Immunotherapy of Malignant Gliomas Using Autologous and Allogeneic Tissue Cells

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Abstract: Immunotherapy of brain tumors is rapidly emerging as a potential clinical option [1-3]. The quality and magnitude of immune responses evoked by the new generation anti-tumor vaccines is in general highly dependent on the source or choice of peptide antigens, and as well, a suitable immunopotentiator. Poorly immunogenic antigens, such as those present in tumor cell lysates, may not reliably provide stimulation like recombinant or DNA-encoded protein antigens might be expected to. In addition, the efficacy of the vaccine may depend on inherent counteracting measures of the tumor which dampen immune surveillance and immune effector activity triggered by immunization [4]. Our body has many means of limiting an immune response to our own (self) proteins. In particular, patients with gliomas exhibit a broad suppression of cell-mediated immunity [5-8]. Unfortunately, for most tumor vaccines the induction of local or systemic immune effector cells does not necessarily translate into objective clinical responses or increased survival [9]. Here we review immunotherapeutic approaches against gliomas and recent pre-clinical and clinical initiatives based on cellular or active immunization of the patient's immune system using autologous and allogeneic tissues or cultured cells. Available evidence shows that single modality cancer therapies likely remain suboptimal. Combination regimens targeting the immune system at multiple coordinated levels must be developed, and possibly combined with strategies to inhibit immune suppressive factors if significant clinical benefit is to be achieved.

Keywords: Astrocytoma, allogeneic, biotherapy, brain tumor, CTL, glioma, immunotherapy, immunization.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common and malignant of all gliomas, with 75% of patients dying within 18 months of diagnosis. Brain tumors are graded according to their likely rate of growth, from grade I (benign) to grade IV (most malignant), with grades III and IV considered high-grade gliomas. Grade IV glioma is also known as glioblastoma multiforme (GBM). The prognosis for patients with this tumor is very poor. Glioblastoma overall survival rates are less than 3.3% at 5 years [10]. In the United States alone over 18,000 primary brain tumors are estimated to occur each year. Of these 18,000, over 60% are diagnosed gliomas. The median survival time of untreated GBM tumors is 3 months, with death most commonly due to cerebral edema or increased intracranial pressure. Even with the best available current therapy, which includes surgery, radiation, and chemotherapy, the median survival time is 14.6 months [11]. Due to their highly infiltrative nature complete surgical resection is difficult. These tumors are, therefore, inevitably recurrent either locally, usually within 2 cm of the original tumor, or at distant sites. Treatment of these recurrent lesions by a second surgery and further chemotherapy may increase the symptom free interval, but the 5-year survival remains 10%. The present review discusses various typical immunotherapeutic strategies in a comprehensive way, with a focus on active therapeutic immune intervention. All these therapies will serve as additions to, rather than a replacement of current medical practice. Due to many examples it is impossible to cover all preclinical approaches and past or ongoing clinical trials. We will, therefore, address only the most promising or remarkable strategies, but omission from or inclusion into this review should not be interpreted as rejection or support of a particular approach; they are examples. We propose that immune adjuvant therapies need to be explored to improve median or overall survival.

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THE LONG ROAD TO CURRENT GLIOMA THERAPEUTIC VACCINE APPROACHES

The first report on cancer therapy dates from the Edwin Smith Papyrus, 1600 B.C., the oldest known historical text on surgery. It mentions primitive surgical excision of a tumor using a knife. It has only been for the past two hundred years that surgical excision became established as the preferred option for treating solid tumors. Radiation therapy was introduced shortly after 1895, when Roentgen first reported the use of x-rays for diagnostic medical purposes, and remained a primary treatment option of certain tumors, especially solid tumors. Today radiation therapy can be tailored to the irregular shape of the tumor, allowing a reduction of intensity. During World War I, exposure of soldiers to nitrogen mustard gases resulted in pancytopenia. This observation triggered interest for chemotherapeutic therapy, especially in patients with hematologic malignancies. Today chemotherapy is a well documented treatment option. For glioblastomas, the alkylating agent temozolomide has become a well-appreciated option [12].

