

The development of dendritic cell vaccine-based immunotherapies for glioblastoma

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Abstract In this review, we focus on the biologic advantages of dendritic cell-based vaccinations as a therapeutic strategy for cancer as well as preclinical and emerging clinical data associated with such approaches for glioblastoma patients.

Introduction: glioblastoma overview

Glioblastoma is the most common primary malignant central nervous system tumor among adults with an incidence rate of 3.19 cases per 100,000 person-years, which translates into approximately 13,000 cases diagnosed in the USA each year [1]. The current standard of care for newly diagnosed glioblastoma patients was established over 10 years ago and includes maximum safe surgical resection, targeted cranial external beam radiotherapy administered with daily temozolomide followed by adjuvant temozolomide chemotherapy administered for 6 months or longer. This multimodality treatment approach is associated with a median progression-free survival of only 7.8 months, a median overall survival of 14.6 months, and a 5-year survival rate of under 10% [2]. Despite marked advances that have led to improved understanding of the biology and molecular complexity of

glioblastoma tumors [3, 4] as well as the evaluation of dose intensive temozolomide chemotherapy [5], anti-angiogenic therapy with bevacizumab [6, 7], and integrin inhibition with cilengitide [8] in large randomized phase 3 clinical trials, none of these efforts has led to an improvement in survival. Furthermore, given that all patients ultimately progress following standard therapy, salvage treatments have been woefully inadequate with a series of meta-analyses evaluating outcome across a wide spectrum of salvage therapy clinical trials revealing progression-free survival rates at 6 months (PFS-6) of less than 15% [9]. Although bevacizumab improves PFS-6 to approximately 40%, this improvement translates into minimal overall survival benefit [10]. Two additional points further underscore the inadequate outcome associated with currently available therapies for glioblastoma. First, survival data quoted for glioblastoma patients reflect outcome for patients well enough to participate in prospective clinical trials. A more accurate representation of outcome derives from population-based studies where outcome for all patients from a given geographic locale is summarized [11]. A recent review of population-based studies reveals a median survival of approximately 8.5 months for newly diagnosed glioblastoma patients. [12]. Second, in a recent analysis across a spectrum of aggressive adult cancers, malignant gliomas including glioblastoma were rated as having the highest rate of years per life lost and earliest age of death, providing further evidence of the short survival time following diagnosis [13]. Novel, innovative treatment strategies are clearly needed for both newly diagnosed and recurrent glioblastoma patients.

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The potential of immunotherapy for glioblastoma

Although a vision of harnessing the immune system to effectively treat cancer dates back over 120 years to initial efforts

pioneered by William Coley in which he attempted to enhance anti-tumor immune responses by administration of bacterial toxins to bone cancer patients (“Coley’s toxins”), [14] disappointing results subsequently associated with various immunotherapy approaches dampened enthusiasm for decades. However, enthusiasm has been markedly revitalized in the past few years based on meaningful survival benefits that have been achieved by a panel of immunotherapeutics across a spectrum of oncology indications. In April 2010, a historical benchmark was achieved in that the U.S. Food and Drug Administration (FDA) approved the first vaccine for the treatment of cancer. This vaccine, sipuleucel-T (Provenge®) was approved for the treatment of prostate cancer and consists of autologous dendritic cells sensitized *ex vivo* to prostatic acid phosphatase linked to granulocyte-macrophage colony-stimulating factor (PAP-GM-CSF). Results of a double-blind, placebo-controlled randomized, phase 3 clinical trial revealed that men with metastatic, castration-resistant prostate cancer who received sipuleucel-T had a 4.1-month longer median overall survival than those who received placebo [15]. Thereafter, an exciting series of reports were unveiled documenting remarkable anti-tumor responses against a multitude of different cancers that translated into survival benefit following treatment with inhibitory immune checkpoint blocking antibodies. Initially, ipilimumab (Yervoy), a humanized monoclonal antibody (MAb) against cytotoxic T-lymphocyte antigen-4 (CTLA-4), was approved in March 2011 for unresectable, metastatic melanoma [16] followed by approval of humanized MAbs against programmed death-1 (PD-1) for metastatic melanoma and advanced lung cancer (nivolumab [Opdivo] and pembrolizumab [Keytruda]), as well as renal cell carcinoma (nivolumab [Opdivo]) [17–20]. Furthermore, additional cancer indication approvals for CTLA-4 and PD-1 targeting immune checkpoint inhibitors are anticipated in the near future based on results of recently completed clinical trials. Finally, talimogene laherparepvec T-VEC (Imlygic) an oncolytic herpes simplex 1 virus was approved in October 2015 for the treatment of patients with unresectable metastatic melanoma based on a significant decrease in lesion size that was maintained for over 6 months [21]. T-VEC can directly lyse cancer cells but is also genetically engineered to secrete GM-CSF which can trigger anti-tumor immune responses as an additional mechanism of action [22].

Based on the dramatic benefit associated with these immunotherapy approaches for other cancer indications coupled with the dire need to develop new treatment approaches to improve outcome for glioblastoma patients, there has been substantial interest to evaluate immunotherapeutics for neuro-oncology patients. Nonetheless, overall enthusiasm to advance immunotherapies for brain cancer has historically been tainted by dogma articulating immunoprivilege of the central nervous system including dated studies demonstrating

an ability to eradicate skin allografts at every organ site except the central nervous system [23]. More recent aggregate data refutes limitations associated with immunoprivilege of the central nervous system and instead supports the presence of a dynamic and effective interaction of systemic immune responses within the brain [24, 25].

Preclinical development of dendritic cell vaccines for glioblastoma

Dendritic cells (DCs) are the professional antigen presenting cells (APCs) of the immune system, and as such, mediate the role of capturing antigens in the periphery and processing and presenting them as antigenic peptides to the T cells of the immune system [26]. The DC was described as a novel stellate cell found in the lymphoid organs of mice by Steinman and Cohn in 1973 [27]. The prevailing concept in immunology through the early 1990s was that CD8+ cytotoxic T lymphocytes (CTLs) were activated directly through recognizing MHC class I-restricted antigens presented on the surface of all nucleated cells [28–31]. The discovery of intermediate professional APCs that were capable of capturing exogenous antigens and presenting them in the class I antigen presentation pathway to CD8+ T cells (called “cross-priming”) and the identification of these professional APCs as the DC described by Steinman and colleagues eventually led to an alternate understanding that most, if not all, CTL responses against tumor cells and viruses required cross-priming by DCs to initiate productive T cell responses [32, 33]. When culture methods for expanding large numbers of DCs *in vitro* from myeloid progenitors and monocytes were developed, interest in using these *ex vivo* cultured DCs as cellular vaccines for cancer and infectious disease emerged shortly thereafter [34].

