Dendritic Cell–Based Immunotherapy: State of the Art and Beyond

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Abstract

Dendritic cell (DC) vaccination in cancer patients aims to induce or augment an effective antitumor immune response against tumor antigens and was first explored in a clinical trial in the 1990s. More than two decades later, numerous clinical trials have been performed or are ongoing with a wide variety of DC subsets, culture protocols, and treatment regimens. The safety of DC vaccination and its ability to induce antitumor responses have clearly been established; however, although scattered patients with long-term benefit were reported, DC vaccines have not yet fulfilled their promise, perhaps mainly due to the lack of large-scale well-conducted phase II/III trials. To allow meaningful multicenter phase III trials, the production of DC vaccines should be standardized between centers which is now becoming feasible. To improve the efficacy of DC-based immunotherapy, it could be combined with other treatments. *Clin Cancer Res; 22(8); 1897–906. ©2016 AACR.*

See all articles in this *CCR Focus* section, "Opportunities and Challenges in Cancer Immunotherapy."

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Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the biology of dendritic cells and clinical application of dendritic cell vaccination in cancer, including strategies currently under investigation to improve the efficacy of dendritic cell vaccines.

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Introduction

Dendritic cells (DC) are the most potent professional antigen-presenting cells of the immune system. In an immature state, they act as the sentinels of the immune system, continuously patrolling the environment in search of antigens. After antigen uptake, exogenous antigens are presented as antigenic peptides in MHC class II complexes on the cell surface and endogenous antigens are expressed in MHC class I. DCs have the unique capability to present internalized antigens derived from exogenous sources, not only in MHC class II molecules, but also in MHC class I molecules, so-called cross-presentation. In this way, for example, tumor antigens can be presented to $CD8^+$ T cells. In a mature state, DCs migrate to the lymphoid organs, where they present the antigen to naïve T cells. The activated T cells subsequently proliferate and leave the lymph nodes in search of and to kill cells in an antigen-dependent manner.

DCs comprise a heterogeneous population of cells. In human peripheral blood, two main populations of natural DCs can be distinguished: myeloid DCs (mDC) and plasmacytoid DCs (pDC). Human mDCs can be further subdivided into two populations, based on their differential surface expression of CD1c [BDCA-1, CD141, and BDCA-3, respectively (1)]. The natural DC

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subtypes differ in function, localization, and phenotype (2). Both mDCs and pDCs express distinct Toll-like receptors (TLR) and respond differently to pathogenic stimuli, suggesting that each subset has a specialized function in directing immune responses (3). Because pDCs produce high levels of type I IFNs in response to viral products, they play an important role in the detection and control of viral infections (4). Besides their antigen-presenting function, pDCs also display tumoricidal activity, although to a lesser extent than natural killer (NK) cells (5). mDCs specialize in mediating immunity against fungi and bacteria (2). BDCA3$^+$ mDCs seem to specialize in detection and uptake of necrotic cells and subsequent cross-presentation of derived antigens to T cells (6). Recent observations suggest that both pDCs and mDCs are important for the induction of antitumor responses and may act synergistically (7).

DC Vaccines

Malignant growth, in the beginning, is often a slow and silent process that either fails to elicit a "danger signal" necessary for the activation of the immune system, or tumor cells efficiently silence an initiated immune response to allow tumor growth. The goal of DC vaccines is to mend this inattention of the immune system by providing it with *ex vivo* "trained" DCs, appropriately activated and loaded with tumor antigen, and thus capable of inducing strong antitumor T-cell responses. With the recent introduction of immune checkpoint inhibitors to stimulate the immune response, the role of DC vaccination becomes more important because it induces tumor-specific T cells and directs the immune response toward antigens of interest.

As natural DCs constitute only about 1% of peripheral blood mononuclear cells (PBMC), several ways to generate DCs from precursors have been investigated for DC vaccination purposes. In 1994, the discovery that DCs can be generated from monocytes or CD34$^+$ progenitors, by culturing them in the presence of IL4 and GM-CSF, allowed the procurement of DCs in considerable numbers to facilitate clinical trials (Fig. 1; refs. 8–10).

Although *ex vivo*–generated monocyte-derived DCs share many phenotypic and functional characteristics with natural mDCs, whether DCs differentiated *ex vivo* from precursor cells are the optimal source of DCs for DC-based immunotherapy remains unclear. The extensive culture period (7–9 days) of *ex vivo*–generated DCs and compounds required to differentiate them into DCs might negatively affect DC function, for example, by reducing proinflammatory cytokine production. Therefore, shorter culture protocols have been developed (11). Furthermore, natural DCs do not require extensive culture, and recently, it has been proven feasible to obtain more than 10 million pDCs and even higher numbers of BDCA-1$^+$ mDCs after a single leukapheresis despite their low frequency in blood (12–14). Even BDCA-3$^+$ mDCs, constituting approximately 0.05% of the PBMCs, can now be isolated from a leukapheresis, but this process has not been applied in clinical trials yet.

Recently, the first clinical trials with pDCs and mDCs have proven the safety and feasibility of this mode of treatment (12–14). Although these phase I studies in melanoma patients

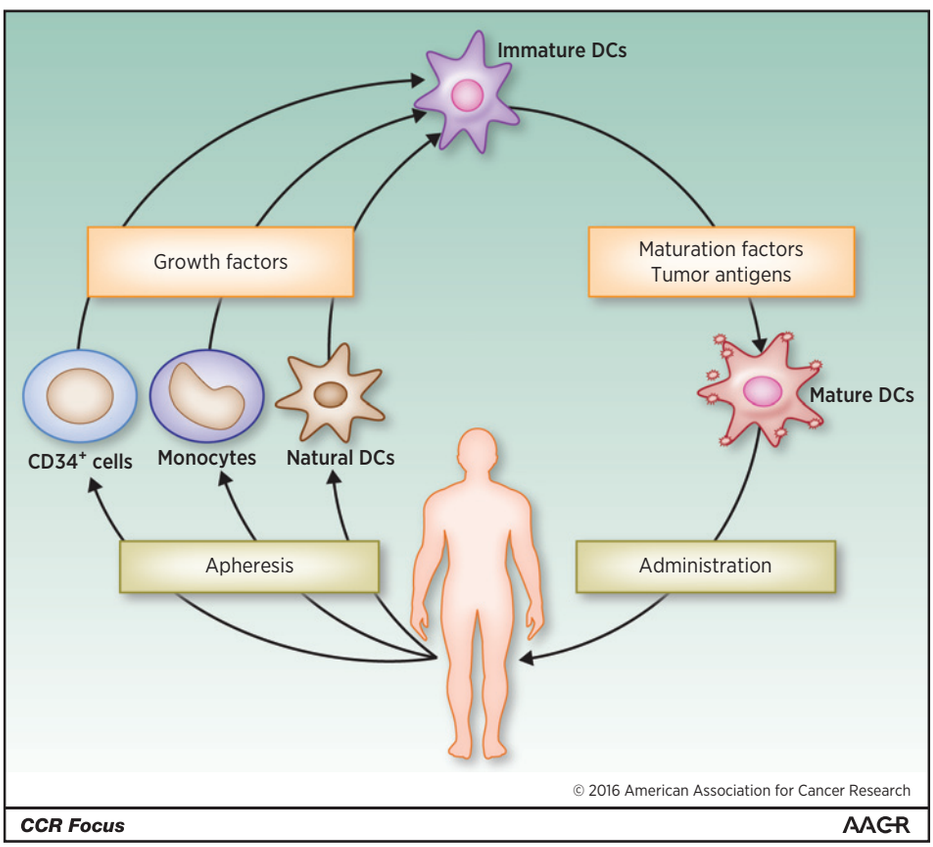

Figure 1.
Ex vivo culture of DCs. Natural DCs or their precursor cells, either CD34$^+$ progenitors or monocytes, can be obtained from the peripheral blood by apheresis. With different culture protocols immature DCs can be obtained and matured. Subsequently, DCs are loaded with tumor-associated antigens and administered to the patient. Refer to Box 1 for growth factors and maturation factors, and Box 2 for tumor antigens.

Box 1. Maturation of DCs

- DC vaccines have been developed using a wide variety of *ex vivo* DC culture conditions. The most widely used method to mature DCs is a cytokine cocktail.
- The optimal DC vaccine should consist of DCs capable of migrating to the lymph nodes, present antigen and costimulation to T cells, and survive long enough for optimal T-cell activation.
- Cytokine cocktails include TNFα, with any of the following cytokines in any combination: IL1β, IL6, prostaglandin E2, or monocyte-conditioned medium (9, 10, 95).
- More recently, TLR ligands that trigger TLRs on DCs, or costimulatory pathways (CD40-CD40L) form a more natural route to induce DC maturation (96).
- TLR ligands can be combined with IFN to obtain type I polarized DCs that produce high levels of IL12 (97).
- Electroporation with mRNA-encoding proteins that modulate DC function has also been explored (98).

Box 2. Methods of antigen loading of DCs (99)

Advantages/disadvantages

- Short peptides: do not require antigen processing/dependent on HLA-type, need for antigen identification
- