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Re-emergence of Dendritic Cell Vaccines for Cancer Treatment

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Abstract

Dendritic cells (DC) are essential in immunity due to their role in activating T cells thereby promoting anti-tumorigenic responses. Tumor cells, however, hijack the immune system causing T cell exhaustion and DC dysfunction. Tumor-induced T cell exhaustion may be reversed through immune checkpoint blockade (ICB), however, this treatment fails to show clinical benefit in many patients. While ICB serves to reverse T cell exhaustion, DCs are still necessary to prime, activate and direct the T cells to target tumor cells. In this review we will provide a brief overview of DC function, describe mechanisms by which DC functions are disrupted by the tumor microenvironment and highlight recent developments in DC cancer vaccines.

Keywords

Dendritic Cells; Tumor Microenvironment; Immune-suppression; Cancer; Immunotherapy; Vaccines

EMERGENCE OF DC BASED VACCINES

Dendritic Cells (DCs) are unique immune cells as they possess the capacity to initiate and regulate both innate and adaptive immunity. As such DCs play a pivotal role in tumor immune-surveillance. Under normal circumstances DCs are maintained in an immature and inactivated state until exposed to optimal stimuli such as inflammatory cytokines, microbial factors or endogenous **alarmins** (see glossary) [1]. Once activated, DCs rapidly mature and process antigens to be presented to T cells on their major histocompatibility (MHC) molecules (Box1). Although all DCs are professional antigen presenting cells (APCs) specific subsets of DCs possess specialized antigen-processing machinery and excel at activating either CD4⁺ or CD8⁺ T cells. Classical conventional DCs are broadly classified into two subsets, namely, CD1c⁺ DCs and CD141⁺ DCs. The CD1c⁺ DCs are migratory cells and mainly recognized for activating CD4⁺ T cells. CD141⁺ DCs, on the other hand, are primarily lymph node (LN) resident with enhanced ability to perform **antigen cross-**

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presentation (see glossary) and activation of CD8⁺ T cells [2–4] (Box 2). Interestingly, in addition to traditional modes of antigen acquisition (Box2), DCs also expand the breadth of immune response by “transferring” their antigens to LN resident DCs using the process of “**cross-dressing**” (see glossary) [5, 6] or by acquiring antigens from exosomes, infected APCs or apoptotic cells [7, 8]. In fact, mice lacking endogenous DCs are unable to mount a T cell response when injected with antigen loaded exogenous DCs, thus, underscoring the requirement for antigen transfer and cross-presentation by endogenous DCs for raising antigen specific T cell immunity [9].

Dendritic cell vaccines against tumor antigens remain an exciting arm of immunotherapy that aim at boosting patient’s own immune response against their tumors [10]. Moreover, cell-based therapies are particularly desirable as they pose low risk of toxicity and hold the potential of activating other immune modulators such as Natural Killer (NK) cells in addition to T cells in anti-cancer mechanisms. Preclinical studies in 1990s first introduced the concept of using autologous bone marrow derived DCs as a viable vaccination option [11]. These studies laid the bedrock for DC vaccines and argued in favor of using ex-vivo generated DCs over peptide vaccination for generating successful CD4⁺ and CD8⁺ T cell mediated tumor immunity [12–14]. However, it was not until protocols were established for generation of DCs from monocyte precursors, **MoDCs** (see glossary) in humans that the use of ex-vivo DCs pulsed with tumor associated antigens (TAA) could really be exploited for clinical intervention [15].

Sipuleucel-T (see glossary) was the first DC-based anti-tumor vaccine to be approved by the FDA for use against asymptomatic or minimally symptomatic castration resistant prostate cancer. Overall the therapy did significantly reduce the risk of death and evidence of immunity against the immunizing protein was observed. However, correlation with an immune response against the PA2024 antigen (a fusion protein between PAP and GM-CSF; see glossary for Sipuleucel-T) was not significantly strong and when compared to the fusion protein, substantially less native PAP specific immunity could be established [16, 17]. Thus, a lack of clear clinical benefits, especially in late stage cancer, lead to a rapid drop in the prescription of Sipuleucel-T in this setting. Deeper analysis of tumor immunobiology indicates that the initial lack-luster performance of Sipuleucel-T can be attributed to the multitude of immune evasion mechanisms deployed by tumor cells in advanced disease. In this review we will describe the immunosuppressive mechanisms that actively dampen DC function in cancer. Furthermore we will provide insights on innovative improvements in DC targeted vaccine platforms and how combination of immunotherapies can be used to overturn tumor-induced immune-suppression and prompt induction lasting anti-tumor responses.

DENDRITIC CELL DYSFUNCTION IN CANCER

The tumor microenvironment (TME) is a specialized niche created by the confluence of tumor cells, supporting stroma and infiltrating immune cells. Within this niche tumor cells adapt their environment to support maximal tumor growth and impede immune detection [18]. Type-I interferon (IFN) signaling and antigen cross-presentation are both considered key functions of DCs in driving anti-tumor immunity in the LNs and in the TME [19].

Indeed, mice deficient for Batf3, a transcription factor intricately involved in differentiation of cross-presenting DCs, are unable to evade tumor establishment [20]. Likewise, **Flt3L** (see glossary) and PolyI:C driven expansion and activation of CD103⁺ DCs is critical for tumor regression in response to immunotherapy [21]. Moreover, DCs isolated from cancer patients often lack maturation markers and fail to activate T cells [22]. These reports are in line with a recent observation made by *Lavin et al*, reporting a decrease in CD141⁺ DCs accompanied by a low number of activated CD8⁺ T cells in the tumors of patients with early stage lung adenocarcinoma [23]. Hence, tumor-derived factors appear to actively suppress normal DC function and recruitment to the TME and additionally have a direct effect on the efficacy of DC vaccines [24]. Below we discuss some tumor-derived factors that can impact DC function in the TME.

Suppressive alarmins

Matrix metalloproteinase-2 (MMP-2) is a gelatinase intricately involved in digesting the extracellular matrix [25]. Increase in MMP-2 expression is found to correlate with progressive disease and poor prognosis in cancer patients [26]. Our group identified a novel role for MMP-2, as a “suppressive” alarmin that inhibits IL12 secretion and Th1 T cell differentiation by facilitating IFN-alpha Receptor 1 cleavage and Toll Like Receptor-2 (TLR-2) stimulation on DCs [27–30]. Another TLR-2 alarmin, Versican, has been reported to induce immunosuppression within DCs [31] and macrophages in the TME [32]. However, targeted disruption of Versican or MMP-2 specifically as a means of cancer intervention has not been tested in humans as yet.