Studies in the early and mid-1990s demonstrated that murine bone-marrow derived DCs pulsed with peptide antigen were potent inducers of CD8+ cytotoxic T lymphocyte (CTL) responses *in vivo* and more effective than direct peptide immunization in strong adjuvants [35]. These observations led to the investigation of the use of DCs pulsed with exogenous tumor antigens in the form of peptides, tumor lysates, tumor RNA, and vectors expressing tumor-associated antigens as a novel form of cellular therapeutic vaccines in experimental model systems. Initial studies were conducted in non-CNS tumor models and established that small numbers of DCs pulsed with tumor antigens were sufficient to protect against tumor challenge, that the effectors generated by DC-immunization were indeed T cells, and that therapeutic responses against established tumors could be engendered by DC immunization [36–41].

Shortly thereafter, bone marrow-derived DCs pulsed with tumor lysates or tumor RNA derived from the B16 melanoma line were demonstrated to be effective in the generation of

therapeutic anti-tumor immune responses against tumors implanted within the CNS of experimental mice [42]. These studies demonstrated that peripheral immunization with tumor antigen-pulsed DCs could engender immune responses that were effective against tumors growing in the brain and importantly, that intolerable immunologic toxicity within the CNS was not induced by this immunization strategy. Further experimentation using syngeneic murine glioma models confirmed the capacity for DC-based immunization to expand therapeutic tumor-specific immune responses against glioma antigens without precipitating autoimmune toxicity against normal brain tissues [43–46]. While these early approaches used unfractionated tumor antigens in the form of tumor lysates or RNA, antigen-specific targeting strategies have also been explored in preclinical model systems. DC vaccines targeting glioma relevant antigens such as the epidermal growth factor receptor variant 3 (EGFRvIII), gp100, survivin, and telomerase have shown efficacy in preclinical glioma models and are suitable antigen targets in subsets of patients with malignant glioma [46–48]. These studies laid the foundation for the advancement of clinical trials exploring both unfractionated tumor antigen loaded DC vaccines as well as DCs pulsed with single or multiple antigenic targets expressed within malignant brain tumors.

Several underlying principles have emerged from preclinical studies of DC-based therapeutics for malignant brain tumors and other cancers, and we now have the benefit of reflection upon the results from the first generation of phase 1 through phase 3 clinical trials of DC vaccines in human patients.

Toxicity concerns

A major concern in the development of cancer immunotherapies is the risk of unleashing intolerable autoimmune or inflammatory toxicity in treated patients through induction of tumor-specific or cross reactive immune responses or the activation of non-specific inflammatory pathways. It is notable that DC vaccination has been well-tolerated in human clinical trials of patients with GBM thus far, including large-scale phase 3 trials. However, it is also notable that GBM tissues contain antigens that can induce lethal experimental allergic encephalitis (EAE) in primates and guinea pigs [49]. In melanoma models and in patients receiving cellular immunotherapy against melanoma, effective anti-tumor immunity can frequently be associated with autoimmune responses against shared melanocyte antigens leading to the induction of vitiligo [50–53]. Thus, there is some risk that autoimmune reactions such as EAE may be concomitant with the induction of truly effective immunization against unfractionated glioma antigens. Preclinical model systems have both demonstrated the capacity for DC immunization against self-

antigens such as myelin basic protein (MBP) to break tolerance and induce robust EAE in mice, as well as for DCs pulsed with unfractionated tumor antigens that contain normal brain proteins to engender potent and effective anti-tumor immunity without evidence of autoimmune toxicity [54, 55]. Thus, the balance between anti-tumor reactivity and autoimmunity appears to lie within an immunologic hierarchy within these systems where tumor-specific antigens dominate the immunologic response over self-antigens, despite DCs possessing sufficient activating capacity to break tolerance to self. In the development of more effective DC-based therapeutics and combinatorial strategies, we will need to remain cognizant of the potential for autoimmune manifestations and the rationale for engineering increasing tumor specificity in parallel with the development of increasing potency.

DC generation and subtypes

Through extensive characterization of murine and human DCs, we now know that several subtypes of these APCs exist in vivo with distinct and overlapping functions and varying lineage-specific differentiation pathways. DCs can be divided broadly into two main cell types, the plasmacytoid DC (pDC), potent anti-viral type I interferon producers, and conventional DC (cDC) that specialize in antigen capture and presentation [56]. While, cancer vaccine strategies in humans have relied largely on monocyte-derived cDCs generated in presence of GM-CSF and IL-4, followed by a maturation cocktail of cytokines or CD40L stimulation, preclinical studies and human in vitro studies have demonstrated a variety of culture systems capable of generating DCs with varying phenotypic markers, cytokine stimulatory capacity, and migratory capacity [57–60]. As an example, Fujita et al. compared mature murine DCs generated from bone marrow under standard conditions or type 1 polarized DCs (DC1) using exogenous culture in the presence of poly-ICLC, IFN γ , IFN α , and IL-4 in a vaccination protocol targeting the murine glioma line, GL261. Polarized DC1s maintained their stimulatory capacity in a superior fashion to standard DCs in the presence of immunosuppressive cytokines and were superior in the induction of therapeutic anti-tumor immunity in the established tumor model, demonstrating that the culture conditions for DC generation can be altered to enhance DC function [45]. Similar studies using genetically engineered DCs have demonstrated the capacity to generate superior DC vaccines through specific modification of DC culture conditions or gene expression [61]. The existence of several DC subsets in vivo and the observation that various culture conditions can alter the phenotype and functional properties of DCs in vitro presents a challenge in determining what conditions are optimal for generation of DCs for therapeutic use in the treatment of cancer. This is further complicated by the fact that parallels between

murine and human DC subsets are not applicable across all molecular markers or APC functions, limiting the utility of murine model systems in the optimization of human DC vaccination strategies [62, 63]. As recent studies have developed the use of humanized mouse models to study human DC function, it may be feasible to construct comparative efficacy preclinical models for human DC vaccines against glioblastoma and other cancers in the near future [64–66].

