Toward Effective Immunotherapy for the Treatment of Malignant Brain Tumors

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Summary: The immunologic treatment of cancer has long been heralded as a targeted molecular therapeutic with the promise of eradicating tumor cells with minimal damage to surrounding normal tissues. However, a demonstrative example of the efficacy of immunotherapy in modulating cancer progression is still lacking for most human cancers. Recent breakthroughs in our understanding of the mechanisms leading to full T-cell activation, and recognition of the importance of overcoming tumor-induced immunosuppressive mechanisms, have shed new light on how to generate effective anti-tumor immune responses in humans, and sparked a renewed and enthusiastic effort to realize the full potential of cancer immunotherapy. The immunologic treatment of invasive malignant brain tumors has not escaped this re-invigorated endeavor, and promising therapies are currently under active investigation in dozens of clinical trials at several institutions worldwide. This review will focus on some of the most important breakthroughs in our understanding of how to generate potent anti-tumor immune responses, and some of the clear challenges that lie ahead in achieving effective immunotherapy for the majority of patients with malignant brain tumors. A review of immunotherapeutic strategies currently under clinical evaluation, as well as an outline of promising novel approaches on the horizon, is included to provide perspective on the active and stalwart progress toward effective immunotherapy for the treatment of malignant brain tumors.
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Despite aggressive, image-guided tumor resection,[1] high-dose external beam radiotherapy or brachytherapy, and advances in efficacious adjuvant chemotherapy, patients with glioblastoma (GBM), which is the most common and deadly malignant brain tumor, still have a median survival of less than 15 months.[2,3] The estimated cost of treatment for each patient with a malignant brain tumor is between $30,000 and several hundred thousand dollars annually. Thus, the annual treatment cost alone for these patients, not accounting for the lost earning potential of affected individuals, is greater than the entire annual budget of the National Institute of Neurological Disorders and Stroke.

In evaluation of the quality-adjusted life-year saved, conventional therapy for patients with malignant brain tumors is the most expensive medical therapy currently provided in the United States.[4] Furthermore, patients treated with the aggressive multi-modality treatments that are used in the standard of care management of this disease are often left with incapacitating damage to surrounding normal brain and systemic tissues.[5,6] Thus, to be more effective, therapeutic strategies for malignant brain tumors will have to precisely target residual invasive tumor cells while minimizing collateral damage to the neighboring eloquent brain. The rationale for using the immune system to target brain tumors is based on the premise that the inherent biologic specificity of the immune system could meet the clear and urgent need for more precise medical therapy.

CANCER IMMUNOTHERAPY: A PERSONALIZED MEDICINE PARADIGM

The immune system is an intricate network of innumerable cellular and molecular mediators that are so exquisitely interconnected and refined that it is capable of recognizing a foreign pathogen within minutes of breaching the outer barriers of the human body, and responding with a myriad of innate defense mechanisms

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and an array of specific humoral and cellular effectors that can control a rapidly expanding and invasive infection and eliminate almost every infected cell from the body. For more than a century now, tumor immunologists have sought to leverage this amazing cytotoxic power and exquisite specificity against malignant cancer cells that spread throughout afflicted hosts with as dire consequences as an uncontrolled microbial infection.

Current efforts in oncology treatment development are directed at the discovery of "targeted therapeutics" that attack specific pathways operative in cancer cells and are key to the maintenance of malignant phenotype. Delineating the phenotype and genotype of tumor cells using the variety of available "omic" technologies, such as genomics and proteonomics to match them most appropriately to treatments known to be effective against cancers that share a genetic or proteomic "profile" constitutes a major current effort to increase the efficacy of oncology treatment regimens through proper patient selection. This quest in drug discovery for more specific and targeted cellular pathway inhibitors, coupled with the molecular interrogation of tumor specimens to match the most effective available pharmacologic treatments, is often descriptive of the era of "personalized medicine" that we are diligently working toward.[7]

