Advances and challenges: dendritic cell vaccination strategies for glioblastoma

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Abstract

Introduction—Glioblastoma is the most common primary brain tumor in adults and prognosis remains poor with a median survival of approximately 15–17 months. This review provides an overview of recent advances in the field of glioblastoma immunotherapy.

Areas covered—Recent advances in dendritic cell vaccination immunotherapy are showing encouraging results in clinical trials and promise to extend patient survival. In this report we discuss current scientific knowledge regarding dendritic cell (DC) vaccines, including approaches to differentiating, priming, and injecting dendritic cells to achieve maximal anti-tumor efficacy in glioblastoma. These findings are compared to recently completed and currently ongoing glioblastoma clinical trials. Novel methods such as 'fastDCs' and vaccines targeting DCs *in-vivo* may offer more effective treatment when compared to traditional DC vaccines and have already entered the clinic.

Expert Commentary—Finally, we discuss the challenges of T-cell dysfunctions caused by glioblastoma immunosuppression and how they affect dendritic cell vaccinations approaches.

Keywords

Glioblastoma; Cancer Vaccine; Dendritic Cell; Immunotherapy; Clinical Trials; Immunosuppression; T-cell dysfunction; fastDCs; *in-vivo* DC vaccine; Recall antigens

1. Introduction to glioblastoma

Glioblastoma (GBM) represents approximately 50% of all malignant glioma and is the most common primary brain tumor in adults. At an incidence rate of 3.2 per 100,000 population, GBM patients have a median survival time of 15–17 months with median diagnosis occurring at age 64 [1]. In spite of the current gold-standard of care — maximal safe resection, radiotherapy, and temozolomide chemotherapy, GBM recurrence is almost always inevitable and the five-year survival rate is estimated at 5.1% [1, 2]. The severity and swift

progression highlight the need for developing novel efficacious treatments for GBM patients.

1.1 CNS Immunocompetence

Literature has traditionally described the central nervous system as an immune-privileged site, marked by a stringent brain-blood barrier, the absence of a classical lymphatic drainage system, and low levels of T-cells, antigen-presenting cells (APCs), and major histocompatibility complexes (MHCs), aside from glial cells [2]. However, recent findings, including the discovery of MHC-1 expression by subsets of neurons, have challenged this viewpoint and credit the CNS with immunocompetence [3]. Of particular note is data by Louveau et al., which shows functional lymphatic vessels lining the dural sinuses in mice and draining cerebrospinal fluid, indicating the presence of a classical lymphatic system in the CNS [4]. In addition, angiogenesis around the growing brain tumor leads to the deterioration of microvasculature and relaxation of tight junction, thus increasing leaking.

The inherent brain immunocompetence, the ability of activated T-cells to enter the CNS [5–8], and the decreased barrier functions around the tumor ensure that the immune system has access to the malignant cells, making it feasible to develop immune-based treatment regimens that will improve the current standard of care.

2. Immunotherapy for GBM

Cancer growth is based on the principle that tumor cells are able to evade normal control of the immune system by using a number of complex mechanisms to escape immune detection, including low immunogenicity, antigenic modulation, and immune suppression [9–14]. Anti-cancer immunotherapies activate the immune system to specifically recognize and destroy malignant cells. Recent advances seen in immunotherapies targeting metastatic melanoma and other cancers show the extraordinary capacity of redirecting the immune system and warrant advancing immunotherapy research for treating glioblastoma [15–18].

2.1 Immunotherapy treatments pursued in GBM

A large portion of current GBM clinical trials are dedicated to testing the safety and efficacy of numerous immunotherapeutic approaches. Two main approaches are being pursued: passive immunotherapy and active immunotherapy. Passive immunotherapy is achieved by using therapeutic agents that activate the immune system and confer an antitumor response, often only for the duration of treatment. These include monoclonal antibodies (mAb), bispecific antibodies (bsAb), and immune checkpoint modulators [19]. Current clinical trials enrolling GBM patients are testing an anti-EGFR mAb (US clinical trial NCT02540161) and an anti-EGFR-CD3 bsAb (US clinical trial NCT02521090). Immune checkpoint modulators make up the majority of current passive immunotherapy trials for GBM (e.g. US clinical trial NCT02311920). Active immunotherapy, or tumor vaccination, is achieved by presenting tumor antigens which stimulate the body's immune system to produce an endogenous anti-tumor response. The goal is to either amplify an existing tumor response or to elicit a *de novo* immune response against new antigens, thereby allowing long-term

recognition and eradication of the tumor. Vaccinations being pursued for treating GBM include whole tumor lysate, peptides, viral vectors, and dendritic cells [19–21].