Route of immunization

Numerous studies in preclinical systems have examined the route of immunization of antigen-loaded DCs and the impact on the induction of anti-tumor immunity. While there is consensus agreement that antigen-loaded DCs must migrate to the T cell-dependent areas of lymphoid organs in order to productively engage the host immune system, the best method for achieving this objective is not readily clear despite several studies examining various routes of DC immunization in preclinical studies and a few clinical studies examining this variable. A review of comparative studies would support the conclusion that subcutaneous injection and intramuscular injection routes for DC vaccination are inferior to either intradermal, intralymphatic, intraperitoneal, or intravenous injection in the induction of T cell-mediated immune responses [57, 67, 68]. Intradermal injection (i.d.) has been the most common route of administration for DC vaccines in patients with GBM. Interestingly, the only FDA-approved cellular vaccine for cancer (Sipuleucel-T) is administered intravenously after exogenous pulsing of blood-derived antigen presenting cells with tumor antigen and GM-CSF. Another interesting variation of DC immunization that has shown promise in preclinical glioma studies has been the intratumoral administration of cytokine-secreting DCs to condition the tumor microenvironment [45]. While intranasal administration has not been explored for DC vaccines targeting gliomas, this route of delivery of other cell-based and vector-based therapeutics in rodent model systems has been shown to convey unique effects on CNS diseases [69, 70]. The deep cervical lymph nodes have been shown to be the tumor-draining lymph nodes for CNS malignancies in murine models, capture tumor-derived antigens that drain from the CNS, and are disproportionately affected by tumor-induced immunosuppression. While it is unclear whether DC vaccines preferentially targeted to the cervical lymph nodes versus other lymphoid organs would be desirable, a recent study examining proximity of vaccination to an intracranial murine glioma demonstrated inferior T cell activation as immunization was delivered more proximal to the primary brain tumor [71]. These findings suggest that route and immunization site may be important variables in the efficacy of DC vaccination in patients with malignant gliomas [72].

DC migration

Regardless of the route or immunization site, DC vaccines presumably must migrate to lymphoid organs and activate resident T cells in order to achieve efficacious anti-tumor responses. Thus, the *in vivo* migratory capacity of administered DC vaccines plays a critical role in their function as a cellular therapeutic. Numerous preclinical and clinical studies have documented that only a fraction of administered DCs successfully migrate to vaccine-site draining lymph nodes (VDLNs) [73–75]. Furthermore, studies aimed at enhancing DC migration to lymph nodes have demonstrated the capacity to enhance anti-tumor immunity in preclinical systems. We recently demonstrated in a preclinical model system and randomized clinical trial in patients with GBM that enhancement of DC migration to VDLNs using a tetanus-diphtheria toxoid vaccine (Td) was a potent modality for improving the immunologic and clinical response to DC vaccination [76]. These observations suggest that further exploration of means to enhance the migratory capacity of tumor-antigen loaded DCs may be a fruitful axis of manipulation to improve the clinical efficacy of this treatment strategy.

Targeting neoantigens

DC vaccination studies that have advanced into clinical evaluation in patients with GBM have utilized DCs pulsed with unfractionated antigens such as tumor lysates, peptides, or tumor RNA, or defined tumor-associated antigens provided as peptides or RNA [77, 78]. However, recent studies have highlighted the importance of tumor-specific mutations or neoantigens as the potential drivers of host anti-tumor immunity and therapeutic immunologic responses to cancer immunotherapy [79–82]. The capacity to rapidly assess and identify neoantigens expressed within glial tumors using the advent of genomic technology and bioinformatics processing to predict immunogenicity, lends the ability to derive patient-specific vaccines tailored to target tumor neoantigens. Murine gliomas have been recently characterized with respect to neoantigen expression laying the foundation for neoantigen-directed DC vaccines to be explored within these experimental systems [83].

A modified view of immune privilege

Our current understanding of immune surveillance of the CNS has shifted considerably from the classic view of the brain as an immune privileged site that is largely ignored by the peripheral immune system. We now know that the CNS is drained by classical lymphatic channels in the meninges and that activated lymphocytes readily gain access to the brain parenchyma and tumor microenvironment [84, 85]. There is consensus agreement that while T cell

priming, or the initiation of immunologic responses, requires antigen-presentation to occur in classical lymphoid organs outside of the CNS, the brain has sufficient mechanisms by which antigens are drained and sampled by the peripheral immune system to initiate productive immunity within the CNS compartment [24, 86]. Further understanding, however, of the role of and origin of APCs involved beyond the initiation of immune responses in the brain may impact on future DC vaccine strategies for malignant gliomas.

Classical DCs are not detected in the normal brain parenchyma, however, MHC class II expressing dendriform APCs are readily recruited into inflammatory lesions within the brain. These DCs may be recruited from the blood, derived from the meninges where classical APCs lie at the interface between the periphery and brain circulatory system, differentiate from perivascular macrophages, or represent brain APCs that are derived from resident progenitor cells such as microglia in the context of inflammation [87, 88]. An understanding of the cellular origin and importance of APC presentation of tumor antigens in situ within the tumor microenvironment may have important implications for continued development of DC vaccines for GBM. Whether intratumoral DC vaccines or vaccines administered in the proximity of the CNS lesions should be prioritized may be contingent on a better understanding on the importance of local APC functions. In contrast, if peripheral T cell activation is sufficient to generate tumor-reactive lymphocytes that can migrate efficiently to malignant gliomas and engage tumor cells and resident APCs in a productive manner, then the expediency of peripheral DC vaccination would take precedent.

Preclinical glioblastoma models for immunotherapy

One of the major challenges in evaluating translational immunotherapy in neuro-oncology (and other cancers) is the limitations imposed by contemporary preclinical GBM models. The majority of preclinical evaluations to date implore the use of syngeneic, immunocompetent murine brain tumor models to examine the efficacy and mechanisms of DC-based vaccine strategies against glial tumors [89]. Because of the nature of cell-based therapeutics requiring the transfer of DCs generated in one host to be used a vaccine in a recipient-host and the desire to have large cohorts of tumor-bearing mice with similarly staged tumors for therapeutic evaluation, the use of genetically engineered spontaneous mouse models has been limited in favor of the evaluation of transplantable intracranial tumor lines. These models likely do not recapitulate the host-immunologic evolutionary process of spontaneous tumor development, and thus may be lacking in certain physiologic conditions that contribute to tumor immunosuppression and immune

evasion. Furthermore, the complex intratumoral heterogeneity that is characteristic of human GBM tumors is not likely recapitulated by current transplantable or spontaneously arising GBM models. Thus, we are aware that current murine glioma models likely oversimplify the complexity of generating productive anti-tumor immune responses against malignant gliomas in humans. It is perhaps, therefore, most useful to utilize murine models as comparative efficacy screens for prioritizing the merits of advancing one DC-based strategy over another approach in stringently controlled model systems and for identifying mechanisms of treatment resistance.

