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Cellular immunotherapy for malignant gliomas

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Abstract

Introduction—Cancer immunotherapy has made much progress in recent years. Clinical trials evaluating a variety of immunotherapeutic approaches are underway in patients with malignant gliomas. Thanks to recent advancements in cell engineering technologies, infusion of *ex vivo* prepared immune cells have emerged as promising strategies of cancer immunotherapy.

Areas covered—Herein, the authors review recent and current studies using cellular immunotherapies for malignant gliomas. Specifically, they cover the following areas: a) cellular vaccine approaches using tumor cell-based or dendritic cell (DC)-based vaccines, and b) adoptive cell transfer (ACT) approaches, including lymphokine-activated killer (LAK) cells, γδ T cells, tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor (CAR)-T cells and T-cell receptor (TCR) transduced T cells.

Expert opinion—While some of the recent studies have shown promising results, the ultimate success of cellular immunotherapy in brain tumor patients would require improvements in the following areas: 1) feasibility in producing cellular therapeutics; 2) identification and characterization of targetable antigens given the paucity and heterogeneity of tumor specific antigens; 3) the development of strategies to promote effector T-cell trafficking; 4) overcoming local and systemic immune suppression, and 5) proper interpretation of imaging data for brain tumor patients receiving immunotherapy.

Keywords

glioma; cellular immunotherapy; dendritic cell vaccine; adoptive cell transfer; CAR-T cell therapy

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Declaration of Interest

H Okada is an inventor in the U.S. Patent Application No. 60,611, 797 (Utility Patent Application) “Identification of an IL-13 Receptor Alpha2 Peptide Analogue Capable of Enhancing Stimulation of Glioma-Specific CTL Response”. An exclusive licensing agreement has been completed on this application between University of Pittsburgh and Stemline, Inc. In this manuscript, the authors discussed a publication using this peptide, but the data interpretation was done by the entire study team and not by Dr Okada himself. Due to the potential conflicts of interest, Hideho Okada did not solely interpret any data in the current manuscript. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

Malignant gliomas are the most common type of primary malignant brain tumor, with more than 18,000 new cases diagnosed each year in the United States[1]. Despite advancements of conventional therapies, including surgery, radiation therapy and chemotherapy, outcomes for these patients remain dismal. Glioblastoma (GBM) is the most common and the most malignant of the gliomas; patients with GBM have a median survival of approximately 15 months following treatment with a combination of chemotherapy (Temozolomide) with radiation therapy[2], pointing to the urgent need to develop novel efficacious therapeutic modalities.

Cancer immunotherapy is aimed at enhancing the systemic and selective immune response against tumor cells. Both innate and adaptive immune responses play complementary roles. In innate immunity, natural killer (NK) and myeloid cells recognize and destroy virally infected cells and a range of tumor cells in a major histocompatibility complex (MHC)-unrestricted manner. Adaptive immune responses are antigen-specific and initiated by presentation of tumor antigens by antigen presenting cells (APCs). Most potent APC are dendritic cells (DCs) which can develop from myeloid cells. DCs present tumor-derived epitope peptides as MHC-peptide complexes to T cells via TCR. Activated T cells clonally expand and then traffic to the tumor-involved organs. T cells recognize antigen epitopes via MHC/peptide complex on tumor cells through the TCR, leading to T cell activation and release of preformed cytotoxic molecules (granzyme and perforin).

The field of cancer immunotherapy has made exciting breakthroughs in recent years. The United States Food and Drug Administration (FDA) approved monoclonal antibodies (mAb) to the inhibitory immune checkpoint molecules cytotoxic T lymphocyte-4 (CTLA-4; ipilimumab) and programmed death 1 (PD-1; pembroluzimab and nivolumab) for metastatic melanoma as well as non-small cell lung cancer (NSCLC)[3–6]. In regard to cellular immunotherapy, the FDA approved the first vaccine against non-viral cancers (sipuleucel-T)[7]. Furthermore, chimeric antigen receptor (CAR) engineered autologous T cells have induced durable remissions among leukemia patients refractory to conventional therapies including bone marrow transplantation[8, 9]. Extensive preclinical and clinical studies are being conducted to extend these successes to other types of cancer, especially, solid cancer.

Cellular immunotherapeutics could be fundamentally classified into vaccine approaches and adoptive transfer of effector cell approaches. In this review, we discuss recent and current efforts in the field of cellular immunotherapy for malignant gliomas.

2. Cellular vaccine approaches

2.1 Tumor cell vaccines

Tumor cell vaccines utilize either autologous or allogeneic tumor cells that are attenuated. Since the inherent immunogenicity of the tumor cells may be limited, they are often genetically engineered to express costimulatory molecules, cytokines chemokines, or those in combination[10]. Especially, to enhance the presentation of tumor antigens, cytokines that

activate antigen-presenting cells (APCs), such as GM-CSF and IL-4[11–13], co-stimulatory molecules, such as CD80[14] have been used.