In many ways, although the tumor immunologists can probably not be credited with coining the phrase of personalized medicine, they very well may be credited with the first attempts to realize such personalized medical treatments for patients with malignancy. Effective cancer immunotherapy captures all of the essential ingredients of a targeted therapeutic that is uniquely matched to the antigenic profile of a given tumor, and is specifically designed to leverage its effects against tumor cells expressing a unique antigenic profile, while limiting "off target" effects on normal cells that do not share expression of these antigens. The inherent premise of immunotherapy does not base treatment strategy on a shared histopathologic appearance and natural disease history as conventional treatment paradigms, but rather bases therapeutic susceptibility almost entirely on the molecular antigenic profile of the tumor, and whether the tumor does or does not express an antigen (biomarker) that can be effectively targeted by the immune system. A cancer vaccine developed against an antigenic target such as epidermal growth factor receptor variant 3 (EGFRvIII), for example, would be expected to be effective only against tumor cells expressing this molecular target. Thus patients with the same pathologic diagnosis of malignant glioma selected upfront as EGFRvIII vaccine candidates based on expression of this biomarker was inherent in its initial clinical evaluation. Cancer immunotherapy may have the added advantage over other cytotoxic or cytostatic mechanisms, in that the immune system kills targeted cells based on the effective activation and recognition of a particular antigen(s) within the target cell, but is not dependent on that target playing a key role in the oncogenic phenotype for executing cytolytic-killing mechanisms. Thus the validation of potential targets for tumor immunotherapy is simplified to expression analysis in normal and malignant tissues and the immunogenicity of the target itself. This is not to suggest that resistance mechanisms to immunologic killing within tumor cells do not exist nor are insignificant, but candidates for immunotherapeutic attack can be evaluated based on their differential expression in tumor cells alone without necessitating a role in tumorigenesis.

Thus, the successful development of cancer immunotherapy has long been based on the now widely accepted concepts captivated under the brand name of "personalized medicine" include the following: 1) that efforts in oncology therapeutics be directed toward the development of treatments that exploit specific molecular differences between normal cells and tumor cells; 2) that treatments are driven by biomarkers that can differentiate susceptible tumor types from nonsusceptible ones sharing the same or distinct pathological diagnosis; 3) that treatments have limited off-target effects against normal tissues by exploiting biological targets operative only in tumor cells; and 4) that treatments ideally can be combined to address the inherent heterogeneity of genetic and epigenetic phenotypes that exist within most malignant cancers. Furthermore, the bioavailability of the immune system's humoral and cellular effector mechanisms have been shown to be system-wide, with even the once believed to be immunoprivileged CNS being effectively surveyed by host immune defenses.[8]

Active immunotherapy (the generation of effective host immunity against tumor-specific antigens) has been shown, at least in preclinical models, to posses an additional benefit that no other targeted therapeutic possesses, and that is the capacity to establish immunologic memory recall responses against immunized antigens that can provide long-term surveillance against tumor recurrence.[9,10]

Suffice to say that with all of the apparent advantages that immunotherapy has to offer, the clinical realization of these advantages is still lacking in the treatment of most human cancers. Notable exceptions to this generalization have been the effective use of adoptive cellular therapy against cancers associated with viral infections, such as Epstein Barr virus (EBV)-associated lymphomas and donor leukocyte infusions against lymphoproliferative diseases and some hematologic malignancies in allogeneic transplant recipients.[11,12] Recent advances in our understanding of the potent effects of tumor-induced immunosuppression and homeostatic regulatory mechanisms that govern lymphocyte expansion and function, however, have provided new insights into how to achieve and sustain potent immune responses in tumor bearing

hosts. These advances have already begun to demonstrate remarkable clinical responses in patients with systemic tumors and hold significant promise for improving immunotherapy for patients with malignant brain tumors.