Improving the predictive value of preclinical studies of DC-based therapeutics or other treatment approaches may be questionable as a laudable goal for translational neuro-oncology research. Recent studies of the predictive value of even phase 2 human clinical trial results in oncology have demonstrated that only about one-third of approaches deemed appropriate for advancement after phase 2 human trials result in positive phase 3 clinical trials [90, 91]. This low predictive value for earlier stage clinical trials in humans suggests that utilization of preclinical models to gain mechanistic insights, identify potential correlative biomarkers and mechanisms of treatment resistance, and to screen treatment approaches for comparative prioritization of clinical development may be nearer term achievements of continued preclinical model development.

Clinical development of dendritic cell vaccines for glioblastoma

Based on the potent ability of dendritic cells to present antigens and induce clonal T cell expansion, [92] a variety of DC-targeting vaccines have been developed for cancer applications including glioblastoma [93, 94]. Proof of concept regarding dendritic cell based vaccination was recently established for oncology with the FDA approval of sipuleucel-T (Provenge®) for the treatment of metastatic, castration-resistant prostate cancer based on a 4.1-month survival advantage demonstrated in a double-blind, placebo-controlled randomized phase 3 study [15].

Clinical trials evaluating dendritic cell-based vaccines for glioblastoma patients initiated over 15 years ago and can be classified based on the type of tumor antigens incorporated to ex vivo sensitize dendritic cells. A major strategy has utilized pooled, non-selected tumor antigens collected via either acid elution or tumor cell lysate for dendritic cell sensitization whereas an alternative strategy has utilized synthesized peptides from defined tumor antigens for dendritic cell sensitization.

Dendritic cell vaccination utilizing pooled, non-selected tumor antigens

Acid-eluted tumor antigens

The rationale for sensitizing dendritic cells to a pool of non-selected tumor antigens is based on the marked heterogeneity present within glioblastoma tumor cells. Although the term “multiforme” was originally associated with glioblastoma based on the marked variability of cell type and morphology observed microscopically among these tumors, we now appreciate the striking molecular genetic heterogeneity within and across glioblastoma tumors based on elegant gene mutation and expression studies [95]. Theoretically, use of pooled, non-selected tumor antigens could sensitize the immune system against a broad repertoire of heterogeneously expressed tumor associated antigens that could lessen the evolution of immune escape. A theoretical disadvantage associated with this approach is that cross reactivity against normal, non-tumor cellular constituents present within the pooled material could lead to potential autoimmunity or experimental allergic encephalomyelitis. The initial studies evaluating this approach utilized pooled non-specific tumor antigens derived from acid elution of tumor cells based on the ability of this approach to effectively segregate tumor surface peptides bound to MHC class I molecules [96]. Liau reported the first glioblastoma patient treated with this approach and utilized tumor antigens eluted from an allogeneic, MHC class I-matched glioblastoma tumor to sensitize autologous dendritic cells pulsed *ex vivo*. [97] Importantly, the dendritic cell vaccinations were well tolerated with no evidence of autoimmune reactivity. Furthermore, peripheral blood T cells demonstrated proliferative responses specific to the allogeneic tumor peptide preparation as well as evidence of increased CD3+ T cell infiltration into the tumor. Despite having a heavily pretreated, recurrent glioblastoma, this patient survived for 6 months after her vaccine therapy. Two, parallel prospective phase 1 studies were then conducted among malignant glioma patients utilizing pooled, non-selected antigens that were acid eluted from autologous tumor samples collected following short-term culture, and then sensitized to autologous dendritic cells *ex vivo*. Yu et al. treated seven patients including five with glioblastoma and two with anaplastic astrocytoma [98] while Liau treated 12 glioblastoma patients [99]. Patients received three biweekly vaccinations of up to 1×10^6 dendritic cells pulsed with 50–100 μg of acid-eluted tumor peptides. Again the dendritic cell vaccinations were well tolerated with no evidence of autoimmunity and no dose-limiting toxicities were observed among treated patients in either study. Over half of the vaccinated patients demonstrated measurable tumor-specific cytotoxic T cell responses, and some of these patients were noted to have a longer survival than those who did not develop positive cytotoxic T cell responses.

Furthermore, intratumoral infiltration of CD3+ T cells, primarily consisting of CD8+/CD45RO+ memory T cells was noted in half of the patients who underwent tumor resection following vaccination therapy.

Tumor lysate-derived antigens: recurrent malignant glioma

While initial studies utilizing acid eluted tumor antigens were ongoing, parallel efforts utilizing whole tumor lysates as a source of tumor antigens began to be explored. The whole tumor lysate approach quickly became preferred due to technical challenges associated with acid elution including requirement for a large tumor sample and difficulties consistently obtaining short-term cultures of resected tumor samples. The tumor lysate approach involves a series of snap freeze/thaws (typically 5–6) of homogenized tumor lysate obtained from the operating room which generates tumor fragments. Following centrifugation, the cell free supernatant is collected and used to pulse cultured autologous dendritic cells. Tumor antigens are phagocytosed and ultimately presented on MHC class I and II molecules. Several neuro-oncology immunotherapy groups have evaluated the tumor lysate dendritic cell vaccination approach for malignant glioma patients. Initial trials focused on patients with recurrent tumors and later including trials for newly diagnosed patients. All results to date derive from primarily single arm, non-randomized clinical trials typically conducted at a single center.

Yu initially reported results of a phase 1 study of 12 recurrent patients in 2004 (nine with glioblastoma and 3 with anaplastic astrocytoma) [100] that was followed in 2008 by results of a phase 2 study that included 23 recurrent glioblastoma patients [101]. There were no grade III/IV adverse events or evidence of autoimmune reactivity noted for either study. Over half of the vaccinated patients demonstrated increased interferon- γ expression by peripheral blood mononuclear cells exposed to tumor lysate after vaccination. Encouraging evidence of prolonged survival was observed, particularly among patients with evidence of interferon- γ cytoresponsiveness.