3. Dendritic cell vaccines

Dendritic cells (DC) play a crucial role in protecting the body from foreign antigens and form a link between the innate and adaptive immune system. Upon encountering foreign antigens, specifically pathogen-associated molecular patterns, DCs act as sentinels of the innate immune response by releasing activating cytokines. As orchestrators of the adaptive immune response, DCs take up, process, and present antigens on their cell surface to T-cells and B-cells, thereby activating naïve, effector, and memory immune cells or maintaining tolerance against self-antigens [22]. Ashley and colleagues describe DCs to be the most potent endogenous activators of *de novo* T-cell and B-cell responses, highlighting their vaccine potential in eliciting potent anti-tumor immune responses [23].

Spurred on by the invaluable efforts of Ralph Steinman, there is now a general consensus that DC vaccines can lead to long-lasting cancer immune responses by inducing tumor-specific T-cells and immunological memory [24]. Clinical trials testing DC vaccinations have, so far, shown encouraging, yet modest, results in patients with advanced cancers [25]. To date, sipuleucel-T is the only APC-based cancer vaccine approved in the United States and has shown an increase median survival of four months in prostate cancer patients [26]. However, several factors may be limiting the efficacy of current DC vaccines, including optimal maturation, tumor antigen loading, and the ability of DCs to migrate to vaccine-draining lymph nodes.

4. Dendritic Cell generation *in-vitro*

The most common approach to generating clinical-grade DCs *in-vitro* uses isolated CD14+ monocytes from patient PBMCs (Figure 1). Over a period of 5–7 days, monocytes are differentiated into immature DCs by culturing with GM-CSF and IL-4 [27, 28]. As originally developed and described by Helmut Jonuleit, DCs are subsequently matured in a cytokine cocktail consisting of GM-CSF, IL-4, TNF-α, IL-1β, IL-6, and, in some instances, prostaglandin E_2 (PGE$_2$) for 16 to 20 hours [29, 30]. However, PGE$_2$ has been shown to skew T-cell differentiation to the Th$_2$ phenotype by blocking the production of IL-12 p70 by DCs, the third signal needed for optimal T-cell expansion and acquisition of effector functions [22, 31, 32].

In order to improve the accessibility of DC vaccines to patients and to decrease cost and labor of DC generation, DC vaccine protocols have been developed that greatly reduce the *ex-vivo* culturing time required. 'FastDCs' have been obtained after mere 2-day culturing periods and retain the essential ability to induce antigen-specific T-cell responses [33, 34]. Important differences of fastDCs compared to standard DCs include higher yields in culture, lower release of IL-12 p70, higher CCL19-induced chemokinesis, better intracellular antigen processing, and more effective priming of tumor-specific cytotoxic T-cells [33, 35, 36]. A currently recruiting phase I/II clinical trial (NCT01734304), is using a 3-day protocol to produce fastDCs loaded with tumor antigens against acute myeloid leukemia and has so far

concluded that vaccination with fastDCs is feasible and safe [37]. Furthermore, techniques that alleviate the need to differentiate DCs *in-vitro* from precursor cells via the direct isolation of myeloid or plasmacytoid DCs from PBMCs have recently proven safety and feasibility in clinical trials [38–40].

Cryopreservation is routinely used to store laboratory as well as clinical samples for extended time periods and has also been used for preserving mature dendritic cells, as first described by Feuerstein et al. [41]. Recent reports highlight that cryopreservation during DC vaccine preparation is feasible and allows patient leukaphoresis to be decoupled from the stringent DC generation process. In a small-scale clinical study, Nair and colleagues use cryopreserved PBMCs obtained from pediatric patients with medulloblastoma to generate functional DCs.[42] Alternatively, Fitzpatrick and colleagues showed that mature DCs can also be cryopreserved, alleviating stringent production timelines. The cryopreserved DCs showed no significant differences in viability and cytokine production when compared to freshly generated DCs [43]. Taken together, cryopreservation of DCs represents a practical approach for alleviating exhaustive production techniques and increasing accessibility of DC vaccines to sites without DC generation capabilities.

5. Tumor antigen targets in glioblastoma

DC vaccination is based on eliciting a potent immune response against malignant tumor cells. After DCs have been generated *ex-vivo*, the cells must be loaded with molecules that will allow the DCs to target harmful cells. So-called tumor antigens represent a set of molecular targets that can be used to direct DCs to initiate an immune response against the malignant cells. Broadly speaking, tumor antigens can be divided into tumor-associated antigens and tumor-specific antigens.

Tumor-associated antigens (TAAs) represent a class of molecules that are overexpressed on tumor cells due to changes in the genome that arise during the course of tumor progression. Based on their high expression level, TAAs can be used as targets for marking malignant cells. However, given the fact that TAAs are overexpressed yet normal proteins, they are also expressed in normal cells, which may lead to limited specificity for targeting tumor cells.