CURRENT EFFORTS IN IMMUNOTHERAPY FOR MALIGNANT GLIOMAS

Tumor-specific rejection antigens and EGFRvIII

Most well-characterized tumor antigens that have been identified represent over-expressed normal proteins. These self-proteins are likely limited in their capacity to generate potent immune responses to vaccination due to some degree of tolerance, and if effectively targeted would likely precipitate some degree of autoimmunity.[13,14] Conversely, tumor-specific antigens derived from tumor-associated mutations in somatic genes would not be as amenable to central tolerance. These mutations would also less likely be associated with autoimmunity if potent immune responses were to be generated against these targets due to absence in normal tissues. In fact, studies examining the immunologic recognition of tumor antigens in patients with malignancy have shown that the majority of the host immunologic response is directed against tumor-specific antigens and not the readily studied over-expressed self antigens and differentiation antigens that have been described in most tumor types.[15] These antigenic mutations, however, arise sporadically during errors in cellular division and tumor expansion,[16,17] and thus would be predicted to be patient-specific and not necessarily linked to the oncogenic process.

EGFRvIII, however, is a rare example of a frequent and consistent tumor-specific mutation, central to the neoplastic process, that consists of an in-frame deletion of 801 base pairs from the extracellular domain of the EGFR that splits a codon and produces a novel glycine at the fusion junction.[18,19] This mutation encodes a continually active tyrosine kinase[20,21] that enhances tumorgenicity[21–24] and migration,[25,26] and confers radiation and chemotherapeutic resistance[27–32] to tumor cells. The EGFRvIII mutation is most frequently seen in patients with GBM,[33–39] but has been found in a broad array of other common cancers.[33,38–45] The new glycine inserted at the fusion junction of normally distant parts of the extracellular domain results in a tumor-specific epitope not found in any normal adult tissues.[46] The exquisite tumor-specificity of EGFRvIII; its clonal expression in GBMs and other common tumors; its absence in any normal tissues; and its importance in the oncogenic phenotype of tumors make EGFRvIII an ideal target for antitumor immunotherapy.[41,42,46,47]

We have demonstrated in clinical studies in both phases I and II that patients with newly-diagnosed GBM who received vaccinations targeting a peptide spanning the EGFRvIII mutated junction induce potent EGFRvIII-specific immune responses and patient survival that significantly exceeds that of historical controls.[48] The efficacy of these EGFRvIII peptide vaccines (CDX-110 [Celldex/Avant/Pfizer, Inc]) in patients with newly-diagnosed GBM is currently being evaluated in large-scale clinical trials to confirm these promising early clinical findings.

Peptide vaccines

Peptide vaccines are of significant interest in the field of immunotherapy due to their ease of manufacturing and administration, and the capacity to induce antigen-specific cellular and humoral responses. In addition to EGFRvIII, a growing list of candidate antigens in malignant brain tumors have been identified that could serve as substrates for peptide-based cancer vaccines. These included melanoma/testis antigens,[49–53] viral antigens,[54–58] cytokine receptors,[59,60] and differentiation antigens expressed in malignant gliomas.[61] Clinical trials evaluating peptide vaccines for malignant glioma have demonstrated capacity to induce tumor-specific immune responses in patients with primary and recurrent gliomas and elicit early signs of clinical responses that await confirmation in larger scale clinical trials.[48,62–64] Several clinical trials evaluating specific antigenic peptides, peptide fractions eluted from tumor cell surfaces or extracts, and peptide-bound chaperone proteins, such as heat-shock proteins, are currently underway to assess the potential efficacy of peptide-based vaccines for malignant brain tumors.