A group led by De Vleeschouwer and van Gool reported an initial series of 12 recurrent malignant glioma patients in 2004 [102], which was followed by their report of 56 recurrent glioblastoma patients treated on a phase 2 study in 2008 [103]. All patients underwent debulking surgery prior to vaccination and they treated patients who were at least 3 years old. Two patients developed grade IV cerebral edema but otherwise the vaccinations were overall well tolerated. Median overall survival on the phase 2 study was 9.6 months and 11% of patients were alive at 3 years. Patients who were younger (<35 years old), and who were able to undergo a gross total resection, appeared to have a better outcome.

Hunn and colleagues recently reported preliminary results of a phase 1 study of temozolomide re-challenge combined with autologous tumor lysate pulsed dendritic cell vaccination among 14 patients who had progressed on standard temozolomide radiochemotherapy [104]. In this study, tumor lysate was derived from surgically resected tumor following progression. There were no grade ≥ 3 adverse events attributed to the study vaccine and two patients (14%) demonstrated positive ELISPOT assays. Two patients achieved a radiographic response and the PFS-6 rate was 22%.

Yamanaka and colleagues utilized a different administration strategy in their approach in that patients received a series of tumor lysate pulsed dendritic cell vaccinations that were administered both intradermally as well as intratumorally through an Ommaya reservoir. They initially reported treatment of seven recurrent patients in 2003 [105] which was followed by a report of 24 additional patients in 2005 [106]. No serious adverse events or evidence of autoimmunity were reported. Evidence of vaccine immunoreactivity was observed in some patients via increased ELISPOT and delayed type hypersensitivity reactions. Overall survival among glioblastoma patients treated on the phase 2 study was approximately 16 months and interestingly patients who received both intradermal as well as intratumoral vaccination appeared to do better than those receiving intradermal vaccine alone.

Tumor lysate-derived antigens: newly diagnosed glioblastoma

The De Vleeschouwer group led efforts to integrate tumor lysate pulsed dendritic cell vaccination with standard temozolomide radiochemotherapy as established by Stupp [107] for newly diagnosed glioblastoma patients. They initially reported outcome of eight patients treated on a feasibility pilot study [108] which was followed by results of a single arm, phase 1/2 clinical trial that enrolled 77 patients. [109] All patients had dendritic cells collected by leukopheresis following definitive surgical resection. Dendritic cells were cultured and tumor lysate vaccinations were prepared while patients underwent the standard 6-week course of external beam radiotherapy during which they received concomitant temozolomide. Thereafter, they received four vaccine doses administered at weekly intervals prior to initiating adjuvant temozolomide. Booster vaccine doses were administered on day eight of the first, second, third, and sixth adjuvant temozolomide cycles. Of note, all patients were off corticosteroids at the time of their dendritic cell collection and subsequent vaccination. For the phase 2 study, the median age was 57 years and 66% of patients underwent a gross total resection. In general, vaccination therapy was well tolerated and there was no clear evidence of increased toxicity relative to that typically experienced following standard temozolomide chemoradiotherapy. Median overall survival on the phase 2 study was 18.3 months and

improved survival was observed for patients with lower recursive partitioning analysis class and MGMT methylation status. Although changes in immune cell subsets were measured before and after vaccination, these data did not correlate with outcome.

Fadul recently reported outcome of ten newly diagnosed glioblastoma patients treated with administration of autologous, tumor lysate pulsed dendritic cells [110]. A total of three vaccinations were administered at 2-week intervals beginning 5 weeks following completion of combined temozolomide chemoradiotherapy. Each vaccine consisted of 1×10^7 dendritic cells which were injected in equal aliquots into two bilateral cervical lymph nodes. Patients underwent leukopheresis prior to beginning vaccination and 2 weeks after the third vaccination at which point they began 12 cycles of adjuvant temozolomide. No serious adverse events were reported for vaccinated patients and the median survival was 28 months. Assessment of immunologic endpoints revealed that the proportion of CD4+ interferon- γ and CD8+ interferon- γ producing cells revealed increases but these values did not achieve statistical significance.

Vik-Mo recently reported preliminary results of an adaptation of the whole tumor lysate approach in which they pulsed autologous dendritic cells with mRNA derived from autologous glioma stem cell cultures established from seven newly diagnosed glioblastoma patients [111]. The rationale for focusing on glioma stem cells is based on data demonstrating this subset of malignant glioma tumors to be associated with treatment resistance, angiogenesis and tumor growth. [112–114] No significant autoimmune or adverse events were noted. Following vaccination, increased lymphocyte proliferation in response to glioma stem cell lysate was observed for all patients but only one patient demonstrated a delayed type hypersensitivity reaction. Progression-free survival was 1.9 years.

Tumor lysate-derived antigens: potential biomarkers of benefit

Recursive partitioning analysis to integrate pre-treatment patient and tumor related parameters has been demonstrated to generate valuable prognostic models for patients with newly diagnosed glioblastoma [115, 116]. De Vleeschouwer developed a four-class recursive partitioning analysis model for 117 adult recurrent malignant glioma patients undergoing tumor lysate pulsed dendritic cell vaccination [117]. The model integrates age, histopathologic grade, Karnofsky performance status, and mental status. Significant overall survival differences were noted among the four classes including percent survival for at least 24 months to be 54.5, 26.7, 11.5, and 0% for classes I, II, III, and IV respectively.

Liau, Prins, and colleagues have reported a series of studies that provide important insight into potential biomarkers that

identify patient subsets more likely to derive significant clinical benefit including improved survival following tumor lysate pulsed dendritic cell vaccination. In a series of 23 glioblastoma patients including 15 newly diagnosed and eight recurrent patients, patients with a mesenchymal gene expression profile, which in general confers a poorer prognosis [118, 119], had significantly prolonged survival compared to a historical control population ($p = 0.0046$) [120]. Median overall survival achieved on this study was also noteworthy including 35.9 months for newly diagnosed patients and 17.9 months for those with recurrent disease.