In preclinical studies, peripheral immunization of rats bearing 9L gliosacroma in the brain with IL-4 transfected 9L cells achieved the most potent therapeutic benefit compared to GM-CSF, IL-12 and IFN-α[15, 16]. IL-4 produced at the local vaccine site appears to promote a T-helper 1-type antitumor immune response[17], and the observed therapeutic response was further enhanced in cooperation with local delivery of IFN-α in the intracranial tumor site[18]. A phase I clinical study evaluated safety and immunological activity of a vaccine with autologous tumor cells admixed autologous fibroblasts that are engineered to express IL-4 in patients with recurrent malignant glioma[19]. While only 2 of 6 enrolled participants received scheduled two vaccinations, both participants demonstrated encouraging immunological and clinical and radiological responses[19]. No significant side effects were observed. However, generating sufficient numbers of IL-4-transfected vaccine cells required 7 to 8 weeks. Most participants were withdrawn from the trial because of tumor progression prior to the first vaccination, which posed a major feasibility issue. A phase I/IIa study evaluating autologous formalin-fixed tumor vaccine in newly diagnosed GBM demonstrated feasibility, tolerability as well as encouraging median overall survival (OS) of 22.2 months and a median progression-free survival (PFS) of 8.2 months[20]. More recently, a phase I study was conducted demonstrating the safety and feasibility of vaccination with irradiated autologous glioma cells mixed with irradiated GM-CSF-transduced allogeneic K562 cells in patients with recurrent malignant glioma [21].

2.2 Dendritic cell vaccines

Dendritic cells (DCs) are the most potent APCs, and establishment of methods to culture DCs from peripheral blood-derived monocytes facilitated developments of DC-based vaccines in a variety of cancer types[22]. DCs can be coupled with a variety of tumor antigen sources, such as, synthetic peptides, autologous glioma lysate or acid-eluted glioma peptides. DCs can be directly fused with tumor cells or transfected with tumor RNA, cDNA, or viral vectors[23].

With the use of whole autologous tumor as the antigen source, DCs can present a wide array of possible tumor antigens to the host immune system[24]. Because DCs present tumor-antigens on their MHC molecules, it seems to make the most logical sense if DCs are loaded with acid-eluted peptides derived from autologous tumor cell surface MHC class I molecules. Vaccinations using DCs loaded with autologous acid-eluted peptides were safe[25], and elicited detectable systemic toxicity and intracranial T-cell infiltration[26]. This method, however, requires 10^8–10^9 tumor cells to derive peptides for loading sufficient numbers of DCs, posing a major feasibility challenge.

Use of autologous, whole glioma cell lysate can alleviate this concern as peptides from proteins in the lysate can still be presented by MHC. Wheeler et al. reported the immune and clinical responses from a phase II trial treating 44 patients (34 GBM) with autologous DC pulsed with tumor lysate. Fifty-three percent of GBM patients exhibited ≥1.5 fold vaccine-enhanced cytokine responses. Vaccine responders exhibited significantly longer survival relative to nonresponders, with 41% of vaccine responders survived at least 2 years

compared with 7% of vaccine nonresponders[27]. In another trial, IL-4-transfected fibroblasts admixed with DCs loaded with tumor lysate were given intradermally in five newly diagnosed GBM patients[19]. The median time to progression (TTP) after surgical resection was 6 months. A more recent phase I/II study evaluating a tumor lysate-loaded DC-based vaccine in 77 patients with newly diagnosed GBM showed the feasibility of integrating this treatment in the standard-of-care treatment with surgery, radiotherapy, and chemotherapy. Median OS was 18.3 months since leukapheresis[28]. DCVax is an autologous DC vaccine pulsed with tumor lysate antigen for the treatment of GBM. Autologous tumor lysate–pulsed DC vaccination in conjunction with TLR agonists was evaluated for safety in newly diagnosed and recurrent glioblastoma patients[29]. In addition to encouraging survival data, patients whose tumors had mesenchymal gene expression signatures exhibited increased survival compared with historic controls of the same genetic subtype. Tumor samples with a mesenchymal gene expression signature had a higher number of CD3+ and CD8+ TILs compared with GBMs of other gene expression signatures, suggesting that GBM with the mesenchymal gene expression profile may be more responsive to immune-based therapies. None of these whole-GBM antigen-loaded DC vaccines demonstrated autoimmune encephalitis.

Use of synthetic peptides encoding tumor antigen epitopes provides “off-the-shelf” feasibility thanks to unlimited availability of synthetic peptides. Furthermore, compared with the whole tumor cell antigen-based approaches, use of synthetic peptides targeting tumor-specific (mostly tumor-specific mutation-derived) or tumor-associated (non-mutated but expressed at higher levels in tumor cells vs. normal cells) antigens may reduce the risk of autoimmunity, although selection of antigens is crucial. There are recent excellent reviews on antigens targeted in immunotherapy of gliomas[30–33].