Gene-modified tumor cell vaccines

One immunotherapeutic strategy is to use entire malignant tumor cells modified to render them more immunogenic and incapable of forming new tumors as the basis for vaccine formulation. These gene-modified tumor vaccines are then inoculated in patients with the aim of inducing immune responses against the myriad of uncharacterized and patient-specific antigens present in transformed tumor cells. Fakhrai et al.[65] used autologous tumor cells genetically modified with a transforming growth factor-beta2 (TGF-β2) antisense vector to reverse the immunosuppressive effects of TGF-β2 and elicit immunologic responses in patients with GBM. The investigators treated six patients with progressive GBM and reported objective clinical responses in two patients and disease stabilization in two patients, as well as the induction of tumor-specific immunity as measured by DTH testing and antibody titers.

Okada et al.[66,67] have used autologous glioma cell vaccines secreting interleukin(IL)-4 or co-injected with IL-4 secreting fibroblasts in patients with recurrent GBM. These vaccines were without any adverse reactions other than local inflammation at the vaccine site (i.e, the thigh). The authors also report induction of sys-

temic tumor-specific immunologic responses and radiographic responses in treated patients, highlighting the potential promise of this treatment, but they also cite the duration of time required for vaccine preparation (i.e., greater than 7 weeks) as a major limitation for usage in patients with recurrent glioma.

Several gene-modified tumor vaccines are under current clinical evaluation using autologous glioma cells modified to secrete a variety of cytokines such as IL-4, granulocyte/macrophage colony stimulating factor (GM-CSF), or IL-12 that have shown promise in preclinical therapeutic models of malignant glioma.

DENDRITIC CELL THERAPY

Dendritic cells (DCs) are potent immunostimulatory cells that continuously sample the antigenic environment of the host and specifically activate CD4+ and CD8+ T-cells and B-cells.[68,69] They are at the crossroads of many of the elegant networks of the immune system, and DCs may represent the most promising contemporary biologic entity for realizing the promise of immunotherapy. Potent immune responses and encouraging clinical results have been seen in phase I and II human clinical trials in systemic cancers,[70–86] and numerous animal studies, including many of our own,[9,10,87] have demonstrated potent antitumor responses using DC-based immunotherapy against CNS tumors.[88,89]

DC VACCINES TARGETING TUMOR-SPECIFIC ANTIGENS

We completed a phase I clinical trial in which 16 patients with malignant gliomas (i.e., 13 GBM, 3 World Health Organization grade III glioma) received intradermal (i.d.) immunizations with autologous DC pulsed with a keyhole limpet hemocyanin (KLH) conjugate of a peptide spanning the mutated region of EGFRvIII after completion of radiation therapy.[90] The i.d. route of administration was supported by evidence that DC delivered in this manner will migrate to lymph nodes[91,92] and subsequently present antigen to T lymphocytes, as well as by prior studies comparing the ability of various routes of administration to elicit strong T-cell-mediated immunity.[93] The enrolled patient population consisted of adults with malignant gliomas who had undergone gross total tumor resection and radiotherapy. Patients underwent leukapheresis to remove autologous peripheral blood mononuclear cells (PBMC), which were cultured in GM-CSF and IL-4 to generate DC. The DCs were pulsed with peptide spanning of the mutated region of EGFRvIII/KLH and matured in a cocktail of tumor necrosis factor-α, IL-1β, and IL-6 (but not prostaglandin E2 [PGE2], due to some concern over a counterproductive effect on DC IL-12 production[94]) before being delivered back to the patient in 3 biweekly i.d. injections.

Immunized patients demonstrated induction of immunologic responses, which were not detectable prior to vaccination, without any evidence of adverse events other than grades I and II local reactions at the vaccination site. For patients with GBM (n = 13), the median survival time was 110.8 weeks, which compares favorably with other published reports in similar patient populations using temozolomide[95] or carmustine wafers,[96] in which median survivals were 63.3 weeks and 59.6 weeks, respectively. These findings suggest that autologous mature DCs loaded with the tumor-specific antigen, peptide spanning the mutated region of EGFRvIII, are safe and may induce beneficial immunologic and clinical responses in patients with malignant gliomas.