About one hundred years ago William B. Coley, an early 20th century New York City surgeon, observed that some of his patients with sarcoma would undergo spontaneous regression of their tumor and that this regression was associated with antecedent bacterial infection (erysipelas, or streptococcus pyogenes in the first patient) which eliminated malignant cells [13, 14]. He intentionally injected cancer patients with a mixture of killed bacterial lysates, later called Coley's Toxins, to stimulate a cytokine storm. William Coley thus became the godfather of cancer immunotherapy [15]. He was the first to recognize the potential role of the immune system in cancer treatment. Activation of the patient's immune system to better recognize the tumor and to eliminate invasive tumor cells that are not adequately removed by surgery will be discussed in greater detail. This area in cancer therapy is based on knowledge accumulated in the vaccine and immunology fields.

Immunization attempts for glioma patients date back to the early 1960s [16]. Two investigative teams hypothesized that brain tumors were hidden from the immune system because of location in the central nervous system. To ensure that immune cells located systemically could "see" the tumor, they placed autologous glioma cells into the thighs of patients. The vaccines given into the thighs

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actually started growing there and metastasized to regional nodes [16, 17]. Ten years later, Bloom and colleagues administered irradiated, whole autologous tumor cell vaccines. Again, there was no effect, but the results may well have been different had they used an adjuvant, such as that used by Trouillas and Lapras [18] who immunized 20 patients with autologous tumor and Freund's complete adjuvant. These early attempts at active immunotherapy had obstacles consisting of potentially radiation resistant cells, and the absences of, or inappropriate, immune adjuvant. Degrees of sophistication in vaccine development have occurred in the past 50 years; and at last the benefits of active immunotherapy are finally documented.

KEY CONSIDERATIONS FOR DEVELOPMENT OF IMMUNOTHERAPY APPROACHES

Tumor Directed Immune Responses

Distinct from classical preventive vaccines, which mostly require the generation of antibodies, the goal of most therapeutic GBM vaccines is to stimulate tumor-specific cytotoxic T cells (CTL) to function at the tumor site. Over the past 20 years we have only begun to learn how to accomplish this. A key factor for the success of a vaccine concerns the requirement of expression of unique tumor antigens, preferably in the context of class I Major Histocompatibility Complex (MHC), on suitable target tumor cells. Upon stimulation of the immune response, the host can specifically target these antigens, by producing antibodies, or by activating immune effector cells which recognize specific antigens in context of MHC Class I antigens. Studies have shown that human glioma cells do express variable levels of class I MHC antigens, and that IFN-γ upregulates these class I antigens [19]. In addition, Fas is expressed on glioma cells [20], providing a possible mechanism for tumor cell death [20]. Therefore under the appropriate conditions, glioma cells can be a target of CTL.

Remarkably, recent animal experiments in various tumor models showed that apart from CD8-positive cytotoxic T cells, which are considered the current "gold standard" effector cell in tumor immunotherapy, CD4 T cells can eliminate tumors that are resistant to CD8-mediated rejection [21]. Indeed, CD4 T cells collaborated with natural killer (NK) cells to achieve this antitumor effect. These findings suggest that adaptive CD4 T cells can also be successful effector cells, and in some cases, even outperform CD8 positive T cells as mediators of cell death. This observation of efficient tumor elimination by CD4 T cells, using indirect mechanisms that do not require MHC expression by the tumor cell, suggests that it might be possible to attack even poorly MHC expressing tumors by designing strategies to elicit CD4 T cell responses against the tumorigenic proteins [21].

Adoptive Transfer of Effector T Cells

Adoptive transfer of lymphocytes into patients is classified as a variant of passive immunotherapy. Adoptive transfer of lymphocytes into brain tumor patients is not a new concept. Indeed, thinking that the blood brain barrier inhibited the passage of immune cells into the brain, one of the first clinical trials involved the adoptive transfer of autologous, unstimulated lymphocytes intrathecally into glioma patients, accompanied with or without interferon [22]. Since the advent of recombinant growth factors, adoptive immunotherapy now in principle, is based on the concept of infusing *ex vivo* expanded effector cells after their *in vitro* activation against tumor antigens and/or culture in growth factor containing media, back into the patient. The whole gamut of effector cells have been tested including autologous or allogeneic cells that are nonspecifically or specifically activated [23, 24]. In newly diagnosed malignant gliomas, this approach revealed several objective clinical responses without adverse effects [25]. Expansion of autologous tumor-specific T lymphocytes *in vitro* with IL-1, IL-2, and/or IL-4, followed by injecting directly into the tumor site gave response rates that were not consistently encouraging [26]. A further variant of this approach included genetic manipulation of such *ex vivo* isolated cells by the introduction of immunostimulatory transgenes [27].