In GBM, studies have found numerous proteins, including survivin, HER2neu, EphA2, EGFR, and telomerase, which are over-expressed and could serve as immunological targets for potential immunotherapies [44–50]. For some of these antigens, clinical potential has been shown [49, 51].

Cancer/testis antigens (CTAs) represent a unique class of TAAs where normal expression is restricted to germ cells in the testis and expression is not found in healthy adult somatic cells. For example, the melanoma associated CTAs (MAGE, CAGE) are expressed across a wide range of cancers [52, 53]. In GBM, CTAs are variably expressed within the tumor and an extensive expression analysis by Freitas and colleagues of 153 CTAs identified 4 CTAs (ACTL8, CTCFL, OIP5, and XAGE3) uniquely expressed within GBM tumors when compared to normal brain.[54]

Tumor-specific antigens (TSAs) arise from mutations in the genome that lead to abnormal proteins found only on the tumor cells or a certain subset of tumor cells. Often, these abnormal proteins have a specific function and confer a survival advantage to the malignant cell. Targeting of TSAs makes it possible to use highly potent therapies without risking damage to normal cells. However, as TSAs may often be expressed in only a subset of tumor cells, treatment may prove to be inefficacious once the targeted cells have been removed, leaving the untargeted malignant cells behind.

In GBM patients there are currently two TSAs that are highly conserved among patients and have shown immunogenicity in multiple studies. 31 to 50% of patients with GBM express a conserved mutation of the epidermal growth factor receptor, called EGFRvIII [55–60]. In patients positive for EGFRvIII, the mutation is expressed in 37 to 87% of tumor cells [57].

The second TSA is a conserved mutation of isocitrate dehydrogenase type 1 (IDH1), which occurs at the critical arginine residue number 132 in the catalytic pocket and results in a neomorphic enzymatic function, genetic instability, and malignant transformation [61]. This mutation, termed IDH1(R132H), occurs in more than 70% of grade II and III gliomas and from a therapeutic viewpoint, represents a promising candidate for a tumor specific treatment of malignant glioma [62, 63].

Given the mutagenic nature of cancer cells, most mutations that arise spontaneously in GBM are patient-specific neoepitopes. Furthermore, since TSAs are only expressed in a subgroup of patients, researchers are pursuing the development of personalized cancer vaccines to develop treatments that benefit all patients.

In addition to mutations of native proteins, viral proteins that are specifically upregulated on malignant cells represent ideal TSAs as they have the unique advantage of being intrinsically foreign to the host. While viral expression is usually not restricted to malignant cells, expression of immunogenic epitopes is often undetectable on healthy tissue in patients with virus-associated cancers. We and others have shown that human cytomegalovirus (CMV) infection is associated with low-level viral gene expression in malignant glioma [64, 65]. Based on the success and safety of cellular immunotherapeutics targeting CMV in immunocompromised patients, immunodominant CMV antigens, such as immediate early 1 (IE1), phosphoprotein 65 (pp65), and glycoprotein B (gB), represent possible tumor specific targets for the development of immunotherapies targeting GBM [66–68].

6. Loading DCs with antigen

In practice, DCs can be loaded with a variety of antigen sources, including peptides, tumor lysate, DNA, or RNA (Figure 1). Antigen loading may occur after maturation or concurrent with cytokine maturation. The variety of antigen loading strategies in current clinical trials testing DC vaccinations in GBM patients reveals that the optimal antigen source for causing tumor regression remains a topic of much interest (see Table 1).

Peptide and tumor lysate loading is achieved by co-incubation, allowing the DCs to scavenge the antigens from their surroundings, process them internally, and present epitopes on their MHC molecules. For peptide loading, either a single peptide or a mixture of

different peptides may be used for generating the DC vaccine. By giving conserved immunocompetent glioblastoma antigen peptides, vaccine preparation can be standardized across a patient subpopulation resulting in a focused immune response. CD133 is an antigen expressed on, albeit not all, GBM stem-like cells, whose expression levels correlate with patient survival in glioma [69, 70]. ICT-121, a DC vaccine trial targeting recurrent glioblastoma, uses a combination of purified peptides from the CD133 protein to load DCs which are injected into the patient over several months with the goal of eliciting an immune response against the tumor (Table 1, NCT02049489).