Tumor lysate or unfractionated peptide pulsed DC vaccines

The first trial of DC vaccination in patients with malignant glioma was published by Yu et al.[88] in 2001. A demonstrable increase in tumor-specific cytotoxicity was successfully developed in four out of seven testable patients who received DC pulsed with major histocompatibility complex-I peptides eluted from the surface of autologous glioma cells. Furthermore, two out of four patients who underwent reoperation demonstrated robust $CD8^+$ and memory ($CD45RO^+$) T-cell infiltrates in areas of the tumor. Based on the small sample size, no reliable data on survival could be generated, but the treatment proved safe.[88]

Parajuli et al.[97] have reported the results of an *in vitro* human DC study that examined the ability of different DC-based strategies to induce effective T-cell responses against malignant astrocytomas. DCs were generated from patient PBMC and were fused with autologous tumor cells or pulsed with total tumor RNA or tumor lysate. They were then assayed for their respective abilities to stimulate tumor-specific T-cell proliferation and cytotoxic T lymphocyte responses *in vitro*. No significant differences were found between the various DC arms in their T-cell stimulatory capacity; all showed enhanced cytotoxicity that was further augmented by addition of CD40 ligand during T-cell stimulation.[98] The data should be helpful in designing protocols for DC-based immunotherapy of malignant astrocytomas.

Liau et al.[99] reported the results of a phase I trial of DC pulsed with peptides that were acid-eluted from the surface of resected, autologous tumor, and were administered to 12 GBM patients in 3 bi-weekly i.d. injections. There were no adverse effects of treatment and evidence of increased immunologic responses against autologous tumor was observed in half of the treated patients. Promising prolongation of survival (median survival, 23.4 months) compared with historical controls was observed,

and a multi-center randomized clinical trial was initiated for further confirmation of these results.

In the largest series of DC vaccinated patients published to date, De Vleeschouwer et al.[100] reported the results of 56 patients (both pediatric and adult) with relapsed GBM who were given at least three vaccinations with autologous mature DCs loaded with autologous tumor lysates. The treatment was well tolerated with a single serious adverse event of vaccine-related edema in a patient with gross residual disease. The median progression-free survival and overall survival of the group was 3 months and 9.6 months respectively, with a 2-year overall survival of 14.8%. Patients were treated in three consecutive cohorts, with progressively shorter vaccination intervals per cohort. The investigators observed a trend toward improved progression-free survival with a shorter vaccination interval (4 weekly injections with boosts of intradermal injections of 1.5 mg of autologous tumor lysate). The vaccination of patients with recurrent disease and likely significant tumor-induced immunosuppression may have contributed to the limited overall clinical responses, although the authors note a small but encouraging 2-year survival rate in some patients with recurrent disease.

Recently, Wheeler et al.[101] reported a statistically significant correlation between vaccine-induced immune responses in patients with GBM receiving autologous tumor lysate pulsed DCs and times to tumor progression and survival. Responders were classified as patients with a greater than 1.5-fold enhancement of interferon-γ production (measured by qPCR in total PBMCs stimulatated by autologous tumor-lysate pulsed DCs) relative to pre-vaccine levels and post-vaccine tumor progression survival was significantly longer in responders (642 $\pm$ 61 days) than nonresponders (430 $\pm$ 50 days) when all patients were analyzed as a group (both recurrent and newly-diagnosed GBM) ($p = 0.041$). Separate analysis of patients with recurrent disease showed a similar trend toward increased survival in responders, but this did not reach statistical significance ($p = 0.067$). This phase II study using a single immunologic response marker (interferon-γ) suggests that the establishment of validated immunologic markers for treatment responsiveness to immunotherapy may be feasible in larger studies of immunotherapy in patients with malignant glioma.

