 fusion protein + sirolimus	Safety	NCT01522820
Vaccine therapy for recurrent GBM	I	50	BTSC mRNA-loaded DCs	Safety	NCT00890032
Phase I study of DC vaccine	I	40	Allogenic stem cell lysate	Safety	NCT02010606
DC vaccine for patients with brain tumors	II	60	Autologous tumor lysate + adjuvant	Most effective combination of DC vaccine components	NCT01204684
ICT-107	IIb	200	TAA	Overall survival	NCT01280552
DCVax-L	III	300	Autologous tumor cell lysate	Progression-free survival	NCT0045968

Table 2. Summary of ongoing DC vaccine clinical trials for malignant glioma. Data obtained from National Institutes of Health. Available at <http://www.clinicaltrials.gov>.

5.4. Adoptive Cell Therapy (ACT)

ACT is the manipulation of autologous cells *ex vivo* to be infused back into the patient. ACT aims to amplify cell lines that best fight cancer including 1) TILs 2) peripheral blood mononuclear cells (PBMCs) 3) lymphokine-activated killer cells (LAKs) and 4) antigen specific CTLs.

ACT originated as a way to restore viral immunity in patients undergoing hematopoietic stem cell transplant and to prevent cytomegalovirus (CMV) reactivation in transplant patients. Early use of adoptive cell transfer to treat non-viral malignancies was seen in attempts to treat hematologic malignancies and melanoma. Steinbok et al. was the first to demonstrate the possibility of ACT in treatment of gliomas in 1984 [118]. Steinbok collected autologous PBMCs to be re-infused into the cavity created post tumor excision. The study, however, showed no significant beneficial outcomes. Newer technologies and a better understanding of the cells involved have made ACT an attractive approach. However, the *ex vivo* generation of large quantities of specifically reacting cells is cumbersome, expensive, and not necessarily available everywhere.

5.4.1. Cytotoxic T-Lymphocytes

Based on the findings that CTLs can bypass the BBB and migrate into brain parenchyma (reviewed here [119]). GAA-reactive CTLs isolated from peripheral blood and glioma tissue might mount a favorable antitumor response in glioma patients. Glioma tissues are infiltrated with GAA-specific CTLs that could be expanded *ex-vivo* using IL-2 and subsequently selected for antigen specificity [120]. Once GAA-specific CTLs have been isolated, *ex-vivo* manipulations include cloning high affinity TCRs, expression of chimeric antigen receptors (CARs), and T-cell subtype selection. Cloning high affinity TCRs is done by isolating CD8⁺T cells with high GAA affinity for particular antigens and cloning TCR α and β genes to be exogenously induced in bulk CD8⁺ cells. ACT using high affinity TCR T-cells has resulted in regression of metastatic melanoma [121], suggesting the potential of applying this method to glioma therapy. ACT of various types using CTLs has a history in glioma trial therapy, but none have shown consistent benefit [122].

Chimeric Antigen Receptors (CARs) are chimeric molecules composed of epitope-binding domain of mAb fused to CD3 signal transduction domain (Gross et al., 1989). CAR is an alternative to transgenic high-affinity TCRs and don't require the expression of MHC molecules. CAR-T cells have just reached clinical trial stage in GBMs (NCT02209376 at ClinicalTrials.gov) targeting EGFRvIII. Other targets include IL13R α 2 [123], HER2/Neu (EGFR2) [124], and 3rd generation anti-EGFRvIII cells [125].

5.4.2. LAK cells

Lymphokine-Activated Killer (LAK) cells are activated prior to exposure to IL-2 *in vitro*. LAK cells possess cytotoxic machinery capable of lysing abnormal clones (allogeneic and autologous) sparing normal cells. As a result of the toxic systemic effect of IL-2 administration, *in vivo* human trials using LAK cells are limited and researchers have resorted to *ex vivo* LAK trials, with an occasional long-term survivor [126]. Administration of LAK cells into post

excision cavity combined with IL-2 therapy has shown to increase median survival from 26 weeks to 53 weeks in patients with recurrent GBM [127]. LAK cells, however, have limited specificity to tumors *in vivo* and their tumor recognition mechanisms are not well understood.

5.4.3. *Lymphodepletion*

Lymphodepletion is the process killing white blood cells to enhance adoptive transfer therapy possibly in vaccination scenarios. Lymphodepletion induces homeostatic proliferation, a situation where lymphocytes proliferate in an enriched cytokine environment and with lowered thresholds of stimulation and decreased numbers of Tregs [128]. Lymphodepletion has a long history in the ACT therapies in melanoma, where there have been objective response rates of greater than 50% [129]. There is also a suggestion that the lymphopenia induced by TMZ chemotherapy may actually benefit anti-tumor vaccination by driving homeostatic proliferation [130], and this may aid in Treg depletion by mAb blockade [131]. Dose-dense TMZ regimens may induce more myelotoxicity, however [132].