In a follow-up study, six specific peripheral blood lymphocyte subsets were analyzed before and after vaccination from 24 newly diagnosed glioblastoma patients who were treated with autologous dendritic cells pulsed with either tumor lysate ($n = 19$) or glioma-associated antigens ($n = 5$) [121]. The subsets included the following: T helpers (CD3+ CD4+); cytotoxic T cells (CD3 + CD8+); natural killer T cells (CD3 + CD16+); natural killer cells (CD3-CD16+); B lymphocytes (CD3-CD19+); and Tregs (CD3 + CD4 + CD25 + cd127^{low}). The expression level of activation (CD25, CD69) and negative (CTLA-4 and PD-1) costimulatory markers for each lymphocyte subset was also assessed. Importantly, across all patients, the median percentage of each lymphocyte subset relative to total isolated cells did not significantly change after vaccination. Although changes in the activation status of any lymphocyte subset did not predict outcome, a poorer outcome to vaccination was associated with two measures of immunosuppression. First, every unit increase in Treg ratio after vaccination was associated with a 2.623-fold increase risk of death ($p = 0.0228$). Second, increased expression of CTLA-4 by either T helper cells or cytotoxic T cells also predicted poorer survival.

More recently, CD8+ T cell cytokine responsiveness after dendritic cell vaccination was evaluated among 21 glioblastoma patients after vaccination with dendritic cells pulsed with either autologous tumor lysate ($n = 17$) or glioma-associated antigens ($n = 4$) [122]. Functional responsiveness before and after vaccination of peripheral blood lymphocytes to the immunostimulatory cytokines interferon- γ and interleukin-2 was measured by phosphorylation of STAT-1 and STAT-5 via phospho-specific flow cytometry. In this study, increased ratios of IL-2 responsiveness/pSTAT5 signaling and decreased ratios of interferon- γ /pSTAT-1 signaling were associated with improved overall survival at 2 years.

Finally, Pellegatta recently evaluated changes in peripheral blood immune cell subsets as well as circulating immune cytokines before and after tumor lysate pulsed dendritic cell vaccinations among 15 patients with recurrent glioblastoma [123]. They noted that improved survival was noted among patients who had an increase in CD56+ natural killer cells as well as a decrease in serum TGF β 2 levels after vaccination. Patients with smaller tumors (defined as <20 cm³ were also

noted to have an improved outcome. Of note, changes in CD8+ T cells did not correlate with survival.

Dendritic cell vaccination utilizing selected tumor antigens

Single antigen vaccination

EGFRvIII is an ideal immunotherapeutic target because it results from an inframe deletion that generates a tumor-specific antigen that is not found in normal tissues [124]. It is present in approximately 30% of glioblastoma tumors [125] and triggers ligand independent, constitutive tyrosine kinase activity [126] that is associated with tumor cell resistance to cytotoxic therapy [127]. Following preclinical demonstration of significant anti-tumor activity against intracranial, EGFRvIII-positive tumors treated with dendritic cells pulsed with an EGFRvIII peptide, an initial phase 1 dose escalation study was conducted [128]. In this study, patients with newly diagnosed glioblastoma received increasing dose levels of dendritic cells pulsed with an EGFRvIII-specific peptide that spanned the fusion junction of EGFRvIII conjugated to keyhole limpet hemocyanin (KLH) for three doses after maximum safe resection and standard radiotherapy. No dose-limiting toxicities or serious adverse events were observed and patients received up to 5.7×10^7 dendritic cells. Most patients demonstrated evidence of EGFRvIII-specific immunoreactivity after vaccination. Specifically, 83% of patients (10 of 12) developed EGFRvIII-specific T cell proliferation responses in vitro while 56% (5 of 9 patients) developed positive EGFRvIII-specific delayed type hypersensitivity skin tests. Median time to progression and overall survival from the date of diagnosis were 10.2 and 22.8 months, respectively. Of note, subsequent clinical trials administering an EGFRvIII-specific peptide conjugated to KLH with granulocyte macrophage colony stimulating factor (GM-CSF) without the use of autologous dendritic cells have shown encouraging anti-tumor activity among newly diagnosed glioblastoma patients [129, 130]. As further proof of principle of the efficacy of targeting EGFRvIII with this approach, recurrent tumor samples evaluated following administration of the EGFRvIII vaccine are no longer EGFRvIII positive [131].

In another phase 1 study, Sakai recently reported results among ten recurrent malignant glioma patients who were treated with autologous dendritic cells pulsed with peptide corresponding to mutant Wilms' tumor 1 (WT1) antigen [132]. Vaccinations were well tolerated and no patients experienced grade ≥ 3 adverse events. Six patients (60%) achieved a >2-fold increase in WT1-specific cytotoxic T lymphocytes by tetramer analysis. Median overall survival for all patients and those with glioblastoma on this study were 19 and 7 months, respectively.

Mitchell et al. recently reported a randomized pilot clinical trial in which 12 patients with newly diagnosed GBM received autologous dendritic cells electroporated with messenger RNA encoding for the immunodominant cytomegalovirus (CMV) antigen, pp65 with or without a tetanus-diphtheria (Td) toxoid booster vaccine selected to enhance DC migration [76]. CMV antigens have been reported to be expressed at low levels in a high proportion of GBM tumors [133]. In an embedded and blinded imaging study examining migration of Indium-111 labeled RNA-pulsed DCs to vaccine-site draining lymph nodes, this study demonstrated that patients who received DC vaccination combined with the Td booster showed increased migration of DCs to lymph nodes, prolonged immunologic responses, and dramatically enhanced progression-free survival (8.5 months vs >32.0 months from time of surgery, $P = 0.01$) and overall survival (18.5 months vs >36.6 months from time of surgery, $P = 0.01$). DC migration to lymph nodes was strongly correlated with clinical responses in this randomized study, suggesting that enhancement of DC trafficking may improve clinical responses to DC vaccination in human patients. A randomized, blinded, and placebo-controlled phase 2 trial is underway to confirm these results (clinicaltrials.gov NCT02465268).

Multi-antigen vaccination

Although glioma associated antigens may induce less robust immune responses than tumor-specific antigens due to co-expression on normal tissues and host immunotolerance, vaccines consisting of synthesized peptides corresponding to a number of such antigens and combined with immunoadjuvant can be readily developed and available as “off the shelf” products for cancer patients. Three studies evaluating autologous dendritic cells pulsed with cocktails of glioma associated antigens have demonstrated encouraging results for malignant glioma patients. In the first study, 22 HLA-A2+ recurrent malignant glioma patients received at least four doses of autologous dendritic cells loaded with four synthetic peptides targeting EphA2, interleukin (IL)-13 receptor- α 2, YKL-40 and gp100, in combination with polyinosinic-polycytidylic acid [poly(I:C)] stabilized by lysine and carboxymethylcellulose (poly-ICLC) [134]. The vaccine was well tolerated and neither grade ≥ 3 adverse events nor DLTs were observed. Encouraging immunogenicity of the vaccine was observed in that 58% (11 of 19 patients) developed positive interferon- γ ELISPOT or tetramer assays after the fourth vaccination. Nine patients (75%) remained progression free for at least 12 months.