RNA-loaded DC vaccines

Tumor material is often limited in patients with malignant brain tumors, and thus vaccine preparations dependent on obtaining sufficient tumor tissue may be limited in broad application to patients. The use of RNA to encode tumor antigens for DCs was pioneered by Drs. Nair and Gilboa, but the ability of RNA-loaded DCs to stimulate potent antitumor immunity has been independently confirmed in murine and human systems.[102,103] In fact, there is accumulating evidence that RNA transfection represents a superior method for loading antigens onto DCs.[104–106] This novel and innovative approach to DC-antigen loading has multiple conceptual advantages over other forms of antigen delivery as well. RNA-based antigen loading does not require knowledge of major histocompatibility complex restriction, and responses are not restricted to single major histocompatibility complex haplotypes or to a narrow B- or T-cell repertoire. This diversity increases the likelihood of inducing effective and sustained antitumor immune responses by simultaneous activation of both cytotoxic T lymphocytes and helper T-cells.[107–109] Using molecular techniques, RNA can be amplified, and *in vitro* it can be transcribed from DNA templates from cloned tumor antigens or from RNA libraries isolated from as few as 100 tumor cells, thus providing a renewable source of tumor antigen for vaccine preparation.[110] Furthermore, in direct comparisons, RNA-loaded DCs have been found to be better stimulators of antigen-specific T-cells than other approaches.[105] Finally, RNA also carries a significant safety advantage, not possessed by other nucleic acid or viral vectors, in that it can not be integrated permanently into the host genome. However, the time required for generation of tumor-specific RNA, either isolated from tumor cells or *in vitro* transcribed from cloned cDNA templates, and the labile nature of RNA molecules are potential limitations to the use of RNA as a source of tumor antigens.

Caruso et al.[111] used tumor RNA-pulsed DCs to vaccinate seven children with recurrent brain cancers (anaplastic astrocytoma [n = 1], GBM [n = 2], ependymoma [n = 2], pleomorphic xanthoastrocytoma [n = 1], and ependymoma [n = 1]) in a phase I clinical study. Induction of tumor-specific immune responses was observed in two patients, and clinical responses were observed in three patients as assessed by MRI (two disease stabilizations and one partial response).

Methods for loading DCs with RNA have been significantly improved since the publication of these trial results, as the investigators used simple co-incubation of DCs with "naked" tumor RNA for loading with tumor antigens. Electroporation of RNA into DC has proven to achieve much higher expression from antigen-encoding RNA and likely will significantly improve immunologic responses against RNA pulsed DCs in humans.[112] We are currently evaluating RNA-electroporated DC vaccines in the context of several clinical trials at our institution.

Although the use of antigen-pulsed DCs appears promising in early clinical trials for treatment of brain tumors, these cells have also been shown to be quite capable of initiating significant autoimmune responses in murine models, and there has been one incident of a spontaneous generalized vitiligo that occurred after intravenous infu-

sion of DCs in a patient with melanoma.[113] Although our group and others have demonstrated that DCs loaded with unselected tumor-derived antigens induce potent, specific, and clinically effective immune responses against brain tumors in rodent models without the induction of autoimmune reactivity,[10,87,89,114] and although no autoimmune reactions have been identified in human DC trials in patients with malignant brain tumors,[88] immunization in preclinical studies has only been effective when given before tumor challenge or in the context of very small established tumors. These data suggest that for DC-based immunotherapy to be effective in the context of large human tumors, a very strong and sustained antitumor immune response will be required.[115] In animal models, when such responses have been generated against tumor-associated antigens that are shared with host cells, severe and clinically significant autoimmune disease has occasionally resulted.[116] Thus, as our capacity to engender more potent immunologic responses in humans continues to advance, careful and long-term toxicity evaluation will be necessary to ensure the safe and effective development of this promising modality.