Alternatively, DCs can be pulsed with whole tumor lysate, which, when compared to peptide loading, has the advantage of containing the full tumor antigen repertoire of conserved and novel patient-specific neoepitopes, allowing DCs to naturally process the proteins and present the most immunogenic antigens. In a recent report, Garg et al. show that inducing immunogenic cell death of the tumor cells, instead of freeze/thawing-based necrosis, markedly improves DC vaccines by increasing the immunogenicity of the dying/dead cancer cells which they link to the exposure/release of potent danger signals [71]. Using autologous tumor cell lysate from each patient to load the DC's could represent an important step towards personalized medicine in the treatment of GBM. The DCVax-L vaccine (autologous dendritic cells pulsed with autologous tumor cell lysate) showed a 3 year overall survival rate, 2.5 times the usual period of survival, in a phase 1/2 clinical trial in patients with newly diagnosed GBM, extended survival by 5 months or more in patients with recurrent GBM (Table 1, NCT00045968), and is currently being tested in a blinded randomized phase 3 trial [72–74].

Antigen presentation on DCs can also be initiated by the transfer of nucleotides which subsequently drive protein expression and processing. DNA-based approaches use retroviral transduction to integrate antigen DNA into the DC genome using retrovirus transduction methods and therefore allow long-term antigen expression [75–77].

However, given the safety implications of genomic integration and the complication of driving efficient antigen expression in DCs, RNA-based approaches have come to be favored over DNA in the laboratory and the clinic. Our lab has shown indications of clinical efficacy in treating GBM patients with mRNA transfected DC's [21]. RNA transfection of DC's has the major advantage that this approach is applicable to a wide range of patients given that little tumor sample is needed to prepare the vaccine since RNA can be amplified from a small number of tumor cells. Furthermore, off-the-shelf RNA-DCs can be generated using conserved GBM tumor antigens such as the CMV antigen pp65. DC loading of whole tumor RNA or antigen-specific RNAs is achieved by electroporation followed by an overnight incubation. In terms of safety, stimulating DCs with RNA is a transient therapy that poses no risk of integration, unlike viral or DNA vectors [22]. In a recent phase 1/2 clinical trial, our group generated a dendritic cell vaccine using pp65 mRNA for treating glioblastoma (US clinical trial NCT00639639). Given the expression of pp65 in glioblastoma, as previously published [64, 78, 79], this viral antigen is ideal for eliciting a specific tumor response. Pre-conditioning patients with tetanus/diphtheria toxoid led to a significant increase in lymph node homing of pp65-specific DCs, anti-tumor immune responses, and patient survival [21]. Confirmatory randomized and double-blinded clinical trials are now testing the effects of Td

preconditioning on survival in patients with newly-diagnosed GBM (Table 1, NCT02366728 and NCT02465268).

7. Overcoming limited DC migration

In addition to developing optimally matured and loaded DC vaccines, DCs must migrate from their injection site to the lymph node to induce immune responses. While previous DC vaccines in GBM have shown limited promise in patients, a recent finding by Mitchell et al. has shown that simply improving DC lymph node homing leads to significantly improved tumor responses in humans and mice [21].

Less than 5% of injected DCs reach the lymph nodes [80]. By preconditioning injection sites with inflammatory molecule TNF-α, researchers have previously shown improvements in DC lymph node homing in mice [81]. Mitchell and colleagues applied the idea of inflammatory preconditioning by injecting tetanus/diphtheria toxoid (Td), a standard vaccine used for the prevention of tetanus and diphtheria, into the vaccine site of GBM patients. Results showed that pp65 mRNA loaded DCs injected bilaterally into unilaterally TD preconditioned patients resulted in a doubling of DC migration bilaterally, indicating a systemic effect, and significantly improved survival. In both humans and mice, the Td preconditioning effects were dependent on chemokine (C-C motif) ligand 3 (CCL3) — knocking out CCL3 or depleting CD4+ T cells in mice abrogated improved survival and giving CCL3 or activated CD4+ T cells intravenously rescued DC migration to the lymph nodes [21]. These results expand our knowledge of the mechanisms that underlie successful DC vaccines by showing the relationship between systemic CCL3, CD4+ T cells, and DC cells. Future research will need to examine the downstream mechanisms of CCL3 to determine which CCL3 receptors are vital and whether other recall antigens or systemic administration of CCL3 will improve DC vaccines in GBM and other cancer patients.

8. Vaccines target DCs *in-vivo*

Given the labor and time intensive process of generating DC vaccines *ex-vivo* in addition to variable response rates, efforts are underway to develop vaccines that target DCs *in-vivo*. Targeting DCs *in-vivo* circumvents the issue of poor migration seen with *ex-vivo* generated DCs and allows the targeting of single or multiple DC subsets via their range of unique and shared pattern recognition receptors [82]. A finding by Allan and colleagues showed injected DCs may act indirectly through DCs already present in the lymph node, further supporting strategies that circumvent *ex-vivo* DC generation [83].