In a small study of nine recurrent malignant glioma patients, α -type-1 polarized autologous dendritic cells were pulsed with synthetic peptides for WT-1, Her2, MAGE-A3, MAGE-A1, and gp100 [135]. Vaccinations were well tolerated with no observed grade ≥ 3 adverse events or autoimmunity.

Positive ELISPOT and skin delayed type hypersensitivity reactions were observed in 6 (67%) and 4 (44%) patients respectively. Anti-tumor activity was disappointing however with only one patient achieving durable stable disease while the remaining patients had a best response of progressive disease.

The third study was conducted among 17 newly diagnosed HLA-A1/A2-positive, glioblastoma patients and pulsed autologous dendritic cells with synthetic peptides corresponding to HER2, TRP-2, gp100, MAGE-1, IL13R α 2, and AIM-2 [136]. All patients underwent a gross total resection and received three vaccinations at 2-week intervals beginning after radiation therapy. Standard adjuvant temozolomide was administered following the final vaccination. Vaccinations were well tolerated and no grade >2 adverse events were observed. Post-vaccination increases in antigen-specific CD8+ T cell interferon- γ secretion was observed in 33% of patients. Median progression-free and overall survivals were 16.9 and 38.4 months, respectively.

Remaining questions

The use of autologous dendritic cells pulsed with tumor antigens represents a robust strategy to generate potent anti-tumor immune responses for malignant glioma patients. The aggregate clinical data to date (Table 1) clearly demonstrates that such an approach is safe and well tolerated. In addition, encouraging evidence of immune responses against targeted antigens has been observed among both newly diagnosed and recurrent patients. Anti-tumor benefit as reflected by encouraging rates of progression-free and overall survival have also been observed although these data typically are derived from small, single arm studies and hence may be affected by selection bias. Additional ongoing trials, including some randomized placebo controlled studies (Table 2) will further define the efficacy of such vaccine strategies for malignant glioma patients. Nonetheless, critical questions remain to be answered. First and foremost is the question whether ex vivo pulsing of dendritic cells are required or can vaccines capable of activating dendritic cells in vivo generate sufficient anti-tumor immune responses. Collection of autologous dendritic cells requires leukopheresis and culturing as well as maturation of these cells ex vivo that can be laborious and technically challenging. There are no planned or ongoing trials designed to compare the efficacy and immunogenicity of ex vivo dendritic cell-based vaccines with vaccines that activate dendritic cells in vivo.

Another highly relevant unanswered question is the determination of which tumor antigens are most likely to generate the most robust and durable anti-tumor immune responses. As reviewed, studies to date have evaluated a full spectrum of tumor antigens including those derived from whole cell lysates to cocktails of tumor associated antigens to single tumor-specific antigens. Along these lines, ongoing studies

Table 1 Published clinical trials of ex vivo tumor antigen pulsed autologous dendritic cell vaccine treatment of malignant glioma patients

Phase	Population	Number of patients	Antigen	Vaccine related grade ≥ 3 AEs	Immunoreactivity	Efficacy	Citation
I	R	56	WTL	2%	DTH: 2/12 (17%)	PFS: 3 months OS: 9.6 months	De Vleeschouwer et al. 2008 [103]
I	R	15	WTL	NS	NS	PFS-6: 27% OS: 8 months	Pellegatta et al. 2013 [123]
I	R	9	WT-1, HER2, MAG-A3, MAGE-A1, gp100	None	ELISPOT: 6/9 (67%) DTH: 4/9 (44%)	SD: 1 (11%)	Akiyama et al. 2012 [135]
I	R	14	WTL	None	ELISPOT: 2/14 (14%)	PFS-6: 22%	Hunn et al. 2015 [104]
I/II	R	10	WTL	None	DTH: 3/6 (50%) ELISPOT: 5/5 (100%)	Minor MRI response: 2/6 (33%)	Yamanaka et al. 2003 [105]
I/II	ND	12	CMV pp65	None	DC migration to lymph nodes 6/12 (50%)	DC vaccine vs DC vaccine + tetanus PFS 10.8 months vs >27.0 months OS: 18.5 months vs >36.6 months	Mitchell et al. 2015 [76]
I/II	R	24	WTL	None	DTH: 8/17 (47%) ELISPOT: 7/16 (44%)	PR: 1 (4%) MR: 3 (13%) SD: 10 (42%)	Yamanaka et al. 2005 [106]
II	R	10	WTL	8%	DTH: 6/8 (75%)	PFS: 3 months; OS: 10.5 months	Rutkowski et al. 2004 [102]
II	R	22	IL-13R $\alpha 2$; EphA2; GP100; YKL-40	None	ELISPOT: 6/10 (60%) Tetramer: 5/9 (56%)	ORR: 2/22 (9%) TTP: 4 months (GBM) and 13 months (AG)	Okada et al. 2011 [134]
II	R	117	WTL	NS	None	OS: 6–48.4 months (varied by RPA class)	De Vleeschouwer et al. 2012 [117]
I	R and ND	14	WTL	None	CTL IFN- γ : 6/10 (60%)	OS: 133 weeks (recurrent GBM)	Yu et al. 2004 [100]
I	R and ND	12	WTL	None	DTH: 6/12 (50%)	TTP: 15.5 months; OS: 23.4 months	Liau et al. 2005 [99]
I	R and ND	23	WTL	None	NS	OS (newly diagnosed): 35.9 months OS (recurrent): 17.9 months	Prins et al. 2011 [120]
I	R and ND	25	AIM-2, MAGE 1, TRP-2, gp 100, HER2, IL-13R $\alpha 2$	None	Cytokine response: 5/15 (33%)	PFS: 16.9 months; OS 38.4 months (newly diagnosed GBM)	Phuphanich et al. 2012 [136]
II	R and ND	34	WTL	None	CTL IFN- γ : 17/34 (50%)	TTP: 261 days (immune responders)	Wheeler et al. 2008 [101]
I	ND	9	WTL	None	CTL activity: 4/7 (57%)	OS: 16.25 month (GBM)	Yu et al. 2001 [98]
I	ND	12	EGFRvIII	None	DTH: 5/9 (56%) T cell proliferation: 10/12 (83%)	TTP: 6.8 months OS: 18.7 months	Sampson et al. 2009 [128]
I	ND	7	Autologous glioma stem cell culture mRNA	None	CTL: 7/7 (100%) DTH: 1/7 (14%)	PFS: 1.9 years	Vik-Mo et al. 2013 [111]
I/II	ND	8	WTL	13%	DTH: 3/6 (50%) ELISPOT: 5/8 (63%)	PFS: 18 months OS: 24 months	Ardon et al. 2010 [108]
I/II	ND	77	WTL	NS	None	OS: 18.3 months	Ardon et al. 2012 [109]
II	ND	10	WTL	None	ELISPOT: 4/10 (40%)	PFS: 9.5 months OS: 28 months	Fadul et al. 2011 [110]