Tumor Associated Antigens (TAAs)

One important step in producing a therapeutic vaccine is the identification of appropriate glioma-associated antigens. Limited knowledge of molecularly defined antigens explains why initial approaches used tumor lysates derived from autologous irradiated glioma cells as the source of tumor antigens. This approach warrants specific activation of the immune response against a broad set of potential tumor associated antigens, which may enhance immune therapy but may also result in immune reactions against unwanted antigens [28]. Glioma associated, rather than glioma specific, appears to be the more correct terminology since the antigens appear to be present at very low levels on normal brain cells, but overexpressed on glioma cells. Importantly, for the majority of studies no evidence of adverse autoimmunity was noted after such immunizations. The premise was that by providing the entire tumor cell, all the appropriate antigens would be made available to the antigen presenting cells of the immune system. Unfortunately, these procedures did not lead to successful therapies [26]. The advent of molecular biology enabled approaches aimed to identify the specific glioma-associated antigens (GAA) at the molecular level [3, 20, 29, 30]. Jadus and colleagues analyzed adult and pediatric brain tumor cell lines and some primary tissues for tumor-associated antigens [30, 31]. The glioma cell lines were characterized for 20 tumor-associated antigens by quantitative reverse-transcriptase real time polymerase chain reactions (qRT-PCR), and where antibodies were available, the protein expressions were confirmed microscopically using flourescently-tagged antibodies or by intracellular flow cytometry. In general, the glioma cell lines had high mRNA expression for the antigens and also made the proteins. Thus, primary malignant brain tumors and the cell lines express many tumor associated antigens; a truncated list of them is given in Table **1**. From these data we conclude that either primary tumor specimens or cell lines could serve as suitable sources for antigens for vaccine development. In general, the surgical specimens from the adult glioblastomas had a more robust antigenic profile than those from the pediatric tumor specimens. Since many of the tumor associated antigens display HLA-restriction, we also conclude that given the known HLA type of the tumor patient, one might predict which antigens might likely be associated with his tumor [30]. Certainly in conjunction with cytogenetic information, especially genomic imbalances, one might predict overexpression of certain antigens.

Interestingly, recent genomic studies have revealed the genes of human glioblastomas and provided insight into associated molecular pathways [32-34]. The genetic subtypes are associated with prognosis. These analyses may provide further refinement on potential glioma-associated target antigens. The Cancer Genome Atlas (TCGA) Research Network Comprehensive genomic characterization defines human glioma genes and core pathways [35].

The tumor antigens most suitable to activating the host specific T cell response are still under investigation. Prominent tumor-associated antigens that others have considered suitable for vaccine development include tenascin (glioma-specific extracellular matrix), gp240 (chondroitin sulfate-associated antigen found in glioma), MAGE 1 and MAGE 3 [36, 37]. For development of their vaccines, Okada *et al*. focused on epitopes that specifically bound to the class I HLA-A2 such as EphA2, IL-13Ra2, YKL-40 and GP-100 [29]. More specifically, the IL-13 Ra2 and EphA2-derived epitopes were shown to stimulate immune activity. The successful use of these antigens may also largely depend on the immunopotentiator system used for switching on and maintaining the specific immune reaction directed towards the antigen of interest [38]. This will be addressed later.

Table 1. Tumor-Associated Antigens Overexpressed in Primary Malignant Brain Tumors

Aim-2	Ezh2	HNRPL	Prame	Sox11	Ube2V
Art-1	Fosl1	IL-13Ra2	PTH-rP	SSX-2	Whsc2
Art-4	Gage-1	Mage-1	Sart-1	Survivin	Wt-1
B-cyclin	Galt-3	Mart-1	Sart-2	Tert	YKL-40
CD133	GnT-V	MELK	Sart-3	TRP-1	
EGFRvIII	Gp100	MRP-3	Sox 10	TRP-2	
Epha2	Her2	NY-Eso-1	Sox 2	Tyrosinase	

Activation of Antigen Presenting Cells

The activation of the cytotoxic T cells generally requires the stimulatory interaction with T helper cells, which recognize antigen in context of Class II expression. Therefore cells that express high levels of class II antigens are most efficient antigen presenting cells (APC) for triggering helper T cells. Although macrophages and microglia express Class II, dendritic cells (DC) are the most efficient antigen presenting cells [39]. Dendritic cells are also called "nature's adjuvant", and represent the key APCs for induction of primary immune responses. As sentinels they sample peripheral tissue for potential antigens and bring them to draining peripheral lymph nodes to present the processed antigens to potential antigen-specific T lymphocytes.