In-vivo DC vaccination is achieved by targeting mAbs to DC-specific cell surface receptors. CD205 is a novel endocytic receptor of the C-type lectin family. It mediates antigen uptake and cross-presentation to T cells and has been a major focus for the development of *in-vivo* DC vaccines. Delivery of tumor antigen bound to anti-CD205 antibodies stimulated CD4+ and CD8+ T cells in mice, resulting in tumor regression superior to *in-vitro* loaded DCs [84–86]. In a recent early-phase study using a vaccine composed of a human CD205 antibody fused to the full-length tumor antigen NY-ESO-1, 45 patients with tumors expressing NY-ESO-1 received various dose levels and adjuvant combinations (US clinical trial

NCT00948961). 13 patients experienced stabilization of disease and two patients experienced tumor regression. This first-inhuman study of a protein vaccine targeting DCs demonstrated feasibility, safety, and biological activity, providing rationale for follow-up studies [87].

Given that DC cross-presentation can activate GBM-specific T cells in the periphery that travel to the brain, as seen in trials using *in-vitro* generated DCs, vaccines targeting DCs *in-vivo* present a promising alternative that use similar mechanisms while bypassing the need for DCs to travel from the injection site to the draining lymph nodes. Future experiments will show whether mAb-mediated delivery of GBM-specific antigens to DCs will lead to tumor regression and increase survival.

9. Remaining Questions

Recurrent tumors differ from primary tumors, since treatment with radiotherapy and chemotherapy promotes genetic changes in the primary tumor allowing it to overcome the treatment regimen. Patients with recurrent GBM have a poor overall performance status and their tumors are unresectable, requiring significant corticosteroid treatment to control cerebral edema. A common strategy in GBM — a strategy that needs to be re-visited — is to test new treatments in patients with recurrent disease and then advance those therapies with indications of efficacy into the newly diagnosed patient group. Testing of novel immunotherapies, such as DC vaccines, in patients with newly diagnosed GBM independently of patients with recurrent GBM, may help reveal novel efficacious immunotherapeutic treatments.

Patients with recurrent GBM have undergone multiple cycles of radiotherapy and cytotoxic chemotherapy resulting in a relative immunosuppression. Although TMZ chemotherapy induces lower CD4+ counts in patients and is linked to worse clinical outcomes, lymphopenia appears to play a complex role in studies testing vaccination strategies. A single-arm phase II trial of rindopepimut found lymphopenia enhances tumor-specific cellular and humoral immune responses [88], and in another phase II trial testing an autologous tumor vaccine, patients with grade 3 lymphopenia had improved outcomes when compared to grade 4 and grade 0–2 lymphopenia [89]. It remains to be seen whether lymphopenia can be leveraged to improve GBM vaccines.

10. Conclusion

GBM remains the most malignant tumor of the CNS and patient outlook is dismal. Novel therapies are needed for effective treatment, and the rise of immunotherapies in the field of cancer treatment is promising to revolutionize GBM treatment. Amongst the multitude of immunotherapeutic approaches, DC vaccines have in the past shown limited benefit in tumor therapy, though recent trials in GBM have shown anti-tumor effects for the first time. Combined with the misconceived dogma of an immune-incompetent CNS, DC vaccine research faces a number of hurdles ranging from optimal production, targeting, and antigen loading to overcoming limited migration.

DCs are immune cells that form a bridge between the adaptive and innate immune system. Acting as sentinels, DCs scavenge for foreign material, which is taken up, processed, and presented on MHC molecules to T-cells and B-cells, thereby activating the adaptive immune system to mount an attack on the foreign antigens. DC vaccine approaches exploit this system by isolating monocytes from patients, maturing them into DCs and loading them with appropriate tumor antigens *in-vitro* before infusing them back into the patient where they will migrate to the lymph nodes and activate the immune system.

The *ex-vivo* maturation of monocytes to DCs is achieved by plating the cells with media containing various cytokines. Depending on the cytokines and the concentrations used, maturation normally takes between 5–7 days. Recently developed fastDC reduce the maturation time to about 2 days; the cells demonstrate comparable characteristics to traditionally matured DCs and are able to stimulate antigen-specific T-cell immune responses [33]. Furthermore, recent findings show that DC injection and subsequent migration to the draining lymph nodes is a critical step in obtaining therapeutic efficacy. By preconditioning the vaccine site with a recall antigen, researchers were able to significantly improve DC migration and show increased survival in patients and mice [21].

Taken together, advancements in DC vaccines and results from recent clinical trials substantiate the feasibility of using DC vaccines for treating GBM and provide support for the continued development of DC vaccines along all stages of vaccine development.

11. Expert Commentary

Optimal DC vaccines mirror the essential signals that dictate effective T-cell activation [90]. *In-vitro* generated DCs need to proficiently present antigens for a period long enough to engage the T-cell receptor (Signal 1) and provide T-cells with co-stimulation via cell surface receptors CD80/86 and CD70 (Signal 2). Finally, the cytokine IL-12p70 is thought to represent the third signal for optimal T-cell activation and proliferation provided by DCs. Given the vital interaction of DCs with T-cells to induce an antigen-specific immune response, T-cell dysfunction seen in GBM may play a major role in dictating efficacy of DC vaccine studies. An improved understanding of the various T-cell dysfunctions could lead to patient-specific combination therapies that will enhance DC-mediated immune activation.