Antigen masking

The TME has been known to alter tumor antigens so as to avoid immune detection. A prime example of this evasion mechanism is the post-translational hypo-glycosylation of Mucin-1 (MUC1) secreted by tumorigenic cells. DCs are unable to process and present the hypo-glycosylated MUC1 (hgMUC1) to T cells [33, 34]. Moreover, hgMUC-1 can act as a chemo-attractant for immature DCs and interfere with DC differentiation and Th1 skewing [35]. A vaccine (TG4010) employing vaccinia virus engineered to express MUC1 and IL2 elicited immunological responses in some patients with metastatic renal clear cell carcinoma but showed no over-all benefit compared to standard treatments [36]. However, immunization of healthy subjects (with a history of adenomatous polyps) with synthetic long peptides corresponding to immunogenic epitopes in hgMUC-1 along with PolyI:C adjuvant lead to a robust vaccine-specific immune response [37]. A clinical trial is underway to compare immune outcome in pancreatic cancer patients vaccinated with autologous DCs pulsed with tumor lysate or MUC1 peptide (NCT03114631). Thus it would seem that hgMUC1 targeting vaccines may have the potential for generating prophylactic and possibly therapeutic tumor-immunity particularly when administered with the right adjuvant and in combination with other therapies.

Immunosuppressive cytokines

Expression of suppressive cytokines such as **VEGF** (See glossary) [38], Transforming growth Factor beta (TGFβ) [39], Macrophage colony stimulating factor (MCSF) [40] and

IL10 [41] in the TME has been directly correlated with advanced tumor stages. These cytokines exert their immunosuppressive influence by; inhibiting DC differentiation and maturation thus hindering Th1 T cell differentiation, promoting expression of **IDO** (see glossary) and PDL1 on DCs thus promoting T cell anergy [42, 43], prompting DCs to differentiate T cells towards a regulatory phenotype [44] and preventing DCs from exiting the TME [45]. Indeed, pharmaceutical interruption of VEGF [38, 46], MCSF [47], TGF β [48] and IL10 (NCT02731742) by way of using small molecule inhibitors or blocking antibodies is being actively explored to treat cancers.

However, it should be noted that mice deficient for IL10 spontaneously develop colitis, characterized by excessive secretion of inflammatory cytokines like IFN γ , IL17 and IL12, thus emphasizing the role of IL10 in limiting pathological inflammation and promoting immune tolerance towards commensal microorganisms in the gut [49]. Furthermore, IL10 has also been reported to promote CD8⁺ T cell activation and proliferation in the TME. Indeed low dose administration of PEGylated IL10 (AM0010) in combination with anti-PD-1 antibody in cancer patients elicited immune activation, tumor shrinkage and good tolerability [50, 51]. Overall, since IL10 appears to play a dual role in tumor immunity, it is unclear at this time under what circumstances IL-10 targeted therapy will be efficacious, as this is likely to be context and dose dependent.

Metabolic Stress

The TME alters the metabolic pathways in tumor associated immune cells to facilitate tumor cell escape and immune detection. A study by *Herber et al* in 2009 first reported that high lipid accumulation could render CD11c⁺CD8⁺DCs and classical tumor associated DCs defective in antigen presentation and T cell activation in both murine models of cancers as well as in cancer patients [52]. Along the same lines of investigation, Cubillos-Ruiz and colleagues demonstrated that an inhospitable TME can cause DCs to accumulate endoplasmic reticulum (ER) stress in the form of reactive oxygen species and lipid peroxidation resulting in divergent activation of the unfolded protein response (UPR). Aberrant UPR activation in turn induces overt expression of transcription factor XBP-1 and consequent inhibition of antigen processing and presentation by DCs. Interestingly, treatment with antioxidants and inhibition of XBP-1 expression using nano-particles successfully relieved the ER stress on DCs restoring their potential to activate T cell and resulting in tumor regression [53, 54]. Taken together these findings indicate that agents that restore DC metabolic health should be developed and tested as a combination therapy cocktails along with immune checkpoint blockade (ICB).

Hypoxia

The unremitting hypoxia in the TME promotes excessive accumulation of immunosuppressive factors like VEGF, adenosine and IDO. Physiological role of adenosine is to reduce overt inflammation in case of tissue injury until homeostasis is restored. However, in a consistently hypoxic TME, adenosine continues to accumulate and interferes with the functions of all anti-tumor immune cells. For DCs in particular, adenosine induces immunosuppressive cytokines and enhances expression of IDO [55] promoting apoptosis, anergy and T cell tolerance [56]. Multiple clinical and pre-clinical studies are underway to

investigate the clinical efficacy of adenosine signaling inhibitors [57] and IDO inhibitors in combination with other therapies [58].

In summary multiple TME generated factors collectively contribute to suppress DC function by inhibiting DC recruitment, egress, activation, antigen presentation and Th1 differentiation (Figure 1). Thus DC vaccine platforms will have to accommodate interventions against these factors as a combination in order to maximize their therapeutic potential, achieve tumor regression and generate immunological memory.

RE-EMERGENCE OF DENDRITIC CELL TARGETING VACCINES

Over the last few years renewed interest in harnessing DCs for therapeutic purposes has rekindled the interest in improving DC vaccine pipeline. These improvements take into the account the severe immune-suppression induced by the TME. As a result the current DC vaccine platforms have innovated means of improving DC maturation, antigen presentation, antigen loading, antigen selection and proliferation. Some of the areas under rapid optimization are listed below.

Alternatives to using MoDCs

Generating MoDCs is a time consuming process with significant logistical challenges. Moreover, the ex-vivo matured MoDCs do not correlate well with in-vivo cross-presenting CD141⁺ DCs [59, 60]. Use of natural or conventional (cDCs), that is DCs isolated directly from peripheral blood, is one way to avoid using MoDCs. cDC vaccines can be prepared quickly as the cells do not need to undergo exvivo differentiation. Following PBMC isolation, cDCs of the preferred subset maybe enriched using commercially available kits or by cell sorting. Thereafter the cells may be matured, activated, loaded with antigens ex-vivo and injected back into the patients. A study testing intra-nodal administration of isolated and purified CD1c⁺ DCs, activated and loaded with HLA-A2.1-restricted tumor derived peptides in patients with stage IV melanoma (NCT01690377) concluded that cDC vaccines were indeed safe and capable of generating tumor specific cytotoxic T lymphocyte (CTL) immunity that correlated with improved progression free survival (PFS) [61]. Similar results were reported using blood isolated plasmacytoid DC vaccinations by the same group [62]. However, no kits are commercially available to enrich for CD141⁺ DC subset and the low yield of pure DC subsets in peripheral blood remains a major roadblock in using blood isolated DCs for immunotherapy.