Several phase I/II trials employing synthetic peptide antigens have been conducted. We have evaluated novel α-type 1 polarizing DCs (αDC1), which were manufactured by maturation of monocyte-derived immature DCs with IL-1β, tumor necrosis factor-α, interferon (IFN)-α, IFN-γ and poly-I:C. αDC1 were loaded with synthetic peptides for glioma-associated antigen (GAAs) epitopes and administered in combination with polyinosinic-polycytidylic acid [poly(I:C)] stabilized by lysine and carboxymethylcellulose (poly-ICLC) in HLA-A2(+) patients with recurrent malignant gliomas[34]. GAAs for these peptides are EphA2, interleukin (IL)-13 receptor-alpha2, YKL-40, and gp100. In 22 recurrent high-grade glioma patients who received at least one vaccine, nine patients remained free from progression for at least 12 months. One patient with a GBM had a complete response, and IL-12 production levels by αDC1 positively correlated with time to progression[34]. In another trial, 19 GBM and one brainstem glioma patient received DCs pulsed with tumor-associated antigens (TAA: HER2, TRP-2, gp100, MAGE-1, IL-13Rα2 and AIM-2)[35]. The median OS was 38.4 months for patients with newly diagnosed GBM. OS was positively correlated with quantitative expression of MAGE-1, AIM-2, gp100 and HER2 in patient tumor samples.

Some of recent DC vaccine studies evaluated combination with immunoadjuvants. These include adjuvant cytokine administration (GM-CSF)[36], and toll-like receptor (TLR) agonists[29, 34]. Chemotherapy or antiangiogenic therapy may also potentiate of DC-based immunotherapy[37, 38]. However, a very carefully designed study has demonstrated that

clinically relevant dosages of standard alkylating chemotherapies, such as temozolomide and cyclophosphamide, profoundly inhibit B and T cell responses to vaccines [39], calling for our cautions designing vaccine studies with concurrent chemotherapy.

Viral antigens may represent particularly attractive targets for immunotherapy because they are foreign to the host immune system and thus are inherently immunogenic. Malignant brain tumors have not been shown to be virally induced, but studies have demonstrated frequent detection of low-level expression of human cytomegalovirus (CMV) genes within malignant gliomas[40, 41]. While the role of CMV in the biology of these tumors is a continued area of study [40], a recent study of a DC vaccine targeting CMV epitopes in GBM demonstrated promising results, especially in combination with the vaccine-site conditioning with tetanus-toxioid[42].

A number of clinical trials are conducted in malignant gliomas (Table 1). DC-based vaccines for brain tumors appear to be safe and can induce anti-tumor immune response. However, objective clinical benefits (objective anti-tumor response and/or extension of survival) remains to be determined.

3. Adoptive cell therapy (ACT) of effector cells

In ACT of effector cells, large numbers (typically $1 \times 10^{6-9}$ orders) of immune effector cells are prepared *ex vivo* and infused to patients. In brain tumor patients, these cells have been administered locally in the brain tumor site or systemically via i.v. In the past, *ex vivo* prepared cells with undefined, broad antigen-specificity were mainly used, such as lymphokine-activated killer (LAK) cells. Recently, antigen-targeted approaches have been developed, such as the use of CAR and TCR-transduced cells (Table 2). Even though some of these approaches are quite successful in other cancer types, it is important to address unique challenges that arise when these approaches are applied for brain tumors.

3.1 LAK cells and NK cells

LAK cells are autologous peripheral blood lymphocytes stimulated with IL-2 *in vitro* [43]. Natural killer (NK) cells are the major effector population in LAK cells. They recognize cancer cells in a non-MHC-restricted fashion. LAK cells may represent a primitive immune surveillance system capable of recognizing and destroying altered cells.

A number of clinical studies have been conducted treating GBM or high-grade glioma patients with local injection of LAK cells[44]. These studies demonstrated the safety of infusing autologous leukocytes into the tumor resection cavity. Some of them also have shown promising results in prolonging disease free survival. However, comparison of LAK cell therapy and IL-2 with IL-2 alone showed no significant difference in response rates in patients with renal cell carcinoma[45]. Also high-dose IL-2 may lead to capillary leak syndrome, including hypotension, oliguria, pulmonary edema and dyspnea, discouraging further study of the approach. Thus, a randomized phase II or III clinical study was never conducted. However, owing to recent advances in the field of NK cell biology[46], there is a renewed interest in NK cell-based immunotherapy for cancer[47]. Recent preclinical advancements of NK cell immunotherapy include augmentation of antibody-dependent

cellular cytotoxicity, manipulation of receptor-mediated activation, and adoptive immunotherapy with ex vivo-expanded, CAR-engineered NK cells (reviewed in [48]).

3.2 γδ T cells

In healthy donors, T cells bearing the γδ T cell receptor constitute 0.5–20% of CD3+ T lymphocytes in peripheral blood and in lymphoid tissues[49]. They can be isolated then expanded by IFN-γ, IL-2, monoclonal antibody against CD3, and IL-1α[50]. γδ T cells express natural cytotoxicity receptor natural killer p 44, and exert their cytolytic activity mainly via the non-MHC-restricted γδ TCR[51]. Activating NK cell receptors such as NKG2D and DNAM1 are also present on most γδ T cells, which recognize stress-induced ligands on tumor cells[50, 52]. Early phase clinical trials have been conducted in non-Hodgkin lymphoma, multiple myeloma, and metastatic solid tumors[53]. In brain tumors, preclinical studies also suggested that γδ T-cell depletion and impaired function occur prior to or concurrent with the growth of the brain tumor[54]. Expanded/activated γδ T-cells from both healthy controls and selected patients have significant cytotoxicity against primary GBM explants[55]. Also there are evidence that γδ T cell therapy may be safe for brain tumor patients who undergo standard cytotoxic therapies[56, 57], opening a previously unexplored approach to cellular immunotherapy of brain tumors.