6. Virotherapy

Oncolytic virotherapy is the use of genetically engineered viruses for the treatment of malignancies. Oncolytic viruses are engineered to specifically infect malignant cells, thus sparing the normal tissue surrounding the tumor [133]. The intracellular replication of the oncolytic virus then results in cancer cell lysis and release of viral progeny that infects additional cancerous cells [134]. The use of oncolytic viruses for GBM treatment has been tested in clinical trials for the last 15 years with multiple phase I trials completed and some ongoing, proving to be a safe option. Two treatment strategies exist in virotherapy 1) replication-incompetent viruses and 2) replication-competent viruses. Replication-incompetent viruses exert their therapeutic effect through the delivery of transgenes that exert their effect through multiple mechanisms, one of which is discussed below [133]. Replication-competent viruses exert their therapeutic effect via replication and lysis of target tumor cells [133].

Concerns about uncontrolled viral infection in hosts led researchers to use replication-incompetent viruses for initial attempts in oncolytic virus therapies. Replication-incompetent adenoviruses and retroviruses are then engineered with the herpes simplex virus thymidine kinase gene (HSV-TK), which produces a cytotoxic metabolite from the drug ganciclovir [135]. Ram et al. used a replication-incompetent retrovirus engineered with HSV-TK for the first phase I clinical trial [136]. Fifteen patients received viral injections to the tumor site and oral ganciclovir with no adverse effects. Median survival time was 8.1 month; however, the lack of statistical significance in phase III trials [137] led researchers to study replication-competent oncolytic viruses (OVs).

OVs are attenuated viruses capable of lysing target tumor cells and activation of the local immune response to tumor antigen release [138]. There currently exist 4 different OVs used in published clinical trials. Herpes Simplex Virus G207 is among the OVs used in clinical trials. G207 is unable to replicate in normal cells because it lacks ribonucleotide reductase. In the first

of 2 separate phase I clinical trials, of the 21 patients injected with G207, 8 patients demonstrated reduction in tumor size shown on MRI scans [139]. In the second trial, investigators demonstrated successful replication of the virus in the tumor [140]. Although safe, OV_s tested to date have proven less efficacious than expected. However, there are efforts to create new viruses equipped with cytotoxic agents and other viruses specifically targeting the stem cell population of GBM [141, 142]. There are also studies showing safety testing of a polio virus-rhino virus chimera that shows high targeting capacities for tumors (due to overexpression of the poliovirus receptor NECL5) and replication within tumor cells due to putative biochemical abnormalities [143, 144]. There is a newly-opened trial based on this virus (NCT01491893 at ClinicalTrials.gov). Other clinical trials involving viral therapy are listed in Table 3.

Trial Name	Phase	Viral Modifications	n	Primary Outcome	Identifier
MV-CEA for recurrent glioblastoma	I	Carcinoembryonic antigen-expressing	40	Safety, MTD, viral propagation and expression	NCT00390299
NDV-HUJ in glioblastoma	I/II	HUJ strain	30	Progression-free survival	NCT01174537
DNX2401 and TMZ in recurrent glioblastoma	I	Mutation in E1A and RGD-related integrin expression	31	No. of patients with adverse events	NCT01956734

Table 3. Selected ongoing oncolytic viral clinical trials for malignant glioma. Data obtained from National Institute of Health. Available at <http://www.clinicaltrials.gov>.

7. Immune checkpoint blockade

As mentioned above, certain molecules expressed on T cells, with ligands or counter receptors expressed on tumors or Tregs, may provide an avenue of control over maintenance of an activated T cell status or denial of a suppressive effect. One such T cell surface molecule is cytotoxic T lymphocyte antigen 4 (CTLA4), which is a receptor that downregulates T cell activity by binding to CD80 and/or CD86 on other T cells, particularly Tregs. Ipilimumab is an antibody that binds to CTLA4 and prevents its binding to CD80/86, thus maintaining the activated state of the T cell (and running some risk for autoimmunity) [145]. This antibody is approved for the treatment of therapy-resistant metastatic melanoma [146], and has been in clinical trials for patients with brain metastases [146]. There is currently a clinical trial open for patients with recurrent GBM utilizing this drug (NCT02017717, at ClinicalTrials.gov) as well as the anti-PD-1 antibody nivolumab (below).

PD-1 and PD-1L/PD-2L are another pair of T cell inhibitory receptors/ligands. The ligands PD-1L or PD-2L are often upregulated on cancer cells, where engagement with PD1 on T cells leads to T cell apoptosis [147]. Nivolumab is an antibody directed against PD-1 that prevents interaction with surface ligands on other cells, thus preventing the immune suppression

induced by the tumor [147]. As mentioned, this antibody is used along with ipilimumab as checkpoint blockades in a clinical trial for patients with recurrent gliomas.

8. Conclusion

Glioblastoma Multiforme (GBM) remains the most common CNS malignancy with abysmal prognosis. Our current GBM therapies are clearly inadequate stressing the need for new treatment modalities. Immunotherapy is an emerging cancer treatment, potentially utilized alongside surgery, radiation, and chemotherapy. The dynamic interactions within the tumor microenvironment dictate the balance between tumor elimination and escape. Additionally, high-grade gliomas employ a multitude of strategies to evade the immune system. Early trials, however, using anti-tumor vaccines and adoptive cell therapy have demonstrated the potential anti-tumor efficacy and feasibility of such approaches. Moreover, several promising phase III trials are underway, and continual research provides more information and more data that indicate immunotherapy is a viable option for the treatment of patients with these devastating diseases.

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