Another approach is to differentiate cross-presenting XCR1⁺Clec9A⁺ DCs (Box2) or Langerhans cells (LCs, a specialized subset of DCs) from CD34⁺ hematopoietic stem cells (HSC) isolated from cord blood [63]. CD34⁺ HSC-derived DCs have been tested in clinical trials in the past with promising success in generating tumor specific immunity [64–66]. Currently a clinical trial is underway (NCT01995708) to specifically test the efficacy of vaccinating with autologous LCs derived from CD34⁺ progenitors transfected with mRNA encoding TAA in patients with multiple myeloma [67]. Lentiviral transduction is also being used to generate CD1d expressing human pluripotent stem cells that could be differentiated into CD1d⁺ DCs with the capacity to activate not just CD8⁺ T cells but also iNKT cells [68].

Advances such as these make it conceivable to use stem cells to generate any DC subset in large numbers to be used for immunotherapy.

The ideal solution for replacing MoDCs as vaccines would be use of off-the-shelf DC-like immortalized cell lines. Access to such cell lines would allow for extensive testing, avoid issues of low cell number, enable easy handling and increase reproducibility. MUTZ-3 cell line, derived from human leukemic myeloid cells, comes close to mimicking a DC-like phenotype and function [69] but has not been tested clinically. A major problem with using a cell line is that these cells are allogeneic and may fail entirely as a vaccine or elicit major adverse events. Having said that, allogeneic DCs have been tested in clinical trials in patients with acute myeloid leukemia (AML) (NCT01373515) and allogeneic DCs generated from cord blood were reported to be immunogenic [70]. Thus, use of allogeneic DCs as vaccines is feasible but further testing is required to optimize this platform.

Optimizing DC maturation Stimuli

The maturation stimulus is a major factor that dictates the success or failure of DC vaccines. TLR agonists (TLR-4 agonist LPS, TLR-3 agonist PolyI:C, TLR-7/8 agonist resiquimod), cytokines (TNF α , IL1 β , IL6, IFN α , IFN γ), co-stimulatory receptor ligands (CD40L), prostaglandin E2 (PGE₂), have been used either alone or in various cocktail formulations to mature and program DCs. The desired maturation outcome is to induce high expression of MHC-I and MHC-II; co-stimulatory molecules CD40, CD80 and CD86; secretion of Th1 inflammatory cytokines like IL12 and IFNs and expression of chemokines such as CCR7 in order to polarize DCs towards Th1 activation [71–76]. More recently mRNA transfection-based delivery of a cocktail of co-stimulatory molecules (CD40L, CD70 and constitutively active TLR-4) called TriMix has emerged as a novel means of maturing DCs (TriMix-DCs) with enhanced T cell activation potential [77–79]. Vaccination with autologous TriMix-DCs co-electroporated with mRNA coding for melanoma-associated antigens was reported to be feasible, safe and has yielded antigen specific immune responses (NCT00074230, NCT01066390) [80, 81]. Combination therapy administering TriMix-DCs in combination with other therapeutics such as anti-CTLA-4 antibody are now being explored (NCT01302496).

Optimizing Antigen Selection

Introducing only select TAAs presents the inherent barrier of activating T cells against a narrow range of antigens in a MHC restricted fashion. Incubating DCs with whole tumor lysate or killed tumor cells allows for a wide range of TAAs to be presented on DCs. In addition, secretion of alarmins and cytokines from tumor cells serves as natural maturation stimuli [82, 83]. A study performed to explore the efficacy of vaccinating with MoDCs loaded with autologous tumor lysate and cytokine induced killer cells (CIKs) in patients with stage IV breast cancer reported a significant increase in PFS and overall survival (OS) over a 10-year follow-up [84]. However, another study reported no particular clinical benefit despite induction of immunological responses [85]. Oxidization of whole tumor cells using hypochlorous acid (HPO) has lately been suggested to improve DC vaccination outcome substantially. Although still awaiting clinical verification, oxidizing tumor lysates with HPO was demonstrated to improve antigen uptake, cross-presentation by DCs and tumor specific

competent CD8⁺ T cell response ex-vivo [86]. Another approach to target DC in-vivo is using the GVAX vaccine platform that consists of irradiated tumor cells modified to secrete GM-CSF. GM-CSF serves to attract and maintain DCs thus promoting tumor immunity [87, 88]. It was demonstrated that dual blockade of both PD1 and CTLA4 along with GVAX administration lead to remarkable tumor rejection in murine tumor models [89].

Fusion of tumor cells with DCs is yet another technique to allow DCs access to all tumor antigens through creation of a tumor-DC hybrid using chemicals such as polyethylene glycol. Tested in a small trial, irradiated tumor cells from resected glioblastoma multiforme (GBM) tumor were fused with autologous MoDCs. Subsequently the fused cells were transfected with PolyI:C and siRNA for IL10 and injected in the patients along with standard chemotherapy. The therapy was tolerated well and appeared to improve patient response to standard chemotherapy [90, 91]. In another clinical trial (NCT01096602) AML patients under remission following ICB therapy were vaccinated with autologous MoDCs fused with their own cancer cells. The results from the trial revealed a successful expansion of helper and CD8⁺T cells specific for TAAs and a remarkable lack of remission in all patients within a median of 57 months of follow-up [92].

Neo-antigens are generated either due to a tumor-related or spontaneous mutations and give rise to novel antigens that differentiate healthy “self” cells from cancerous cells [93, 94]. Several studies are exploring neo-antigens for immunotherapy (as examples, NCT02035956, NCT01970358, NCT02149225, NCT 02348320, NCT02316457) [95]. Although not all neo-antigens are immunogenic, the concept that mutational load correlates with ICB response has been supported in a number of independent studies and clinical trials [93, 96–98]. For example, high number of mutations accrued due to **MSI** (see glossary) in solid tumors has been positively correlated with an increased tumor immunogenicity and improved overall response to PD1 blockade [99, 100]. As a result the FDA has approved use of PD1 inhibitor for treatment in adult patients with unresectable and MSI-high and MMR-deficient solid tumors who do not respond to other treatments [101]. However, not all tumors with high mutation load respond to ICB and inversely patients with modest mutation frequency may respond robustly. Emerging data suggests that high mutation burden alone is not sufficient to ensure ICB response. Other factors such as CTLA4 and PDL1 expression on the tumor cells or immune cells as well as tumor cell intrinsic signaling mechanisms like the Wnt/βCatenin pathway (reported to inhibit infiltration and activation of CD103⁺ DC and T cells in the TME) may also influence a patient’s ability to respond to ICBs [102–104]. Perhaps the most important aspect of neo-antigen vaccine is the vast difference in neo-antigen repertoires between patients. Therefore, efforts are being made to generate vaccines that target patient-specific mutations. Indeed, vaccination with DCs loaded with personalized neo-antigenic epitopes has been shown to elicit clear evidence of priming and boosting of CD8⁺ T cells in cancer patients [105] (Box3). Our group has recently launched a clinical trial (NCT02721043) to test the safety and immunogenicity of a personalized vaccine in patients with advanced solid tumors. Under this platform tumors will be sequenced to identify unique mutation-associated neo-antigens to generate personalized peptide vaccine. Thereafter the patients will be vaccinated with their personalized genomic peptide vaccine along with PolyI:C:LC adjuvant and monitored for adverse events and anti-tumor immunity. It is hoped

that the data will provide novel insights into how neo-antigens maybe predicted and used to design anti tumor vaccines.