During carcinogenesis, the tumor microenvironment induces T-cell tolerance, contributing to tumor growth. Although T-cell tolerance is an integral component of T-cell maturation that prevents autoimmunity, T-cell tolerance toward malignant cells is detrimental and leads to tumor immune escape [91]. Direct induction of T-cell tolerance occurs via down-regulation of MHC molecules and costimulatory molecules and the upregulation of inhibitory receptors such as PD-1 ligand, HLA-G, and HLA-E [92–95]. Blocking of PD-1 ligand *in-vivo* has been shown to enhance the host's anti-tumor response, highlighting the potential of altering the tumor receptors [96]. In addition to altered surface reception molecules, tumor cells secrete various immunosuppressive factors, such as TGF-β, VEGF, IL-10, and CCL21, leading to an immunosuppressive microenvironment that results in the recruitment or promotion of immunosuppressive immune cells including regulatory T-cells (Tregs), immature DCs, and tumor-associated macrophages [97–103]. In GBM patients,

immunosuppression is particularly severe, resulting in a shift toward Th2 cytokine production, increased Treg recruitment, and inhibiting T-cell proliferation [13, 104, 105]. Fecci and colleagues found that GBM patients had decreased CD4+ T-cell counts but that the fraction of Tregs in the remaining CD4+ T-cells was increased. Treg reduction removed T-cell proliferative defects and reversed Th2 cytokine shifts [13]. Breaking of T-cell tolerance in GBM patients may be required to obtain durable DC vaccine efficacy in patients. This could be done by changing the tumor microenvironment signals or decreasing Treg numbers.

In addition to tolerance, T-cell exhaustion in cancer patients is marked by the upregulation of negative regulators, a distinct regulation of transcription factors, and the hierarchical loss of effector functions on tumor-infiltrating lymphocytes [106]. T-cell inhibitory receptors prominently include LAG-3, PD-1, CTLA-4, and TIM-3, some of which are already being targeted in successful immunotherapies [15, 107]. Although trials testing checkpoint inhibitors in GBM have commenced, they may ultimately not prove advantageous when given as stand-alone therapy, but instead will prove successful as a way reverse immune suppression while treating with tumor-specific immunotherapies such as DC vaccines. Given the complexity of tumor-mediated effects on patient immune systems, researchers developing DC vaccines will need to consider tumor-mediated immunosuppression and how to intelligently design co-therapies to boost DC vaccine efficacy.

12. Five-year view

Dendritic cell vaccinations for glioblastoma have already shown promising anti-tumor effects in clinical trials. Determining whether DC vaccinations have the potential to become standard-of-care remains to be elucidated. Results from ongoing clinical trials, including the phase 3 DCVax-L and ICT-107 trial (Table 1), will reveal which DC vaccine protocols are efficacious. Based on recent findings that tetanus toxoid preconditioning acts as an adjuvant to boost systemic DC migration and increase survival, it is probable that over the next few years DC vaccination strategies for GBM will include novel recall antigens as adjuvants to increase efficacy and enhance immune responses. Furthermore, it is expected that DCs loaded with immunogenic neoepitopes will soon enter trials, mirroring current GBM peptide vaccinations strategies and bringing us closer to designing patient-specific tumor treatments. In parallel, immune checkpoint modulators are likely to be used in combination with DC vaccinations in an effort to 'release the immunologic brakes' and thereby enhance the immune response and magnitude of antitumor DC vaccines.

Acknowledgments

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Reference annotations

*Of interest

**Of considerable interest

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13. Key Issues

- Dendritic cell vaccinations have shown anti-tumor effects, although limited, in glioblastoma patients.

- Efforts to improve DC vaccines focus on optimal maturation, tumor antigen loading, and the ability of DCs to migrate to vaccine-draining lymph nodes.

- Fast-DC maturation protocols promise improvements in DC vaccine preparation, helping to reduce time and labor needed when compared to traditional DC vaccine protocols.

- A variety of tumor antigen sources can be used to load DCs, including peptides, tumor lysate, DNA, and RNA. To date, no one antigen source has been found to be significantly superior, despite numerous ongoing GBM clinical trials testing antigen sources.

- Tetanus toxoid has been used as an inflammatory adjuvant to boost DC migration and overall survival in patients and mice, hinting at a possible pervasive strategy to boost DC vaccine efficacy using recall antigens.

- Vaccines targeting DCs in-vivo allow targeting of specific DC subsets and promise more effective immune responses.