Therefore events that trigger the maturation and activation of these cells under natural conditions at the tumor site, or in the cervical lymph nodes are considered valuable for natural tumor surveillance. By contrast, maturation of APCs at the site of the vaccine inoculums or in the local draining lymph node are likely important for producing an effective vaccine [38]. There is substantial evidence that DCs exposed to tumor antigens under the right circumstances are potent stimulators of cytotoxic T cells.

Dendritic Cell Vaccines

In recent years there have been numerous attempts to use dendritic cells in therapeutic "vaccines" [3, 40-42]. The isolation of dendritic cells by elutriation enabled the handling and *in vitro* loading with antigens. Unfortunately, there is no consensus on the best protocol to isolate and use DCs; different DC subtypes may exert distinct functions and efficacy. Recently, several dendritic cell vaccines have been tested in glioblastoma immunotherapy. These were based on either crude tumor lysates or acid-eluted peptides from cell cultures derived from surgically removed glioblastoma multiforme [9, 43-45]. In some patients, a peripheral CTL response was detected. However, only sporadic objective responses and modest increases in the patient's survival were observed, with no long-term survivors, from these approaches [9, 43-45]. Importantly, no evidence of adverse autoimmunity was noted after these immunizations.

Siesjo *et al.* showed that co-administration of autologous tumor cells with autologous DC decreased tumor growth [46]. Liau *et al.* demonstrated that autologous DC incubated with autologous tumor protein increased survival, and increased tumor-specific cytotoxic T cells within the tumor [9]. These initial studies that were performed in only a few patients led to clinical trials. Different methods to activate these DC were explored. Expanding the immune response was first approached by using non-specific immunomodulators, such as BCG, IL-2, interferon (IFN)-α, which had been registered as approved immunostimulants.

Immunopotentiators Co-Administered with Tumor Antigens

As mentioned earlier the success of glioma immunotherapy will depend on better understanding of glioma biology but also from lessons learned from the vaccine and immunology fields [47]. It is beyond the scope of this review to mention the plethora of distinct immunopotentiators used in various pre-clinical and clinical settings, in most cases with different types of antigen formats.

Here we briefly mention a few types which employ in most cases immunostimulants that were approved for human application. In a rat model, IL-4 was shown to have the most potent therapeutic results, mediating local endothelial cell activation, recruiting immune T cells, and stimulating antibody production [45]. IFN-α was also a possible candidate because this cytokine stimulated endothelial cells to produce CXCL-10, a chemokine shown to induce homing of cytotoxic T cells (Tc1) to the tumor site [48]. Granulocyte macrophage-colony stimulating factor (GM-CSF) has also been used as an effective adjuvant to attract large numbers of antigen presenting cells to the vaccination site [25]. The heat shock protein (HSP) 70, in particular, has been shown to directly activate NK cells and indirectly stimulate the dendritic cell population; in addition, this extracellular secreted product induces the release of IFN-γ from peripheral blood leucocytes of tumor patients thereby further stimulating the immune response [49]. Other studies showed that poly I:C, the ligand for the toll-like receptor (TLR)3, was effective in triggering the maturation and functional activity of DC [50]. Further studies used lysine and carboxymethyl cellulose stabilized poly I:C (poly ICLC) to activate cells, however no significant advantage to survival in humans was reported [29]. Yet another approach used CpG-oligonucleotide (ODN), which binds to TLR9, and thereby stimulates immune responsiveness [7]. Although no registered effective treatments are currently available, sporadic positive clinical responses were observed following immunization with vaccines prepared from the patients' autologous irradiated glioma tumor cells mixed with GM-CSF, irradiated GM-CSF secreting K562 cells, or IL-4-secreting fibroblasts (www.clinicaltrials.gov NCT00694330) [25, 45, 51]. In general, increases in patient survival were observed, however these approaches yielded no long-term survivors [9, 43, 45]. Importantly, no evidence of adverse autoimmunity was noted after these immunizations.

Collectively, these studies emphasize the importance of both the correct activation of the cell population presenting the antigen, as well as the accurate delivery of the targeted antigen, as part of the vaccine approach. It should be realized that a potent new antigen is not enough to make a vaccine. The antigen should be presented to the immune system in a formulation which activates the desired immune pathway required for tumor eradication. Vaccine immunopotentiators comprise a diverse group of molecules or formulations, which have been used routinely as critical components of inactivated antimicrobial vaccines for almost a century. Advances in vaccine research are expected to emerge from better knowledge of immune pathways and antigen delivery [52]. Apart from the historic examples of immunopotentiator technologies a variety of other options have not been explored as yet because of restrictions for use in human subjects [47].