Optimizing Antigen Loading on DCs

The nature of T cell immunity generated by DCs depends heavily upon the mode of antigen uptake [106]. Antigens bound to antibodies against endocytosis receptors such as C type Lectin Receptors (like Clec9A [107] and DEC-205 [108]), Mannose Receptors and CD40 [109] are more likely to undergo cross-presentation. Indeed a vaccines comprising of a fusion protein of DEC-205 and TAA NY-ESO-1 (CDX-1401) is being tested to enhance NY-ESO-1 cross-presentation by DCs in-vivo (NCT02166905, NCT01834248). Vaccination with a combination of systemic Flt3L (CDX-301) and CDX-1401 along with PolyIC:LC (NCT02129075) has shown evidence of priming T cell immunity to the vaccine antigen and makes a case for using Flt3L for mobilizing DCs in-vivo to improve vaccine response [110]. Similarly, Mannose Receptor engaging vaccine, CDX1307 has yielded promising results [111]. CD40L, either in the form of recombinant protein, targeting antibodies, electroporated mRNA or in fusion with tumor lysate, is being investigated for facilitating tumor lysate uptake and cross-presentation by DCs in-vivo (NCT00053391) [112, 113]. Furthermore, anti-CD40 antibodies were demonstrated to substantially improve T cell immunity in non-human primates [114] and CD40 antibodies are now being explored in clinic as a tumor therapeutic (NCT02376699, NCT02482168, NCT01103635). Lentiviral transduction is being used to genetically over-express desired tumor antigens in DCs differentiated from hPSCs to avoid the additional step of antigen loading [115]. In addition, monocytes are being modulated by lentiviral transduction to express growth cytokines like GM-CSF and IL4 along with TAA thus giving rise to “smart DCs” with capacity to self-differentiate (not requiring exogenous addition of cytokines) and express TAAs on MHC molecules [116]. A particularly exciting new avenue for DC vaccine therapy is the advent of RNA based DC vaccine such as TriMix-DCs discussed above [78]. Under this module DCs are transfected with mRNA coding for selected TAA and cytokines so that each DC naturally expresses, process and presents self-antigens on its MHCs [117, 118].

Optimizing DC mobilization

Once a DC vaccine is administered, the extent of immunological response is strongly influenced by capacity of the antigen bearing DCs to traffic to the draining LNs and tumor sites. *Mitchel et al* demonstrated that pre-conditioning the tumor site with tetanus/diphtheria (Td) toxoid vaccine significantly improved the survival and antigen specific T cell responses in GBM patients receiving autologous MoDCs vaccines loaded with GBM antigen pp65. The authors surmised that as most people have received Td toxoid vaccines in their childhood, re-exposure would recall the CD4⁺ memory response thus activating immunity and aiding in DC migration to LNs [119]. GM-CSF is known for its function in DC recruitment and maturation. In addition, it also facilitates homing of cytotoxic T lymphocytes (CTLs) to the tumor site [87, 88]. GM-CSF secreting genetically modified tumor cells under the GVAX vaccine platform, (GVAX-Pancreas NCT00084383 or Melanoma-GVAX-NCT01435499) have yielded promising antigen specific protective immune responses specially when administered along with supporting drugs like cyclophosphamide or innate immune ligands (STINGVAX) [120–123]. Flt3L is also being

actively tested for DC expansion both ex-vivo and in-vivo with encouraging results [21]. Future trials are set to explore the reach of rhFLT3L as a combination therapy for treating cancers (NCT01811992, NCT02839265). Incoming data from early clinical trials (NCT0219075 and NCT01976585) suggests promising future for combination rhFLT3L therapy in early stage tumors (unpublished, personal communications with Dr. Brody).

Combing DC vaccine with other cell-based therapies

DC vaccination protocols are being designed to include other cell-based therapies such as cytokine induced killer cells (CIK) therapy and adoptive cell transfer therapy (ACT) therapy. CIK are autologous T cells, NK cells and NKT cells activated and expanded ex-vivo under the influence of anti-CD3 stimulation and cytokines. The DC-CIK combined vaccine has been shown to elicit far less adverse events as compared to standard chemotherapy and has shown promising potential in terms of improving over all survival and quality of life when tested in patients [124, 125]. Adoptive transfer of tumor specific T cells or T cells with engineered T cell receptor [126] in combination with TAA loaded DCs is yet another attractive immunotherapy option that has gained momentum due to its anti-tumor effects and usefulness even in advanced tumors [127]. A summary of multiple variables in DC vaccine platforms is depicted in Figure 2 (Key Figure).

Concluding Remarks

Many modern immunotherapy platforms have been developed to treat established cancers such as the ICBs and more recently, personalized CAR T cell therapy for treatment of B-cell acute lymphoblastic leukemia [128]. However, none of these therapies have been effective in all patients, indicating a need for combining different immunotherapy platforms. The future of cancer immunotherapy is likely to be built upon combination of two arms. One arm shall focus on inhibiting tumor-induced immunosuppression by using inhibitors of checkpoint molecules CTLA4 and PD/PDL1; blocking antibodies against immunosuppressive cytokines like VEGF, inhibitors of hypoxia, IDO inhibitors, adenosine antagonists and inhibitors of regulatory T cells like anti-CD25 antibodies and cyclophosphamide. The second arm shall be aimed at activating anti-tumor immunity through DC vaccines utilizing novel techniques of DC generation, activation, maturation, in-vivo targeting, antigen loading and personalized neo-antigen mapping (Figure 3). In line with this view many cancer immunotherapy platforms are currently testing DC vaccinations in combination with therapies such as ICB, chemotherapy, radiation therapy, IDO inhibitors, etc (Table1). Indeed, the Sipuleucel-T vaccine approach that had initially failed to garner clinical efficacy is now being re-assessed as a combination therapy, and also being tested in earlier stage disease (Table2). Another example favoring the two-arm approach or combination therapy is the promising early results of DC targeting Flt3L combination therapy (with TLR ligands and TAAs-fused with DEC-205) over Flt3L monotherapy (Table3).