3.3 TIL transfer

TILs are obtained from tumor tissue, draining lymph nodes or malignant effusions. They contain high numbers of tumor-specific T cells that presumably have already been selected for their ability to recognize and respond to the tumor antigens. While TILs may not possess sufficient antitumor activity in the highly immunosuppressive microenvironment established by tumors, activation and expansion of TILs *ex vivo* can overcome these immunosuppressive effects and allow for the generation of sufficient numbers of TILs for adoptive immunotherapy. These TILs are expanded *ex vivo* with high dose IL-2, then transferred back to the patient.

Adoptive cell therapy with TILs in combination with lymphodepletion and high-dose IL-2 has mediated durable, complete regressions in patients with melanoma, with reproducible objective response rates of approximately 50% in patients with highly advanced, refractory metastatic melanoma, probably by targeting somatic mutations exclusive to each cancer[58]. However, in brain tumors only few attempts have been made[59–61]. This may be because obtaining and expanding enough numbers of TILs require highly immunogenic, large, and accessible tumors. For malignancies other than melanoma, it has been very difficult to expand TILs from tumor tissues[62]. Also T cells present at the tumor bed are often exhausted, limiting their functions and their proliferative capacity. To overcome this issue for gliomas, a clinical trial was performed first vaccinating patients with irradiated autologous tumor cells, then harvesting tumor-draining lymph node T cells, expanding them *ex vivo* with anti-CD3 antibody and bacterial superantigen Staphylococcal enterotoxin A, and systemically infusing these cells[63, 64]. Three out of ten patients with recurrent malignant gliomas[63] and four out of ten patients with newly diagnosed malignant gliomas[64] showed radiographic partial response. However, no study has proven prolongation of the survival of glioma patients.

3.4 Adoptive transfer of genetically engineered T-cells (CAR and TCR)

3.4.1 αβT-Cell Receptors—The cDNAs for the α- and β-chains of the TCR are cloned from class I HLA-restricted TCRs of tumor-reactive cytotoxic T cells and transferred to fresh T cells. Several TCRs have been cloned for several HLA-restricted epitopes encoded by TAAs[65–68]. Genetic modification of T cells with α/β TCRs also requires high expression and correct pairing of two different receptor molecules from a single vector, which has proved problematic for transgenic α/β TCRs, especially because mispairing between transgene- or endogenous TCR-derived α and β chain can occur. A variety of gene-engineering technologies have been evaluated, such as small interfering RNA constructs that specifically down-regulate endogenous TCR;[69] a disulfide bridge in the α/β constant (C) regions by the extra cysteine residues; substituting human with murine C regions; codon optimization to enhance protein synthesis; TCR chain leucine zipper fusions; and a single chain TCR (reviewed [70, 71]).

In the first reported trial to examine the *in vivo* efficacy of TCR-transduced T cells in patients with cancer, the adoptive transfer of autologous T cells that were transduced with a MART-1–reactive TCR lead to tumor regression in 2 of 15 treated patients with metastatic melanoma[65]. Another study using autologous T-cells transduced with TCR treated 36 patients with metastatic melanoma using high-avidity TCRs that recognized either the MART-1 or gp100 melanoma-melanocyte antigens[67]. Objective cancer regressions were observed in 30% and 19% of patients who received the MART-1 or gp100 TCR, respectively, but severe off-tumor, on-target toxicity was seen in the skin, eyes, and ears due to the presence of melanocytes in these organs. The use of a high-affinity TCR against the carcinoembryonic antigen (CEA) in patients with metastatic colorectal cancer that expressed high levels of this antigen[72] was halted when all 3 patients experienced life-threatening colitis and colonic hemorrhage. Unexpected toxicities can also present when previously unknown cross-reactive targets are expressed in healthy vital organs. For example, while MAGE-A3 is not known to be expressed in any normal tissues, targeting an HLA-A2.1-restricted peptide in MAGE-A3 caused severe damage to brain gray matter, resulting in 2 deaths because this TCR homed to a different but related epitope expressed by MAGE-A12 at very low levels in the brain[73].

A TCR directed against NY-ESO-1, a cancer germline antigen expressed in a variety of solid cancers holds promise. Objective responses were observed in 11 of the 18 patients (61%) with synovial cell sarcoma and 11 of the 20 patients (55%) with melanoma who received autologous TCR-transduced cells. The total number of T cells and the number of antigen-reactive T cells administered to patients correlated with response to therapy. However, there was a lack of a correlation between clinical responsiveness and persistence of infused T cells, possibly resulted from a failure of the T cells to persist for longer time periods[75]. NY-ESO-1-specific TCR-engineered T cells also showed encouraging clinical response in 16 of 20 patients with myeloma, in which engineered T cells expanded, persisted, trafficked to bone marrow and exhibited a cytotoxic phenotype. Disease progression was associated with loss of T cell persistence or antigen escape[76]. For brain tumors, however, no clinical study with αβ TCR T cells has been initiated.