AEs adverse events, CTL cytotoxic T lymphocyte, DTH delayed type hypersensitivity, ELISPOT enzyme-linked immunosorbent spot assay, IFN- γ interferon-gamma, MR minor response, MRI magnetic resonance imaging, N newly diagnosed, NS not specified, OS overall survival, PFS progression-free survival, PFS-6 progression-free survival rate at 6 months, PR partial response, R recurrent, RPA recursive partitioning analysis, SD stable disease, TTP time to progression, WTL whole tumor lysate

Table 2 Ongoing clinical trials of ex-vivo pulsed dendritic cell vaccines for adult glioblastoma patients

Phase	Population	Number of patients	Design	Antigens	Sponsor	Clinicaltrials.gov
I	Recurrent	20	Open label	Whole tumor lysate	University of Miami, Miami FLA USA	NCT01808820
I	Recurrent	20	Open label	CD133	Immunocellular Therapeutics	NCT2049489
I	Recurrent and newly diagnosed	40	Open label	Allogeneic glioblastoma stem-like cell lysate	Cedars Sinai, Los Angeles, CA USA	NCT02010606
I/II	Newly diagnosed	20	Open label	WT-1 RNA	University Hospital, Antwerp, Belgium	NCT02649582
II	Newly diagnosed	116	Randomized, single blind	CMV pp65-LAMP mRNA	University of Florida	NCT02465268
II	Newly diagnosed	100	Randomized, double blind	Glioma stem-cell like antigens	Fudan University, Shanghai, China	NCT01567202
III	Newly diagnosed, HLA-A2+	414	Randomized, double blind	AIM-2, MAGE-1, TRP-1, gp100, HER-2, IL-13R α 2	Immunocellular Therapeutics	NCT02546102

are evaluating targeting tumor antigens by peptides, DNA- and RNA-based strategies and it remains to be seen whether a specific tumor antigen formulation may sensitize autologous dendritic cells more effectively.

There is also much debate regarding choice of vaccine adjuvant and it remains unclear whether there is an optimal adjuvant strategy for dendritic cell-based vaccines. A broad array of adjuvants are currently being evaluated in multiple vaccine formulations including toll-like receptor (TLR) agonists against TLR3 (polyI:CLC), TLR4 (monophosphoryl lipid A), TLR5 (flagellin), TLR7 (Imiquimod), TLR7/8 (resiquimod), and TLR9 (CpG) [137]. In addition, GM-CSF has frequently been added based on its ability to attract and activate dendritic cells [138], although a severe but reversible hypersensitivity reaction associated with anti-GM-CSF autoantibodies was recently reported in a glioblastoma patient undergoing autologous RNA-pulsed dendritic cell vaccination combined with GM-CSF and dose-intensified temozolomide [139]. Choice of vaccine site and dosing schedule have also not been carefully evaluated and represent additional factors of potentially significant impact. Which patient populations with regard to underlying tumor status (newly diagnosed versus recurrent), degree of associated tumor burden (gross total resected or subtotaly resected) and use of concurrent corticosteroids may also be relevant variables that could impact vaccine efficacy and these considerations also remain to be investigated.

A critical question going forward is whether robust tumor-specific immune responses generated by potent vaccination strategies can overcome the myriad mechanisms of immunosuppression aggressive tumors such as glioblastoma exploit [140]. Recent clinical studies have demonstrated highly encouraging rates of durable tumor response following administration of immune checkpoint inhibitors as a strategy to overcome immunosuppression mediated by PD-1 signaling [141].

Combination strategies evaluating potent mechanisms of immunosensitization such as dendritic cell vaccination combined with immune checkpoint blockade are highly relevant next-generation clinical trials which may significantly enhance therapeutic efficacy relative to that achieved with either approach alone [142].

Finally, an exciting adaptation for future vaccines includes the possibility of targeting specific subsets of dendritic cells in order to enhance particular aspects of anti-tumor immunoreactivity. For example, sensitization of tumor antigens to CD141+ DCs would be expected to generate particularly potent cytotoxic T lymphocytes whereas antigen targeting to CD1c+ dendritic cells would be expected to expand CD103 + CD8+ T memory cells that could persist to prevent relapse in the CNS microenvironment.

Conclusion

The use of autologous dendritic cells pulsed ex vivo against tumor antigens continues to be a very promising avenue of immunotherapy for malignant glioma patients. Preclinical studies have validated this approach and results of clinical studies conducted to date are highly encouraging with regard to safety, immunogenicity and anti-tumor efficacy. Nonetheless, many variables related to patients, vaccine components, and methodology for vaccine preparation and administration remain to be carefully evaluated. Furthermore, given the remarkable diversity of immunosuppressive mechanisms employed by many cancers including malignant gliomas, combination immunotherapy strategies that incorporate potent treatments to induce immunosensitization with blockade of critical immunosuppressive mediators will likely be required in order for the full potential of immunotherapy approaches such as dendritic cell vaccination to be achieved.

Compliance with ethical standards

Conflict of interest (current and at time of the research): David A. Reardon received honoraria from and has a consulting or advisory role with Abbvie, Bristol Myers Squibb, Cavion, Celldex, Inovio, Juno Pharmaceuticals, Merck, Novartis, Roche/Genentech, Amgen, Novocure, Oxigene, Regeneron, and Stemline Therapeutics; is involved in speakers' bureaus with Roche and Merck; and received research funding from Incyte, Midatech, and Celldex.

Duane A. Mitchell received honoraria from and has a consulting or advisory role with Tocagen, Inc.; has patents that have been licensed to the Celldex Therapeutics, Inc.; and received research funding from Immunomic Technologies, Inc.

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