Immunotherapeutic Concepts

The human body has evolved a complex immune system to eliminate pathogens and abnormal cells with minimal damage to

healthy tissues. The immune system has multiple levels of regulation to guarantee the appropriate balance between immune activation and immune suppression. Over the last two decades the fundamentals of this regulation have become clearer. This knowledge now provides new opportunities for rational intervention based on a tailored response of the immune system against tumors that may lead to clinical benefit.

Since the observations by William Coley there has been a long held belief that the immune system can prevent the emergence and growth of cancer. Indeed, Galon and coworkers elegantly demonstrated that an adaptive immune response influences the behavior of human colon cancer tumors by *in situ* analysis of tumor-infiltrating immune cells [53, 54]. Also, in ovarian cancer the presence of intraepithelial tumor infiltrating lymphocytes is associated with significantly longer clinical remission after chemotherapy and with improved overall survival [55]. Similar observations have been made for other tumors, including renal carcinoma [56], prostate cancer [57] and breast carcinoma [58]. The observation that improved survival is linked to the capacity to mount natural immune responses supports the concept to stimulate the patient's immune effector responses by active immune programming.

As for most traditional vaccines the quality and magnitude of immune responses evoked by new generation immunotherapeutic anti-tumor approaches is in general highly dependent on the choice of vaccine antigen and the provision of a suitable immunopotentiator or concurrent immune activation strategy; especially for poorly immunogenic antigens, such as tumor cell lysates, recombinant or DNA-encoded protein antigens [38, 47].

Despite decades of failed attempts to develop effective cancer vaccines, several candidates are now progressing towards commercial approval. Several distinct technological concepts have been pursued to achieve immunotherapeutic cancer treatment, which are summarized in Table **2**.

i) Classical vaccines consisting of tumor antigens, isolated either from tumor cell lines or *ex vivo* tumor tissue obtained by surgical removal, and formulated or co-administered together with an effective immunopotentiator into a vaccine formulation. This vaccine is injected parenterally and able to unlock the required immune response [1, 29]

ii) The source of tumor associated antigens (TAA) may also be generated by recombinant biotechnology procedures, such as laboratory-based expression from mammalian, bacterial, yeast, insect or plant cells, or as synthetic protein peptides.

iii) The loading of TAAs, either contained in surgically removed tumor cells or isolated as tumor-derived peptides, or as synthetic or recombinant molecular mimics, on the patient's own dendritic cells, which are isolated from the patient by leukapheresis, and after a culture period in the laboratory, transfused back into the patient [9, 40]. These autologous dendritic cells are activated in the culture dish by a mixture of recombinant cytokines or immunostimulatory ligands.

iv) Instead of protein antigens, investigators have used the genes encoding these antigens, either in RNA or DNA format or inserted in replicating viral or bacterial vectors which produce the TAAs in the patient after parenteral injection. Such genetic vaccines contain immunostimulatory sequences which directly activate immune cells after recognition by specific receptors. In addition, the provision of gene-encoded antigens allows for intracellular expression and correct processing of candidate antigens by the patients antigen presenting immune cells.

v) As a variation of the above strategy, the same genes can be delivered directly into isolated dendritic cells by transfection in culture dishes before infusion. This approach assures the correct targeting of the gene-encoded TAAs into the patients dendritic cells in the laboratory before infusion.

vi) Instead of providing TAAs in the context of a favorable immune system activating conditions, clinical trials have been designed with patient-derived tumor-specific T cells. These cells have been developed in the patient and are isolated and expanded in the laboratory in specialized culture media supplemented with T cell growth promoting molecules before reinfusion back into the patient.

vii) Another strategy involves the administration of immunostimulatory agents which strengthen the endogenous immune response of the patient in order to better attack the tumor. For example, this approach comprises intravenous administration of recombinant cytokines (IL-2, IL-12) or microbe-derived immunostimulants, including toll-like receptor agonists, like synthetic CpG motifs [59], stabilized synthetic RNA oligonucleotides (ODN) [60] or resiquimod® [61].