With constant influx of new information the above-mentioned parameters will need to be accordingly tweaked and adjusted (see Outstanding Questions) [129–131]. Indeed, a host of novel adjuvants are being rapidly developed and stand to further improve the DC vaccine platform [10]. Moreover, past few years have witnessed technological advancements making it possible for patients to receive treatment best suited to complement his/her immune

system. Furthermore new technologies such as DC differentiation from stem cells and deep sequencing technology and CRISPR/Cas9 genome editing are predicted to change the landscape of personalized immunotherapy.

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Glossary

Alarmins

Alarmins are endogenous ligands secreted by dying, necrotic or stressed tumor cells that can activate immune cells even in the absence of infections. Examples of activating alarmins include HMGB1, HSPs and ATP [1]. Several RNA species are now emerging as novel alarmins. Long non-coding RNA such as HSATII (up regulated in human epithelial cancers such as pancreatic cancer) was shown to act as an alarmin by inducing secretion of IL12 and TNF α in human monocyte derived DCs [132]. Similarly tumor secreted exosomes laden with non-coding Y RNA, hY4, were demonstrated to activate TLR-7 on monocytes and elicit inflammation [133]. Furthermore, pharmacological epigenetic modulators have been shown to enhance expression of “activating” alarmins such as double stranded (ds) RNA encoded by endogenous retroviruses [134, 135].

Antigen Cross-presentation

Antigen Cross-presentation is a unique ability of DCs to internalize exogenous antigens into phagosomes and present them on the MHC-I molecules to activate CD8⁺ T cells [136, 137]. Interestingly, TLR-4 engagement has been shown to induce cross-presentation by facilitating lysosome clustering and delaying cargo degradation in the phagosomes [138, 139]. In addition, early TLR stimulation has also been reported to promote transfer of reserve MHC-I molecules from the endosomal recycling compartment to the phagosomes to support cross-presentation [140].

Cross-Dressing

Cross-dressing is a cellular process through which receiver LN-DCs may directly take up pre-antigen loaded MHC-I complexes from donor migratory DCs and express this complex on their cell surface [141].

Flt3L

Fms-related tyrosine kinase 3 ligand is a cytokine needed for DC mobilization and proliferation.

IDO

Indoleamine 2,3-dioxygenase is an enzyme that induces depletion of tryptophan, a metabolite important for T cell activation.

MoDCs

Monocyte derived dendritic cells are generated by isolating CD14⁺ monocytes from patient blood and differentiating these into immature DCs under the influence of IL4 and GM-CSF.

MSI

Microsatellite instability is manifested as aberrant repetitions in DNA sequences that are introduced when the tumor cells harbor mutations in DNA mismatch repair (MMR) genes.

Sipuleucel-T

Sipuleucel-T is a vaccine comprising of an enriched preparation of white cells containing a significant fraction of antigen presenting cells, including DCs. These are pulsed with prostatic acid phosphatase (PAP) fused with GM-CSF (PA2024) ex-vivo and then re-introduced in the patient intravenously to induce immunity [142, 143].

VEGF

Vascular endothelial growth factor is a cytokine induced in response to stresses such as hypoxia, under the regulation of hypoxia inducible factor and aids in tissue healing and wound repair.

Highlights

- Dendritic cells are key mediators of tumor immunity due to their unique capacity for cross-presenting self-tumor antigens to CD8⁺ T cells.
- Tumor-derived factors actively suppress normal DC function and have a direct effect on the efficacy of DC vaccines.
- Effective anti-cancer vaccines will need to be administered as a combination of two main components; a) inhibitors of TME-induced immunosuppression and b) improved DC vaccines loaded with most immunogenic tumor associated antigens.

Box1**T cell activation by DCs**

Activation of immature DCs is characterized by MHC up-regulation, increased expression of lymph node (LN) homing chemokine receptors such as CCR7, T cell co-stimulatory molecules like CD80 and CD86 and secretion of cytokines like IL12, IL15 and type I IFNs [144]. DCs may take up antigens from malignant cells through multiple mechanisms namely, phagocytosis, pinocytosis and receptor mediated endocytosis and migrate to the draining LNs [145]. Within the LNs, guided by CCR7 and LN stroma, both DCs and naïve T cells make their way into the paracortex region where DCs and T cells are crowded together [146]. The use of advanced imaging techniques has demonstrated that migratory DCs tend to disperse in the peripheral paracortex whereas the LN-resident DCs tend to accumulate in the central paracortex [147]. Complimenting this observation, migratory DCs bearing viral antigens were found to travel to the LN, activate CD4⁺ T cells in the paracortex and recruit active CD4⁺ T cells to promote cross-presentation by XCR1⁺ DCs to CD8⁺ T cells in the deep cortex [148]. DC-mediated T cell priming is a three-step process. In phase I, naïve T cells sample DCs in short bursts. In the phase II, T cells establish and maintain prolonged contact with DCs, initiating activation and generation of memory CD8⁺ T cells [149]. In the phase III, T cells resume transient DC contact and commence proliferation [150]. This entire process may take up to days accounting for the delay in appearance of adaptive immune response. However, a dedicated subset of LN-DCs within the lymphatic sinus epithelium (LS-DCs) is speculated to capture; LN draining antigens, vaccine components or microbial factors and activate T cells rapidly [151].

Box 2**DC cell subsets and classifications**

The CD1c⁺ DCs in humans (equivalent to CD11b⁺ DCs in mice) are migratory cells characterized by high expression of CD11c, CD172α (Sirpα) and TLRs1-8, and are the most common type of DCs outnumbering CD141⁺ DC by several folds in peripheral tissues. CD141⁺ DCs in humans (comparable to CD103⁺CD8⁺ DCs in mice), while present in peripheral tissues, are primarily resident in the lymph nodes (LNs), and are marked by expression of XCR1, Clec9a, CADM1 and TLRs 3 and 8. In addition, monocyte derived inflammatory DCs (iDCs), are characterized by CD1c, CD11c, CD11b, CD172α (Sirpα) and CD206. Recently single cell RNA sequencing on cells derived from healthy donor blood indicated six DCs subsets; DC1 (Clec9A⁺), DC2 (CD1c⁺_A), DC3 (CD1c⁺_B), DC4 (CD1c⁻CD141-CD11c⁺), DC5 (Axl⁺SIGLEC6⁺) and DC6 (pDCs). However, the exact physiological role of these subgroups *in vivo* remains to be determined [2]. The iDCs and CD1c⁺ DCs are exceptional at MHC-II antigen presentation and CD4⁺ T cell activation [4, 152, 153] where as the CD141⁺ DCs, are recognized for producing type I IFNs and cross-presenting antigens to CD8⁺ T cells [2–4].