- Neoepitope-loaded DCs and combination approaches with immune checkpoint modulators are likely to be pivotal research areas in the next years.
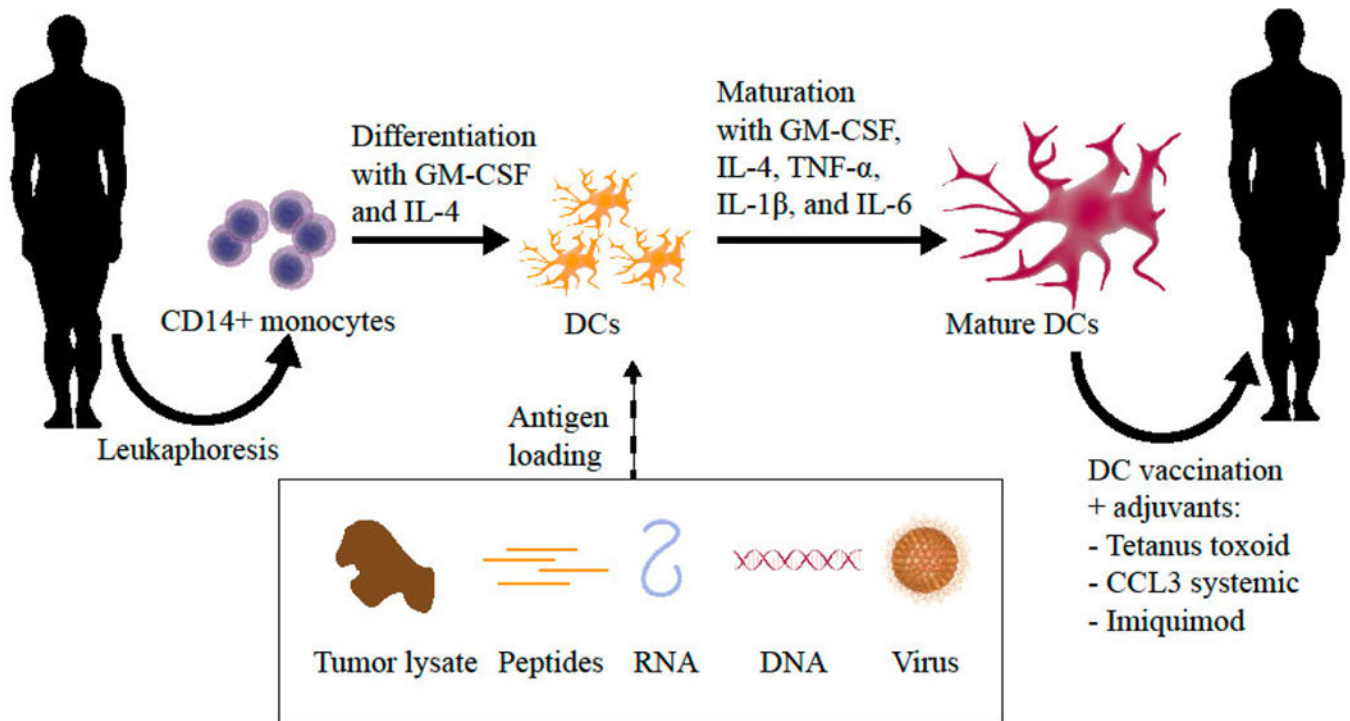
Figure 1. Dendritic cell vaccine production

Dendritic cells (DC) vaccines for glioblastoma (GBM) immunotherapy are generated *in-vitro* using CD14+ monocytes isolated from patient PBMCs. Monocytes are typically differentiated into immature DCs by incubating with GM-CSF and IL-4 for a period of 5–7 days. Alternatively, monocytes can be differentiated in as little as 2 days using novel 'fastDC protocols. DCs are subsequently matured in a cytokine cocktail for 16 to 20 hours and loaded with tumor antigen. DCs can be loaded with various formats of tumor antigen, including peptides, tumor lysate, DNA, and RNA. Finally, the DCs are injected back into the patient where they travel to vaccine-draining lymph nodes to elicit a tumor-specific immune response. Injection with adjuvants such as the tetanus toxoid can be used to increase DC migration to the lymph nodes and augment vaccine efficacy.