3.4.2 CAR-T cells—CAR engineering involves transgene-expression of single chain variable fragment (scFv) of a monoclonal antibody (Ab), which is specific for a tumor cell surface protein, at the surface of T cells, allowing the T cells to recognize tumor directly and not through the MHC complex. The Ab is linked to the CD3ζ chain and other T cell activation pathways, allowing T cell activation and target cell killing[77, 78]. In addition, Abs bind antigens with much greater affinity than do TCRs, resulting in the formation of a more stable immunological synapse[79]. CARs have evolved over the last decade, with progressively increasing co-stimulatory activity. In addition to a single signaling unit derived from the CD3ζ chain or the high-affinity IgG receptor Fc1RIg[80], second-generation CARs incorporate the intracellular domain of a co-stimulatory molecule, CD28. Subsequent incorporation of both CD28 and a tumor necrosis factor receptor family member CD137 (4-1BB), CD 27, CD134 (OX40), CD244, or ICOS has enhanced the ability of these receptors to stimulate cytokine secretion and T cell proliferation and persistence in preclinical studies[81]. Compared with TCR engineered T cells, CAR engineered T cells are applicable to all patients irrespective of their HLA alleles expressed, and circumvent tumor evasion through HLA down-regulation.

CARs have been generated for the glioma cell surface antigens, including IL-13Rα 2[82], HER2[83], EphA2[84], and EGFRvIII[85–87]. It should also be noted that CARs can induce toxicity against self-antigens as well. Acute pulmonary toxicity resulting in death was observed after infusion of CAR-T cells specific for ERBB2, likely due to the recognition of low levels of this antigen on pulmonary epithelium[74]. These observations underscore the need for selecting tumor-specific antigens, such as tumor-specific mutation-derived antigens (i.e., neoantigens), for effective and safe ACT. Among these targets, only EGFRvIII is tumor-specific, while others are TAAs. In preclinical animal models, T cells expressing these EGFRvIII-specific CARs showed potent antitumor activity[85, 86]. Phase I clinical trials of CART cells that are engineered to target HER2 (NCT02442297) or EGFRvIII mutation (NCT01454596 and NCT02209376) in patients with GBM are ongoing. A phase I clinical study with T cells expressing IL13Rα2-specific CAR has demonstrated safety in patients with recurrent GBM[88].

Most individuals naturally have high-avidity T-cells against viral epitopes at high frequencies. Transduction of viral antigen-specific T-cells with CAR may allow re-stimulation of CAR-transduced T-cells via the endogenous viral antigen-specific TCR (e.g. CMV-specific TCR) and the corresponding epitope (bispecific T cells). A phase I clinical studies with CMV-specific “bispecific” T-cells transduced with anti-HER2 CAR has been initiated (NCT01109095). A study with T cells expressing HER2-specific CARs showed that these cells had potent antitumor activity against HER2-positive, CD133-positive glioma stem cells[83]. A similar bispecific T cell, anti-GD2 CAR EBV-specific T cells, is being conducted as a phase I study in patients with refractory/relapsed neuroblastoma (NCT00085930). EBV-specific T cells, expressing a GD2-ζ CAR, persisted significantly longer than control, non-viral specific GD2-ζ T cells. Infusion of GD2-specific T cells resulted in tumor necrosis or regression (including a complete remission) in four out of eight patients[89].

A number of ways have been explored to increase the specificity of CAR-T cells to achieve a more promising, safer targeting. CAR-T cells can be genetically modified to recognize two or more tumor- associated antigens, which can enhance discrimination between abnormal and healthy tissue. One can transfer two CARs[90]; split-signal CARs, which can limit full T cell activation to tumors expressing multiple antigens[91, 92] ; tandem CARs (TanCARs), which contain ectodomains with two scFvs[93], also limiting the risk of immune escape; or co-expression of inhibitory CARs (iCARs) directed against molecules in healthy organs together with their activating counterparts (reviewed with a schema in[94]). Furthermore, a novel approach has been developed to engineer the T cells with dual-receptor circuits, in which a synthetic Notch receptor for one antigen induces the expression of a CAR for a second antigen[95].

4. Conclusion

We discussed recent developments of cellular immunotherapy for malignant brain tumors. While some of novel cellular therapeutics, such as CAR therapy, have demonstrated remarkable successes in other cancer types, translation of those successes to brain tumors will not be achieved unless we gain in-depth understanding of the unique immunological environment of brain tumors and develop strategies that are adequate to overcome challenges associated with the environment. In the “Expert Opinion” section below, we will further discuss our perspective.

5. Expert Opinion

After decades of efforts to revise the longstanding dogma that the brain and tumors arising therein are “immunologically privileged”, immunotherapy, including cellular immunotherapy, for brain tumors has been emerging as a promising approach. However, the ultimate success of cellular immunotherapy in brain tumor patients would require advancements in the following areas: 1) feasibility of timely production of cellular therapeutics; 2) paucity and heterogeneity of tumor specific antigens; 3) better strategies to promote antigen-presentation and effector T-cell trafficking; 4) local and systemic immune suppression, and 5) proper interpretation of imaging data for brain tumor patients receiving immunotherapy.