ADOPTIVE CELLULAR THERAPY

Adoptive immunotherapy encompasses treatments that involve transfer of autologous lymphocytes that have been expanded ex vivo against tumor specific antigens. Treatment approaches have differed in the types of cells administered, the route of administration, and the activation status of the cells. Cell types that have been used in adoptive immunotherapy for malignant brain tumors include 1) PBMC;[117,118] 2) lymphokine-activated killer cells;[119–121] 3) mitogen-activated killer cells;[122,123] 4) tumor-infiltrating lymphocytes;[124] and 5) antigen-specific and unselected cytotoxic T-cell lymphocytes.[125,126] Routes of administration have generally been either systemic or into the tumor cavity (also known as. intralesional or loco-regional). Clinical studies evaluating these approaches have demonstrated the safety of these treatments in patients with malignant brain tumors and a minority of patients was observed to have achieved objective clinical response to treatment.

Recent advances using genetically engineered lymphocytes with redirected specificity for antigens expressed in malignant brain tumors provides an opportunity to generate large numbers of tumor-specific lymphocytes from easily accessible pools of peripheral blood lymphocytes and shows exciting potential as a novel treatment strategy.[127] However, due to the complexity and labor intense nature of adoptive cellular therapy protocols, in general, this modality has lagged significantly behind effort in development of active vaccinations using DCs, tumor cells, or defined antigens delivered as peptides or through viral and nonviral expression vectors.

However, striking advances have recently been made in the success of treating advanced metastatic melanoma using nonmyeloablative and myeloablative chemotherapy conditioning regimens for lymphodepletion prior to adoptive T-cell transfer and exogenous IL-2 support.[128,129] The mechanistic principles underlying these treatment advances have also been begun to be elucidated in experimental mouse models.[130,131] Thus, adoptive cellular therapy after lymphodepletion has recently emerged as the most effective treatment strategy to date for advanced refractory melanoma with objective responses achieved in greater than 50% of treated patients.[132] Leveraging the concepts elucidated in this treatment strategy toward effective immunotherapy against malignant brain tumors seems to be of paramount interest, especially given that complete regressions of metastatic lesions within the CNS have been observed in patients treated with this approach.

EMERGING CONCEPTS IN IMMUNOTHERAPY FOR MALIGNANT BRAIN TUMORS

Lymphopenia and homeostatic proliferation

After periods of lymphopenia, there is a homeostatic proliferation of the remaining lymphocytes of the host, which is designed to recover normal lymphocyte counts.[133] Probably as a result of a surge in cytokines (IL-7, IL-15) in response to lymphopenia, lymphocytes undergoing homeostatic proliferation enjoy a reduced activation threshold[133,134] and differentiate directly into effector memory T-cells capable of rapid and intense response to antigen.[135] Still, lymphocytes must encounter their cognate antigen and compete for limiting amounts of these homeostatic cytokines to proliferate even under these conditions.[133] Thus, B- or T-cells specific for antigens that predominate during this recovery period, such as those provided in the form of a vaccine or adoptively transferred after ex vivo expansion against specific antigens, have a competitive advantage and become disproportionately over-represented in the recovering lymphocyte population both in murine models[136,137] and in humans.[138] These skewed homeostatic responses have been shown to enhance antitumor immunity,[136,137,139] but can also increase the risk of autoimmunity.[140,141] Futhermore, lymphodepletion may remove inhibitory regulatory T-cells, further accentuating the effectiveness of antitumor immunotherapy.[142,143]

Leveraging this principle, Dudley et al.[144] have used intentional nonmyeloablative lymphodepletion to enhance the preferential expansion and maintenance of adoptively transferred, tumor-specific T-cells. This has resulted in dramatic clinical responses,[145–149] along with some autoimmune toxicity, in patients with advanced malignant melanoma.[132,144] These studies have shown

that under these conditions, transferred T-cells can expand dramatically in the lymphopenic host to constitute up to 90% of the T-cell repertoire of the host, and can be maintained for months after adoptive transfer.[132,150] These studies demonstrated that clinical regression of systemic disease correlates with the frequency of tumor-specific T-cells achieved in the peripheral blood and persistence of these cells *in vivo*.[145-149,151] At least in murine models, this antitumor effect was also significantly enhanced by autologous stem cell support.[152]