viii) The above approach activates the systemic immune activity and may enforce the critical local anti-tumor effector responses to a limited extent. Therefore, alternative strategies involve local immune manipulation by local administration of immune activating agents in combination with local destruction of tumor tissue, and associated liberation of TAAs following surgical ablation or tumor targeting by "magic bullets" such as TAA-specific or tumor cell DNA-targeting antibodies . Examples include the radiotherapeutic strategy, called Cotara®, that employs the isotope 131Iodine conjugated to an antibody that binds the necrotic core found in all solid tumors [62], or antibody-targeted delivery of chemokines by chemokine/antibody fusion proteins, which results in high local tumor-associated concentrations of chemokines that attract monocytes, neutrophils and lymphocytes [63]. Yet another variant of this concept includes locoregional or intratumor application of oncolytic viruses, e.g. NDV [64] or HSV [65], which cause a pro-inflammatory immune response in the vicinity of the tumor tissue.

ix) All above-mentioned approaches are based on expanding tumor–specific immune effector elements. In addition, it is known that during immune surveillance reactions, local or systemic immunosuppressive regulation contributes to the escape of solid brain tumors and that such processes may also contribute to the inhibition of suboptimal, vaccination-triggered immunotherapy [5-7, 66]. Hence, inhibition of immunosuppressive molecules, such as CTLA-4 [67] PD-1 [68-71], TGF-β [72], IL-10, or immunosuppressive regulatory T cell [50, 73, 74], may also contribute to anti-tumor immunity. Abrogation of immunosuppression can be achieved by a stand–alone approach, or as a push-and-pull tactic in combination with one of the active immunotherapeutic approaches mentioned earlier [75].

x) Combinations of two or more of the above-mentioned approaches.

Tumor Mediated Immune Suppression

Despite stimulatory strategies, the production of active immunotherapy in cancer patients at an advanced stage of disease may be hampered by the suppression of systemic immune responsiveness in these patients [4]. Radiation and chemotherapy can induce generalized immune suppression because these treatments reduce the production of immune competent cells. Such therapies can provide the natural selection process resulting in the development of resistant tumors [4, 76, 77]. Furthermore, the administration of highly immunosuppressive glucocorticoids to control brain edema would tend to point toward vaccine administration early after diagnosis rather than later, after patients develop systemic immune suppression. Evidence collected long ago showed that glucocorticoids such as dexamethasone transcriptionally inhibit IL-2 synthesis in T lymphocytes. They interfere with nuclear factor activating protein-1 binding to the IL-2 promoter and also with calcineurin dependent pathways for T cell activation [78]. Since endogenous immune cell activation and proliferation must be engendered upon successful immunization and is reliant upon mRNA and protein synthesis [79],

Table 2. Survey of Immunotherapy Approaches

	Tumor Associated Antigens (TAA)	Immunopotentiation	Category
1	From lysates extracted from cell lines or tumor tissue	Classical vaccine adjuvants, or cytokines such as interleukin (IL)-12, GM-CSF, TNF, haptens, BCG, etc	Classical vaccine
2	Recombinant proteins or peptides	Classical vaccine adjuvants, or cytokines such as interleukin (IL)-12, GM-CSF, TNF, haptens, BCG, etc	Modern vaccine
3	Antigens from 1 or 2 loaded on patient-derived autologous dendritic cells	Activating cytokine (or cocktail)	Dendritic cell vaccine
4	RNA, DNA, or viral/microbial vector encoding the TAA	Direct parenteral injection, without or with immunopotentiators	Genetic vaccine
5	RNA, DNA, or viral vector encoding the TAA	Transfection into dendritic cells	Dendritic cell vaccine
6	Endogenous TAAs expressed/released by the tumor	Patient-derived or allogeneic T lymphocytes cultured in medium facilitating T cell survival and expansion, sometimes gene modified T cells expressing transgenic T cell receptors specific for defined TAA	Adoptive T cell transfer
7	Endogenous TAAs expressed (released) by the tumor	Cytokine or immunostimulant administration (IFN, IL-2, IL-12, TLR agonists etc.)	Non-specific stand-alone immunostimulation
8	Endogenous TAAs released from the tumor after local destruction of tumor tissue by ablation	Local inflammatory responses resulting from apoptosis or necrosis of tumor tissue, optionally combined with immunostimulants.	Tumor necrosis therapy (TNT) by non-specific local immune activation
9	One of the categories of exogenous or endogenous TAAs described above	Blockade of endogenous immunosuppressive cells or molecules (PD-1, CTLA-4, TGF-β, IL-10, regulatory T cells, etc.)	Inhibition of local or systemic immune suppression
10	Two or more combinations of the above approaches		Multi-modal

vaccination would be optimal if given to patients who are not steroid-dependent.