5.1 Feasibility of Producing Cellular Therapeutics

Production of autologous cell products inevitably involves lengthy and intensive processes, such as leukapheresis, engineering, expansion, as well as quality-assurance tests and assays. There require substantial costs, infrastructure of the institution as well as invasive procedures for patients, such as leukapheresis. These are important issues to address, especially because we hope that cellular immunotherapy will become effective, standard-of-care therapy in future. Ongoing efforts are directed to development of efficient bioreactors and automated processing systems. Another way to solve the issue is to develop “off-the-shelf” allogeneic cell products that can be safely administered without being rejected by the host immune-system. While this requires multiple immuno-genetic engineering of cells, developments are underway in this direction.

5.2 Paucity of tumor-specific antigens and heterogeneity of antigen-expression

Although the list of antigens that could be used for immunotherapy of brain tumors has expanded over the last decade[31], there are not many truly brain tumor-specific antigens, except for those derived from EGFRvIII and mutant IDH1[96]. Use of tumor-associated, but non-specific antigens (TAAs as referred in this manuscripts) can cause life-threatening and fatal events by on-target[72] or off-target[97] cross-reactivity of T-cells against normal cells. Furthermore, due to marked heterogeneity of genetics and protein expression in solid cancers, targeting a single antigen may result in the evolution of variants that lack the target antigen [98]. These observations underscore the need for expanding the list of available tumor-specific antigens, such as mutation-derived antigens (i.e., neoantigens), for effective and safe immunotherapy. Extension of these approaches should foster broader availability of target antigens for immunotherapy of brain tumors.

5.3 Better Strategies to Promote Antigen-Presentation and Effector T-cell Trafficking

While brain tumors are heavily infiltrated by myeloid cells, the vast majority of them are suppressive for effector T-cell functions but not effective APCs[99]. Efforts are being undertaken to modulate the function of these cells and promote their function as type-1 APCs. In regard to T-cell homing to brain tumors, although T-cells are able to traverse the blood-brain-barrier via chemokine axes and multistep adhesion processes, homing of effector CTL is weaker in brain tumors compared with cancer in other organs[100, 101]. To date, there have not been many immunotherapy regimens for brain tumors incorporating therapeutic agents that can facilitate T-cell homing to the brain tumor site. Our regimens using poly-ICLC have been among the first to address this issue and are expected to enhance T-cell homing to the glioma site [34, 100, 102]. In other organ sites, Kershaw et al. have demonstrated that engineering the chemokine receptor CXCR2 into T cells enabled the T cells to efficiently migrate toward melanoma[103]. Transgenic co-expression of CCR4 improved the homing of CAR-CD30-modified T cells to CD30+ Hodgkin lymphoma that secreted CCL17 (the ligand for CCR4)[104]. Enhanced CCR2b expression from mesothelin-reactive CAR-T cells and CAR-GD2 T cells led to improved anti- tumor effects against malignant pleural mesothelioma and neuroblastoma[105, 106]. Some of these inventions may be applicable for brain tumors as well.

5.4 Local and Systemic Immune Suppression

Brain tumors mediate a variety of immunosuppressive mechanisms to escape from immunological attacks. These include expression of check-point molecules and immunosuppressive cytokines as well as recruitment of regulatory T-cells and immunosuppressive myeloid cells. Furthermore, it is important to recognize that significant levels of systemic immunosuppression are likely caused by treatments for these patients. These include chemotherapy, such as temozolomide[39] as standard-of-care, corticosteroids as well as radiation therapy. Grossman et al have suggested that lymphocyte counts alone are predictive of prognosis, with lower counts correlating with shorter survival in patients with GBM[107]. It is important to address how we can minimize the impact of treatment-induced immunosuppression by the time the patient receives immunotherapy, although for adoptive

transfer of T-cells, lymphopenic conditions induced by prior treatments may serve as a proper “conditioning”, thereby promoting post-infusion expansion of T-cells.

In CAR T cells, post-infusion *in vivo* activity is mainly supported through addition of costimulatory molecules in the CAR construct. Recently, TRUCK T cells, also called fourth-generation CARs, were developed involving two separate transgenes, with the CAR gene and a T cell activation responsive promoter linked to a cytokine[108]. Studies have shown that therapy with T cells engineered to express IL-12 could change the tumor microenvironment and enhance anti-tumor function[109, 110]. IL-12 secretion by engineered T cells expressing CARs resulted in the destruction of antigen negative cancer cells that may escape from T cell therapy[111]. Also antigen-specific T cells expressing dnTGF-βRII were resistant to the anti-proliferative effects of TGF-β and retained their effector function *in vivo*[112]. On the other hand, CAR-T cells also express PD1, and are susceptible to PD1/PDL1 interaction-mediated suppression[113]. It has been shown that blocking PD1 immunosuppression can boost CAR-T cell therapy, likely representing a fruitful area for future study[114, 115].