Box3**Personalized neo-antigen Vaccines**

Carreno et al performed whole exome sequencing on tumors from three patients with Stage III resected cutaneous melanoma to identify somatic mutations and generated HLA-A*02:01 restricted neo-antigenic peptides. Autologous MoDCs loaded with selected neo-antigen peptides were injected into the patients. The results showed evidence of priming and augmentation of CD8⁺T cell response to multiple neo-antigens and provided a proof of concept for the future of personalized neo-antigen based therapies [105]. Following their remarkable success with first three patients, the authors have extended their study to include 17 patients (NCT00683670). Two recent clinical studies have explored the efficacy of vaccinating with predicted personalized HLA matched neo-antigens with favorable results in stage II and IV melanoma patients. One of these studies utilized an RNA based vaccine by engineering the ten highest-ranking neo-antigens into two RNAs and administering intranodally (NCT02035956) where as the second study explored the benefits of vaccinating with long peptides representing twenty neo-antigens along with TLR-3 agonist, PolyIC:LC as an adjuvant (NCT01970358). In both studies (conducted over a period of 12–26 months), the patients displayed an immune response to the vaccine and most patients registered significantly reduced recurrence of melanoma either due to vaccination alone or in combination with PD1 blockade [154, 155].

Open Questions

1. Despite the progress in the field of therapeutic cancer vaccines, there is a pervasive need for preventive cancer vaccines. How to accurately detect and define pre-diagnostic cancer markers and how to target these markers for prophylactic cancer vaccines?
2. Manufacturing personalized neo-antigen vaccines is a time consuming and expensive process. Hence, it is worth considering, if the use of better adjuvants and improved DC targeting may improve the clinical response to shared antigen vaccines thereby making cancer vaccines more accessible.
3. As discussed in the review how to generate and/or target the most beneficial DC subset, ie, XCR1⁺ DCs in humans for DC vaccines is still an open question.
4. Furthermore, it is still unclear whether exogenous DC vaccines perform better than in situ DC vaccines.
5. In view of recent findings indicating that checkpoint molecules prevent effective T cell priming and activation [129, 130], it might be worth combining vaccination with ICB to enhance immune responsiveness [131]. Finally there needs to be a better understanding of which combinations of ARM1 and ARM2 treatments best complement a patients' immune constitution to ensure tumor regression.

Fig 1. DC dysfunction in the tumor microenvironment

The tumor microenvironment is enriched in immune suppressive elements that serve to promote tumor growth by directly or indirectly inhibiting DC functions. These elements include suppressive cytokines, suppressive alarmins, hypoxia, metabolic stress and evasive mechanisms like antigen masking. Cumulatively these elements impair multiple aspects of DC functions and result in absence of adequate Th1 differentiation in the tumor microenvironment.

Fig 2. Variables in DC vaccine platform

A summary of current aspects of DC vaccine pipeline undergoing optimization. **A) Differentiating immature DCs from precursors:** patient derived monocytes and CD34⁺ Hematopoietic stem cells could be used to generate MoDCs or XCR1⁺Clec9a⁺ DCs or CD1d DCs or Langerhan cells, respectively. Or natural/conventional DCs could be directly isolated from patient blood. In future perhaps DC like cell lines could be optimized for generating a universal immature DC line. **B) Maturing DCs:** DCs would be matured using cytokines, TLR ligands, PGE₂, CD40 ligand or a combination of above. Maturation signals can be provided as exogenous stimuli or transfected/transduced into the immature DCs as in the case of Trimix DCs and SMART DCs. **C) Selecting and loading antigens:** Whole tumor lysates or tumor cell fusion with DCs may be used. TAAs or neo-antigens (personalized or shared) may be selected. Antigens are loaded on DCs in form of short or long peptides through traditional DC priming, as DNA through lentiviral transduction or in RNA form through electroporation. Antigens could be introduced with CLRs, mannose receptors, CD40L or CD40 activating antibodies to improve cross-presentation. **D) Combinations:** To maximize DC vaccine clinical efficacy the vaccine should be administered with most suitable adjuvant in combination with, i) CTLA4 or PD1/PDL1

inhibitor, ii) inhibitors of immune suppression, iii) facilitators of DC mobilization like Td toxoid vaccine, GM-CSF or Flt3L, iv) other cell based therapies like CIK, ACT and CAR T cell therapy and v) in combination with standard chemotherapy and radiation therapy.

MoDCs: Monocyte derived DCs; nDCs: natural DCs; PGE₂: prostaglandin E₂; TAAs: Tumor associated antigens; CLR: C type lectin receptors.

Fig 3. Two-arm approach for treating cancer with anti-tumor DC targeted vaccines

A “two-arm” approach enables DC vaccines to overcome tumor-induced immune-suppression and successfully induce anti-tumor immunity. Under “ARM-1” of this approach patients would first undergo tumor de-bulking by undergoing surgery and/or followed by radiation/chemotherapy. Thereafter, patients would be treated with checkpoint blockade inhibitors to reverse T cell exhaustion. At the same time other T cell inhibitory elements like regulatory T cells and myeloid derived suppressor cells (MDSCs) would need to be neutralized by use of drugs such as cyclophosphamide or anti-CD25 antibodies, or agents that modify the TME e.g. IDO inhibitors, adenosine antagonists etc. Under ARM-2, the patient would be pre-conditioned with adjuvants and with agents like Flt3L to mobilize DCs before being vaccinated with autologous DCs loaded with shared or personalized antigens. At this stage other immunotherapies such as adoptive T cell transfer, CAR T cell therapy, insitu tumor vaccination with TLR ligands and Oncolytic virus therapy could also be co-administered to boost the efficacy of DC vaccines.

Table 1

Current clinical trials testing DC vaccines in combination with other therapies for treating cancer