In addition to drugs, the tumor itself can regulate immune reactivity. It has been recognized for some time that patients with malignant gliomas demonstrate a profound immune suppression when compared to normal persons, suggesting that gliomas produce an immune inhibitory environment [20, 80]. Such immune suppression is mediated by soluble cytokines and growth factors. For example, transforming growth factor (TGF)-β, and interleukin (IL)-10 have been reported to be secreted by glioma cells [81, 82]. These cytokines functionally impair T cell activity and are responsible for the development of immunotolerizing T regulatory (T reg) cells [83]. Gliomas also produce prostaglandin E2 (PGE_2), and IL-6, both potential immune suppressive agents, as well as macrophage chemoattractive protein (MCP-1) [29, 84, 85]. In addition, local synthesis of Vascular Endothelial Growth Factor (VEGF) inhibits DC maturation on the one hand, and induces tumor-promoting angiogenesis on the other hand [86]. Apart from these soluble factors, gliomas may express membrane-bound Fas ligand (FasL), which induces apoptotic cell death of infiltrating immune cells when interacting with Fas, and/or PD-1, an inhibitory co-receptor, a B-7 family member, which attenuates T cell receptor signaling of infiltrating lymphocytes [29]. These mechanisms can shut down anti-tumor immunity and can be viewed as a counterattack by the tumor.

CD8- and CD4-positive T cells become inhibited after expression of CTLA-4 and subsequent interaction with its ligands, CD80 or CD86. Hence, the use of the anti-CTLA-4 antibody has resulted in better and more sustained anti-tumor responses [87]. Another recent approach to reversing this immune suppression is the use of small molecule inhibitory drugs to block the common signaling pathway to these suppressive activities, including the Signal Transducers and Activators of Transcription 3 (STAT3) activation pathway [80]. Thus it has to be appreciated that multiple factors must be taken into account when considering an immune therapy approach. Regardless of the stimulatory concepts attention should be given to factors which promote immune escape.

In addition, immunosuppressive enzymes may provide another way for tumor cells to evade immune responses. Ninety percent of human glioblastomas are positive for indoleamine 2,3, dioxgenase (Ido-1) [4, 88]. Ido expression is accompanied by a lack of accumulation of specific T cells at the tumor site.

Recent Encouraging Pre-Clinical Results

As mentioned earlier, initial tumor vaccine approaches used tumor lysates derived from irradiated glioma cells as the source of tumor antigens or whole irradiated tumor cells themselves. However, while the tumor cells contain a plethora of tumor associated antigens, they may be present at relatively low levels. Additionally, tumor specimens can contain normal, nonmalignant cells as well as tumor cells. Nevertheless, in a rat model where systematic subcutaneous administration of either allogeneic or xenogeneic tumor cells, or that combined with syngeneic cell lysates, proved safe and protective in early and advanced malignant glioma growth [89]. These results suggest that injections of allogeneic cells and/or lysates, or xenogeneic cell lines, can activate the immune system and can break anti-self/tumor. Also, these cells likely contained critical antigenic determinants shared with the implanted tumor, leading to a reduction in tumor growth. These data therefore, support the potential viability of this cancer vaccine strategy as an adjuvant treatment to prevent tumor relapse in cancer patients after standard surgical removal of the tumor. The impact of such data may be far reaching when translation of this strategy to patients proves possible. Indeed, alloresponsive effects may prove to be powerful.

Immunotherapy Approaches Utilizing Allogeneic Cells for Cancer Treatment

Table **3** provides examples of open or pending clinical trials utilizing allogeneic cells for immunotherapy of cancer. There are cellular therapy trials using allogeneic effector T cells a) sensitized to tumor associated antigens or patient human leukocyte antigen, b) genetically modified T cells with targeting elements for brain tumor antigenic receptors as well as to T cell receptor (TCR) signaling, or T cells sensitized to highly antigenic viral proteins. For the latter, cytomegalovirus-specific T cells are used because of subclinical reactivation in CMV-exposed brain tumor-bearing individuals [90-92]. Since the allogeneic effector cells are administered directly into the brain they are protected, at least for a short while, from destruction by the host's immune cells. The use of allogeneic cells also obviates the use of immune cells from immunosuppressed