5.5 Proper interpretation of imaging data for brain tumor patients receiving immunotherapy

Early phase immunotherapy clinical trials in brain tumor patients have revealed unique challenges associated with assessment of radiological changes reflecting delayed responses or therapy-induced inflammation[116]. Neuroimaging often reveals temporary worsening of abnormal findings and even appearance of new lesions. Clinical benefit, including long-term survival and tumor regression, can still occur following initial apparent progression. A multinational and multidisciplinary panel of neuro-oncology immunotherapy experts recently described immunotherapy response assessment for neuro-oncology (iRANO) criteria [117] that are based on guidance for determination of tumor progression outlined by the immune-related response criteria (irRC)[118] and the response assessment in neuro-oncology (RANO) working group [119]. The iRANO guidelines specifically address interpretation of initial progressive imaging findings in the context of neuro-oncology patients with a goal of decreasing the likelihood of premature discontinuation of potentially beneficial therapies while ensuring maximum patient safety. Prospective evaluation of the iRANO criteria in brain tumor immunotherapy trials for neuro-oncology patients will be required to improve their ultimate clinical utility.

To address above discussed issues, it is apparent that our ultimate success will largely hinge upon effective collaboration across multiple disciplines. Scientifically, we have to integrate cutting edge progresses in both cancer immunology and central nervous system immunology. To implement novel combination strategies, it is essential to promote effective collaboration across companies and regulatory authorities. Encouraged by recent success in cancer immunotherapy for other cancer types, we believe that we are on the right direction and hope that we will develop truly effective immunotherapies for patients with malignant brain tumors.

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Article Highlights Box

- Cellular immunotherapeutics could be classified into vaccine approaches and adoptive transfer of effector cell approaches.
- Cellular vaccine approaches utilize tumor cells and/or antigen presenting cells, such as dendritic cells.
- Adoptive cell transfer (ACT) approaches can utilize a variety of effector cell types, including lymphokine-activated killer (LAK) cells, γδ T cells, tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor (CAR)-T cells and T-cell receptor (TCR) transduced T cells.
- The ultimate success of cellular immunotherapy in brain tumor patients would require improvements in the areas including feasibility in producing cellular therapeutics as well as strategies to promote effector T-cell trafficking to the tumor site and to overcome local and systemic immune suppression.
- With advances of technologies allowing antigen-specific targeting of ACTs, it is critical to expand the list of glioma-speicific antigens that can be safely targeted in future immunotherapies.

Table 1

Open Studies of cellular vaccine therapy in patients with primary brain tumors (as of April 5, 2016 in clinicaltrials.gov)

NCT#	Study Name	Responsible Party	type of cells used	Disease	phase
NCT01792505	Dendritic Cell Vaccine With Imiquimod for Patients With Malignant Glioma	Cedars-Sinai Medical Center	Tumor lysate loaded DCs	Malignant Glioma	I
NCT02010606	Phase I Study of a Dendritic Cell Vaccine for Patients With Either Newly Diagnosed or Recurrent Glioblastoma	Cedars-Sinai Medical Center	DCs Pulsed With Lysate Derived From an Allogeneic Glioblastoma Stem-like Cell Line	Glioblastoma	I
NCT00639639	Vaccine Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme (ATTAC)	Duke University Medical Center	Human CMV pp65-LAMP mRNA-pulsed DCs	Newly diagnosed glioblastoma	I
NCT00890032	Vaccine Therapy in Treating Patients Undergoing Surgery for Recurrent Glioblastoma Multiforme	Duke University Medical Center	BTSC (brain tumor stem cells) mRNA-loaded DC vaccine	Recurrent Glioblastoma	I
NCT02529072	Nivolumab With dendritic cells Vaccines for Recurrent Brain Tumors (AVERT)	Duke University Medical Center	Human CMV pp65-LAMP mRNA-pulsed DCs, in combination with anti-PD-1 Monoclonal Antibody (Nivolumab)	Recurrent high grade glioma	I
NCT02709616	Personalized Cellular Vaccine for Glioblastoma (PERCELLVAC)	Guangdong 999 Brain Hospital	Tumor antigen mRNA-pulsed DCs	Glioblastoma	I, II
NCT01567202	Study of dendritic cells Vaccination Against Glioblastoma	Huashan Hospital	DCs Loaded With autogeneic glioma stem-like cells (A2B5+)	Glioblastoma	II
NCT01204684	Dendritic Cell Vaccine for Patients With Brain Tumors	Jonson Comprehensive Cancer Center	Tumor lysate loaded DCs	high grade glioma	II
NCT01635283	Vaccine for Patients With Newly Diagnosed or Recurrent Low-Grade Glioma	Jonson Comprehensive Cancer Center	Tumor lysate loaded DCs	low grade glioma	II
NCT01759810	Proteome-based Personalized Immunotherapy of Glioblastoma	NeuroVita Clinic	Hematopoietic stem cells (HSCs), dendritic vaccine (DV) and cytotoxic lymphocytes (CTLs)	Glioblastoma	II, III
NCT00045968	[DCVax®-L] to Treat Newly Diagnosed GBM Brain Cancer (GBM)	Northwest Biotherapeutics	Tumor lysate loaded DCs	Glioblastoma	III
NCT02649582	Adjuvant Dendritic Cell-immunotherapy Plus Temozolomide in Glioblastoma Patients (ADDIT-GLIO)	University Hospital, Antwerp	Autologous Wilms' tumor 1 (WT1) messenger (m)RNA-loaded DCs	Newly diagnosed glioblastoma	I, II
NCT01326104	Vaccine Immunotherapy for Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor (Re-MATCH)	University of Florida	Total tumor RNA (TTRNA)-loaded DCs (dendritic cells), ex vivo expanded Autologous Lymphocyte	Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor	I, II
NCT02465268	Vaccine Therapy for the Treatment of Newly Diagnosed Glioblastoma Multiforme (ATTAC-II)	University of Florida	Human CMV pp65-LAMP mRNA-pulsed DCs	Newly diagnosed glioblastoma	II
NCT01902771	Dendritic Cell Vaccine Therapy With In Situ Maturation in Pediatric Brain Tumors	University of Miami	Tumor lysate loaded DCs	Pediatric Brain Tumors	I