Immunosuppression in GBM and IL-2Rα^+ regulatory T-cells

A substantial barrier to the activation of antitumor immune responses in patients with GBM is their well-documented impairment of T- and B-cell immunity. Although immunosuppressive factors secreted by the tumor clearly play a role, we have recently demonstrated that a major contributor to depressed cellular immunity in patients with GBM is an increased level of regulatory T-cells (T_{Regs}).[153] T_{Regs} are a physiologic subset of $CD4^+$ T-cells that normally comprise 5 to 10% of this compartment and serve to thwart pathological responses toward self antigens. They constitutively express high levels of the high-affinity IL-2Rα (CD25) on their surface,[154] and they can be identified even more specifically by expression of the intracellular transcription factor, FOXP3. T_{Regs} potently inhibit T-cell cytokine secretion and proliferation,[155–159] directly curtail the generation and expansion of endogenous or induced immune responses,[154,160–168] and appear to play a significant role in hindering immunity to normal and tumor-associated antigens.[169,170] Accordingly, increased levels of T_{Regs} have been found in the tumors and peripheral blood of patients with various tumors including GBM.[153,171–175] The relative importance and precise interactions between systemic and intratumoral T_{Regs} has not been established.[176–178] Strategies to inhibit or deplete T_{Regs} including CD25 blockade using MAbs, CD25-binding immunotoxins, or pharmacologic inhibition of T_{Reg} activity are under current clinical and preclinical evaluation in our laboratory and others.[179–181]

BEYOND PROOF-OF-CONCEPT AND TOWARD CLINICAL EFFICACY

The debate as to whether human tumors express proteins capable of serving as tumor rejection antigens that pre-occupied much of the early decades of tumor immunology and immunotherapy research has now been settled. It is clear that the immune system can recognize and mount significant cellular and humoral responses against over-expressed self antigens, as well as novel tumor-specific antigens in human tumors, including malignant brain tumors. Also, it is well-accepted that the once believed to be "immunoprivileged," and therefore inaccessible CNS, is readily surveyed by activated effector cells of the immune system, although less extensively and perhaps with attenuated function compared with lymphocytes surveying tissues in the periphery.

Therefore, continued advancement of immunotherapeutic efforts in the treatment of malignant brain tumors will be dependent on a concerted effort to push past the now-established feasibility and safety demonstration of immunotherapeutic treatments, and even beyond basic immunologic monitoring efforts using nonstandardized and noncomparable immunologic assays. Future efforts will need to focus on methods to significantly enhance the magnitude of immune responses and proportion of responding patients against targeted glioma antigens. These responses will need to be evaluated using standardized immunologic assays that can be compared across clinical trials within a given institution and ideally among different institutions. Although a myriad of newer targets and interventional strategies will undoubtedly continue to spring forth, investigators will need to conquer the difficult, but essential, task of quickly and objectively evaluating what likely constitutes a significant advance in treatment strategy, and therefore justifies a change in direction versus continuing to forge forward with incremental improvements in existing treatment strategies against identified antigenic targets.

An effort in understanding the molecular differences between clinical and immunologic responders and non-responders to immunotherapeutic interventions will be very helpful in guiding further improvements and also in patient selection, and innovative trial designs are needed to rapidly evaluate complex biological therapies in larger scale and more definitive clinical studies.

The current emphasis on targeted molecular therapies and use of biomarkers and tumor signatures to guide treatment selection is optimally suited to match the exquisite specificity and precise targeting capacity of immunologic treatment regimens. Therefore, in our opinion, cancer immunotherapy represents a contemporary treatment modality whose time has finally come for a comprehensive and forward-looking clinical evaluation and rational investigational plan for the successful integration into the mainstay of effective therapies for treatment of malignant brain tumors.

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