Study Start	Brief Title	Condition	NCT Identifier	Intervention	Phase	Status as of December 2017
2004	Vaccine Therapy With Either Neoadjuvant or Adjuvant Chemotherapy and Adjuvant Radiation Therapy in Treating Women With p53-Overexpressing Stage III Breast Cancer	Breast Cancer	NCT00082641	Drug: Doxorubicin and cyclophosphamide, Drug: Paclitaxel Procedure: Surgery Drug: Radiotherapy Biological: Autologous dendritic cell-adenovirus p53 vaccine	1 and 2	Active, not Recruiting
2004	Vaccination of Patients With Renal Cell Cancer With Dendritic Cell Tumor Fusions and GM-CSF	Renal Cancer	NCT00458536	Drug: GM-CSF Biological: Dendritic Cell Tumor Fusion Vaccine	1 and 2	Active, not Recruiting
2006	Lymphodepletion Plus Adoptive Cell Transfer With or Without Dendritic Cell Immunization in Patients With Metastatic Melanoma	Melanoma	NCT00338377	Drug: Chemotherapy Biological: T-Cells infusion + high dose IL2 + MART-1 loaded DC vaccine.	2	Recruiting
2008	To Immunize Patients With Extensive Stage SCLC Combined With Chemo With or Without All Trans Retinoic Acid	Small Cell Lung Cancer	NCT00617409	Drug: Paclitaxel Biological: Ad.p53-DC vaccines Drug: All -trans Retinoic Acid (ATRA)	2	Ongoing but not Recruiting
2009	Dendritic Cell (DC)-Based Vaccines Loaded With Allogeneic Prostate Cell Lines in Combination With Androgen Ablation in Patients With Prostate Cancer	Prostate Cancer	NCT00970203	Biological: Androgen ablation (AA). Biological: DC1 vaccine (alpha-type-1-polarized dendritic cells loaded with apoptotic allogeneic tumor)	2	Recruiting
2009	Study of Gene Modified Immune Cells in Patients With Advanced Melanoma (F5)	Metastatic Melanoma	NCT00910650	Drug: Chemotherapy Biological: Autologous MART-1 TCR CTLs + MART-1 peptide pulsed dendritic cells	2	Recruiting
2010	Vaccine Therapy and 1-MT in Treating Patients With Metastatic Breast Cancer	Breast Cancer	NCT01042535	Drug: 1-methyl-d-tryptophan (IDO inhibitor) Biological: adenovirus-p53 transduced dendritic cell vaccine	1 and 2	Ongoing but not Recruiting
2010	Blockade of PD-1 in Conjunction With the Dendritic Cell/Myeloma Vaccines Following Stem Cell Transplantation	Multiple Myeloma	NCT01067287	Drug: CT-011 (anti-PD1 antibody) Biological: Dendritic Cell Fusion Vaccine	2	Ongoing but not Recruiting
2012	Gene and Vaccine Therapy in Treating Patients With Advanced Malignancies	Malignant Neoplasm	NCT01697527	Drug: Chemotherapy Biological: IL2+ NYESO-1 reactive TCR retroviral vector transduced autologous PBL B+NY-ESO-1 peptide pulsed dendritic cell	2	Recruiting
2013	Dendritic Cell Vaccines + Dasatinib for Metastatic Melanoma	Metastatic Melanoma	NCT01876212	Drug: Dasatinib (tyrosine kinase inhibitor) Biological: autologous type-1 polarized Dendritic Cell pulsed with HLA-A2-presented tumor blood vessel antigen (TBVA)-derived peptides (DLK1310-318, EphA2883-891, HBB31-39, NRPI433-441, RGS55-13 and TEM1691-700)	2	Recruiting

Study Start	Brief Title	Condition	NCT Identifier	Intervention	Phase	Status as of December 2017
2014	Dendritic Cell-based Immunotherapy for Advanced Solid Tumours of Children and Young Adults	Sarcoma, Central Nervous System Tumor	NCT02496520	Procedure: Surgery Drug: Chemotherapy Radiation therapy Biological: Autologous dendritic cells pulsed with tumor lysate	1 and 2	Recruiting
2014	Treatment of Patients With Progressive and/or Refractory Solid Malignancies	Progressive Solid Malignancies Refractory Solid Malignancies	NCT02224599	Drug: Cyclophosphamide Biological: Tumor associated peptide antigens (TAPA) - pulsed DCs	1 and 2	Recruiting
2014	Phase III Study of DCVAC Added to Standard Chemotherapy for Men With Metastatic Castration Resistant Prostate Cancer (VIABLE)	Metastatic Castrate Resistant Prostate Cancer	NCT02111577	Drug: Docetaxel Drug: Taxotere Biological: Dendritic Cells vaccine-DCVAC	3	Ongoing but not Recruiting
2014	αDC1 Vaccine+ Chemokine Modulatory Regimen (CKM) as Adjuvant Treatment of Peritoneal Surface Malignancies	Peritoneal Surface Malignancies	NCT02151448	Drug: Experimental chemokine modulatory regimen (Interferon Alfa-2b+Celecoxib +Rintatol inmod Biological: Autologous type-1 polarized Dendritic Cell pulsed with tumor antigen	1 and 2	Recruiting
2015	MiHA-loaded PD-L-silenced DC Vaccination After Allogeneic SCT (PSCT19)	Hematological Malignancies	NCT02528682	Biological: PD1/PDL1 silenced and Minor histocompatibility antigens (MiHA)-loaded DC Vaccination	1 and 2	Recruiting
2015	myDC/pDC in Stage III Melanoma Patients	Melanoma	NCT02574377	Combination of peptide loaded myeloid and plasmacytoid DCs	1 and 2	Ongoing but not Recruiting
2016	Dendritic Cell/Myeloma Fusion Vaccine for Multiple Myeloma (BMT CTN 1401)	Multiple Myeloma	NCT02728102	Procedure: Autologous Stem Cell Transplant Biological: DC-Myeloma fusion vaccine with GM-CSF	2	Recruiting
2016	Autologous Dendritic Cell-Vaccination in Mesothelioma (MESODEC)	Malignant Pleural Mesothelioma	NCT02649829	Drug: Chemotherapy Biological: Dendritic cell vaccination	1 and 2	Recruiting
2016	Sequential Intranodal Immunotherapy (SIIT) Combined With Anti-PD1 (Pembrolizumab) in Follicular Lymphoma (Lymvac-2)	Follicular Lymphoma	NCT02677155	Drug: Radiotherapy Biological: Rituximab(anti-CD20 antibody) Biological: Autologous dendritic cells Biological: GM-CSF Biological: Pembrolizumab	2	Recruiting
2016	Adjuvant Dendritic Cellimmunotherapy Plus Temozolomide in Glioblastoma Patients (ADDIT-GLIO)	Glioblastoma Multiforme of Brain	NCT02649582	Drug: Chemotherapy Biological: Dendritic cell vaccine	1 and 2	Recruiting
2017	Avelumab Plus Autologous Dendritic Cell Vaccine in Pre-treated Metastatic Colorectal Cancer Patients (AVEVAC)	Colorectal Carcinoma	NCT03152565	Biologic: Autologous DCs vaccine Biologic: Avelumab (anti-PD-L1 antibody)	1 and 2	Not Recruiting
2017	Autologous Dendritic Cells Pulsed With Tumor Lysate Antigen Vaccine and Nivolumab in Treating Patients With Recurrent Glioblastoma	Glioblastoma	NCT03014804	Biologic: Autologous dendritic cells pulsed with tumor lysate antigen Vaccine Biologic: Nivolumab (anti-PD-1 antibody)	2	Not open for Recruiting