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Table 2

Open Studies of adoptive cell transfer therapy in patients with primary brain tumors (as of April 5, 2016 in clinicaltrials.gov)

NCT#	Study Name	Responsible Party	type of cells used	Disease	phase
NCT02100891	Phase 2 STIR Trial: Haploidentical Transplant and Donor Natural Killer Cells for Solid Tumors	Medical College of Wisconsin	Donor NK cells	Solid tumors, including high risk malignant brain tumors	II
NCT01759810	Proteome-based Personalized Immunotherapy of Glioblastoma	NeuroVita Clinic (Russia)	Dendritic vaccine, hematopoietic stem cells, cytotoxic lymphocytes	Recurrent glioblastoma	II, III
NCT01326104	Vaccine Immunotherapy for Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor	University of Florida	Total tumor RNA (TTRNA)-loaded DC + ex vivo expanded TTRNA loaded autologous lymphocyte transfer	Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor	I, II
NCT02661282	Phase II Autologous Cytomegalovirus (CMV)-Specific Cytotoxic T Cells for Glioblastoma (GBM) Patients	M.D. Anderson Cancer Center	Autologous CMV-Specific Cytotoxic T Cells	Glioblastoma	I, II
NCT01109095	CMV-specific Cytotoxic T Lymphocytes Expressing Chimeric Antigen Receptors(CAR) Targeting HER2 in Patients With GBM (HERT-GBM)	Baylor College of Medicine	Anti-HER2 CAR transduced autologous CMV-specific cytotoxic T-lymphocytes (CTL)	Glioblastoma	I
NCT02442297	T Cells Expressing HER2-specific CAR for Patients With Glioblastoma (iCAR)	Baylor College of Medicine	Anti-HER2 CAR transduced T Cells	Recurrent or refractory GBM	I
NCT02541370	Treatment of Relapsed and/or Chemotherapy Refractory Advanced Malignancies by CART133	Chinese PLA General Hospital	Anti-CD133-CAR modified T Cells	Relapsed and/or Chemotherapy Refractory Malignancies	I
NCT02208362	Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma	City of Hope Medical Center	Anti-IL13Rα2 CAR and a truncated CD19 transduced central memory enriched T cells	Recurrent/Refractory Malignant Glioma	I
NCT02209376	Pilot Study of Autologous T Cells Redirected to EGFRVIII-With a Chimeric Antigen Receptor in Patients With EGFRVIII+ Glioblastoma	University of Pennsylvania in collaboration with University of California, San Francisco	Autologous T cells transduced with a lentiviral vector to express a CAR specific for EGFRvIII	Newly diagnosed and recurrent glioblastoma	
NCT02664363	EGFRvIII CAR T Cells for Newly-Diagnosed GBM (ExCeL)	Duke University Medical Center	Anti-EGFRvIII CAR transduced T Cells	Newly diagnosed glioblastoma	I
NCT02575261	CAR-T Cell Immunotherapy for EphA2 Positive Malignant Glioma Patients	Fuda Cancer Hospital, Guangzhou	Anti-EphA2 CAR transduced T cells	EphA2 Positive Recurrent and Metastatic Malignant Glioma	I, II
NCT01454596	CAR T Cell Receptor Immunotherapy Targeting EGFRvIII for Patients With Malignant Gliomas Expressing EGFRvIII	National Cancer Institute (NCI)	Anti-EGFRvIII CAR transduced PBL	Malignant Gliomas Expressing EGFRvIII	I, II
NCT02617134	CAR-T Cell Immunotherapy in MUC1 Positive Solid Tumor	PersonGen Biomedicine (Suzhou) Co., Ltd.	Anti-MUC1 CAR transduced T cells	MUC1 Positive Advanced Refractory Solid Tumor	I, II

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NCT#	Study Name	Responsible Party	type of cells used	Disease	phase
NCT02331693	CAR T Cells in Treating Patients With Malignant Gliomas Overexpressing EGFR	RenJi Hospital	Anti-EGFR CAR transduced T cells	EGFR-overexpressing malignant glioma	I
NCT02713984	A Clinical Research of CAR T Cells Targeting HER2 Positive Cancer	Southwest Hospital, China	Anti-HER2 CAR transduced T Cells	HER2 positive cancer	I, II