Table 1

Recent clinical trials employing dendritic cell vaccination immunotherapy for glioblastoma

NIH clinical trial	Type	Location / Sponsor	Phase	Eligibility Criteria	Primary Outcome	Dosing regimen	Treatment groups
NCT 02010606	DC loaded with lysate	Cedars-Sinai Medical Center	1	Newly diagnosed or recurrent GBM	Safety, adverse events, treatment-related toxicities	Autologous DCs loaded with lysate from allogeneic GBM stem-like cell line given once every week for 4 weeks followed by once every 8 weeks	Cohort A: Newly diagnosed GBM Cohort B: Recurrent GBM
NCT 01808820	DC loaded with lysate, Imiquimod, Tumor lysate	University of Miami	1	Recurrent GBM	Number of adverse events	DCs loaded with tumor lysate are injected into imiquimod-treated skin given once every week for 4 weeks followed by tumor lysate injections during weeks 8, 12, 16, and 28	Single-arm
NCT 02049489	DC loaded with peptide antigens	Multi-center / Immuno-Cellular Therapeutics	1	Recurrent GBM	Safety study	At least 4 doses of DCs loaded with CD133 peptides given (ICT-121), followed by additional maintenance vaccines	Single-arm
NCT 02649582	DC loaded with RNA	Antwerp University Hospital	1/2	Newly diagnosed GBM	Overall survival	WT1 mRNA loaded DC vaccine given weekly for 3 weeks followed by maintenance vaccine on day 21 of every TMZ cycle	Single-arm
NCT 01567202	DC loaded with lysate	Huashan University, Fudan University	2	Histologically confirmed GBM	Overall survival	8–10E6 DCs loaded with autogeneic glioma stem-like cells associated antigens given once a week for 6 weeks	Triple-blind Arm I: DCs Arm II: Placebo
NCT 02366728	DC loaded with RNA	Duke University Medical Center	2	Newly diagnosed GBM	Overall survival	CMV pp65-loaded DC vaccines #1–3 given every two weeks followed by vaccine #4 (only Arm I-II). Td given to all during vaccine #1. Arm III patients receive basilixumab 1 week before vaccine #1–2. Before vaccine #4, Arm I receives unloaded DCs, Arm II-III Td dose.	Arm I: Unloaded DCs + loaded DCs Arm II: Td + loaded DCs Arm III: Td +loaded DCs + basiliximab
NCT 02465268	DC loaded with RNA	University of Florida	2	Newly diagnosed GBM	Overall survival	CMV pp65-loaded or influenza-loaded DCs matured with GM-CSF and injected ID into Td preconditioned patients at day 22–24 after first TMZ cycle then at 2 week intervals. Doses 4–10 given on day 22–24 of each TMZ cycle.	Arm I: pp65 DC + GM-CSF + Td Arm II: Influ. DC + GM-CSF + Td Arm III: PBMC + GM-CSF + Td Arm IV: pp65 DC + GM-CSF + Sal.

NIH clinical trial	Type	Location / Sponsor	Phase	Eligibility Criteria	Primary Outcome	Dosing regimen	Treatment groups
NCT 01204684	DC loaded with lysate	University of Los Angeles / Jonsson Comprehensive Cancer Center	2	Newly diagnosed or recurrent grade 3 or 4 glioma	Most effective combination of DC vaccine components	DCs loaded with tumor lysate given alone or with adjuvants 0.2% resiquimod or polyICLC	Arm I: DCs Arm II: DCs + resiquimod Arm III: DCs + polyICLC Arm V: Influ. DC + GM-CSF + Sal. Arm VI: PBMC + GM-CSF + Sal
NCT 01759810	DC loaded with protein, HSC, CTL	NeuroVita Clinic / Numerous sponsors	2/3	Histologically confirmed GBM	All-cause mortality	DCs loaded with recombinant proteins analogous to key tumor antigens, CTLs, and autologous proteome-modified HSCs or allogeneic HSCs given at different intervals to patients over the first couple of months.	Arm I: allogeneic HSCs Arm II: autologous HSCs
NCT 02546102	DC loaded with peptides	Multi-center / ImmunoCellular Therapeutics	3	Newly diagnosed GBM	Overall survival	DCs loaded with immunogenic peptides from 6 tumor-associated antigens (ICT-107) given once a week for four weeks ID followed by maintenance injections once a month for 11 months and once every 6 months therafter.	Arm I: ICT-107 Arm II: Placebo
NCT 00045968	DC loaded with lysate	Multi-center / Northwest Biotherapeutics	3	Newly diagnosed GBM	PFS	Autologous DCs pulsed with tumor lysate antigen, called DCVax-L, or autologous PBMCs (placebo) given ID twice daily on days 0, 10, 20, and at weeks 8, 16, 32, 48, 72, 96, and 120	Arm I: DCVax-L Arm II: Placebo

CTL, cytotoxic T-lymphocyte; DC, dendritic cell; ID, intradermal; GBM, glioblastoma; GM-CSF, granulocyte macrophage colony-stimulating factor; HSC, hematopoietic stem cell; PBMC, peripheral blood mononuclear cells; PFS, progression-free survival; pp65, phosphoprotein 65; Sal., saline; Td, tetanus/diphtheria toxoid; TMZ, temozolomide