Study Start	Brief Title	Condition	NCT Identifier	Intervention	Phase	Status as of December 2017
2017	Vaccination With Dendritic Cells Pulsed With Autologous Tumor Homogenate in Combination With HD-IL2 and Immunomodulating Radiotherapy in Metastatic RCC (RENALVax-2)	Metastatic Renal cell carcinoma	NCT03226236	Radiation: boost radiotherapy (XRT) Biological: Autologous DC vaccine Drug: High-Dose IL-2	2	Recruiting
2017	Dendritic Cell Therapy After Cryosurgery in Combination With Pembrolizumab in Treating Patients With Stage III-IV Melanoma That Cannot Be Removed by Surgery	Cutaneous Melanoma	NCT03325101	Procedure: Cryosurgery Biological: Pembrolizumab (anti-PD-1 antibody) Biologic: Therapeutic Autologous Dendritic Cells	1 and 2	Recruiting
2017	Dendritic Cell Therapy, Cryosurgery, and Pembrolizumab in Treating Patients With Non-Hodgkin Lymphoma	Non-Hodgkin lymphoma	NCT03035331	Procedure: Cryosurgery Biological: Pembrolizumab (anti-PD-1 antibody) Biologic: Therapeutic Autologous Dendritic Cells Biologic: Pevnar	1 and 2	Recruiting

Source: clinicaltrials.gov. List of current combination trials on clinicaltrials.gov under search terms "Dendritic cells", "Phase 2-3" and "Interventions". Sipuleucel and Flt3L trials are not included in this list and can be found in Table2 and Table3, respectively. Trials that have not been updated in past one year were excluded.

Table 2

Sipuleucel-T combination therapies in clinical trials for Prostate Cancer

Study Start	Brief Title	Condition	NCT Identifier	Intervention	Phase	Status as of December 2017
2012	Phase II Study of Sipuleucel-T and Indoximod for Patients With Refractory Metastatic Prostate Cancer	Metastatic Prostate Cancer	NCT01560923	Sipuleucel-T + Indoximod (IDO pathway inhibitor)	2	Active, not recruiting
2013	A Study of Sipuleucel-T With Administration of Enzalutamide in Men With Metastatic Castrate-Resistant Prostate Cancer	Metastatic Prostate Cancer	NCT01981122	Sipuleucel-T + Enzalutamide (Synthetic non-steroidal antiandrogen)	2	Active, not recruiting
2013	Sipuleucel-T With or Without Radiation Therapy in Treating Patients With Hormone-Resistant Metastatic Prostate Cancer	Adenocarcinoma of the Prostate; Bone Metastases; Hormone-resistant Prostate Cancer; Recurrent Prostate Cancer; Soft Tissue Metastases Stage IV Prostate Cancer	NCT01807065	Sipuleucel-T + External beam radiation therapy	2	Active, not recruiting
2013	Sipuleucel-T and Stereotactic Ablative Body Radiation (SABR) for Metastatic Castrate-resistant Prostate Cancer (mCRPC)	Metastatic castration-resistant Prostate Cancer	NCT01818986	Sipuleucel-T + Stereotactic Ablative Body Radiation	2	Recruiting
2013	Provenge With or Without pTVG-HP DNA Booster Vaccine in Prostate Cancer	Prostate Cancer	NCT01706458	Sipuleucel-T + DNA Vaccine (Plasmid DNA encoding human prostatic acid phosphatase)	2	Active, not recruiting
2013	Radiation Therapy in Treating Patients With Metastatic Hormone-Resistant Prostate Cancer Receiving Sipuleucel-T	Hormone-Resistant Prostate Cancer; Metastatic Malignant Neoplasm in the Bone; Recurrent Prostate Carcinoma; Stage IV Prostate Cancer	NCT01833208	Sipuleucel-T + Radiation Therapy		Recruiting
2014	A Randomized Phase 2 Trial of Combining Sipuleucel-T With Immediate vs. Delayed CTLA-4 Blockade for Prostate Cancer	Prostate Cancer	NCT01804465	SipT Treatment + Ipilimumab (Anti-CTLA-4 antibody)	2	Recruiting
2015	Ph 2 Study of Sipuleucel-T W/ or W/O Radium-223 in Men With Asymptomatic or Minimally Symptomatic Bone-MCRPC	Prostate Cancer	NCT02463799	Sipuleucel-T + Radium-223	2	Recruiting
2015	Men With Metastatic Castrate-Resistant Prostate Cancer Treated With Either Sipuleucel-T (Provenge®), Abiraterone Acetate (Zytiga®) or Enzalutamide (Xtandi®) Undergoing Cardiopulmonary Exercise Testing	Prostate Cancer	NCT02353715	Sipuleucel-T + Enzalutamide (Synthetic non-steroidal antiandrogen) or Abiraterone acetate (Androgen synthesis inhibitor)	1	Recruiting
2017	Clinical Study of Atezolizumab (Anti-PD-L1) and Sipuleucel-T in Patients Who Have Asymptomatic or Minimally Symptomatic Metastatic Castrate Resistant Prostate Cancer	Metastatic Prostate Cancer	NCT03024216	Sipuleucel-T + Atezolizumab (Anti-PDL-1 antibody)	1	Recruiting

Source: clinicaltrials.gov.

Table 3

Flt3L combination therapies in clinical trial for cancer

Study Start	Brief Title	Condition	NCT Identifier	Intervention	Phase	Status as of December 2017	Results
2013	Combined Cytotoxic and Immune-Stimulatory Therapy for Glioma	Malignant Glioma; Glioblastoma Multiforme	NCT01811992	Ad-hCMV-Flt3L + AdhCMV-TK	1	Recruiting	N/A
2013	In Situ Vaccine for Low-Grade Lymphoma: Combination of Intratumoral Flt3L and Poly-ICLC With Low-Dose Radiotherapy	Low-Grade B-cell Lymphoma	NCT01976585	rhFlt3L/CDX-301 + Poly-ICLC	2	Recruiting	N/A
2014	CDX-1401 and Poly-ICLC Vaccine Therapy With or Without CDX-301 in Treating Patients With Stage IIB-IV Melanoma	Resected Melanoma	NCT02129075	rhFlt3L/CDX-301 + DEC-205/NY-ESO-1 + Poly-ICLC	2	Completed	Higher tumor specific immune responses observed in subjects who received FLT3L (unpublished data, personal communications with Dr. Nina Bhardwaj)
2016	FLT3 Ligand Immunotherapy and Stereotactic Radiotherapy for Advanced Non-small Cell Lung Cancer	Non-small Cell Lung Cancer	NCT02839265	rhFlt3L/CDX-301 + Stereotactic Body Radiotherapy	2	Recruiting	N/A

Source: clinicaltrials.gov. Ad-hCMV-Flt3L: Replication defective adenoviral vector expressing soluble Flt3L under transcriptional control of the CMV promoter. Ad-hCMV-TK: replication defective adenoviral vector expressing Herpes Simplex Virus thymidine kinase gene under transcriptional control of the CMV promoter. rhFlt3L: Recombinant human Flt3L.