Table 3. Immune Therapies Using Allogeneic Cells or Tissue

Site/Investigator	Description	Disease	Study Phase-Enroolment	References
City of Hope, Duarte, CA/ B Badie	Allogeneic T Cells modified with chimeric IL-13α2 - TCRζ	Brain tumor	I - 10	[97]
Penn State Univ, Hershey, PA/ K Lucas	Allogeneic, CMV specific CTL	Brain tumor	I/II - 10	[98]
UCLA, Los Angeles, CA/L Liau & C Kruse	Alloreactive CTL and IL-2	Brain tumor	I - 15	[93, 99]
NovaRx, San Diego, CA/H Fakhrai	Allogeneic Tumor Cell Vaccine with TGF2 knockdown	Non-small cell lung carcinoma and brain tumor	II -75	[100]
Baylor, Houston, TX/ J Fay	Autologous DC pulsed with Allogeneic Melanoma Tumor	Melanoma	I/II - 33	Clinicaltrials.gov, NCT00313235
IDM Res Lab, Sanofi- Aventis, Paris, France/ M Salcedo	Autologous DC pulsed with Allogeneic Melanoma Tumor	Melanoma	I/II - 15	[101]
Univ Pittsburgh, Pittsburgh, PA/ G Chatta	Autologous DC pulsed with Allogeneic Prostate Tumor	prostate cancer	I - 12	Clinicaltrials.gov, NCT00970203

cancer patients. The peripheral blood mononuclear cells of glioma patients has been documented to contain higher numbers of T regulatory cells [8]; as well, the CD4 to CD8 ratios of T cells are about 1:1 instead of 2:1 as it is in normal individuals [93].

Other immunotherapy approaches listed in Table **3** involving allogeneic cells employs whole tumor cell vaccines using allogeneic tumor cells with TGF-β knockdown, or the use of autologous dendritic cells that are pulsed with antigens derived from allogeneic tumor cells. While the majority of clinical trials still remain in the Phase I or I/II arena, no phase III clinical trials have been completed at this time. The results from a handful of immunotherapy trials are finally being reported. A prominent example is DCVax®-Brain, an immunostimulant cancer vaccine, based on experimental autologous cellular therapy, produced by the American pharmaceutical company Northwest Biotherapeutics, Inc., which exhibits promising efficacy (www.clinicaltrials.gov NCT00045968) [9]. DCVax-Brain vaccine is manufactured using a patient's dendritic cells loaded with a tumor cell lysate prepared from surgically resected tumor tissue. The clinical data from a cohort of 141 newly-diagnosed glioblastoma patients treated in a Phase II study is still being collected, however, assessments from their Phase I trials suggest overall safety, with delayed time to disease recurrence and increased survival, especially in glioblastoma patients with stable disease at entry [see http://www.nwbio.com/clinical_dcvax_brain.php]. Indeed, for those patients treated in the Phase I trials, the company is reporting that the median survival is 33.8 months, with 9 of 19 patients still alive at 8-82 months from initial surgery.

A multiinstitutional Phase II trial where 82 patients were treated was supported by Pfizer Pharmaceutical company, Celldex Therapeutics [see http://www.celldextherapeutics.com/]. The trial is described at (www.clinicaltrials.gov NCT00458601) and they report interim positive results from the Phase 2b study. This involves a non-cell based vaccine using an EGFRviii peptide conjugate, CDX-110, given in conjunction with temozolomide [94, 95]. Newly diagnosed WHO Grade IV glioma patients were treated. The trial is still active for follow-up, but not currently recruiting patients.

The company ImmunoCellular Therapeutics, Ltd (http://www.imuc.com/) has supported a Phase I study using ICT-107 for glioblastoma. This a dendritic cell-based vaccine that targets multiple glioma associated antigens [44, 96]. In a June, 2010 company report they say that the median overall survival had not yet been reached at the 26.4 months analysis point, with 12 out of 16 treated newly diagnosed patients alive (http://www.imuc.com/pdf/Brain-Cancer-Vaccine-Looks-Promising-in-Small-Trial-1.pdf#zoom=100). Other clinical trials involving immunotherapy for brain gliomas can be found at www.clinicaltrials.gov by refining search terms using key words at that site.

CONCLUSION

To conclude, the results of the most recent clinical trials suggest that systemic immunotherapy using dendritic cells or peptide vaccines are capable of inducing an immune response in malignant glioma; increased patient survival has been reported, though no phase III clinical trials are completed to this time. Apart from better targeted radiotherapy and more fine tuned surgery, we will experience a gradual continuing increase of immunological insight that will enable novel intervention strategies. Successful vaccine approaches will likely result from the "golden" combination of antigen(s) and immunopotentiators [97-101].

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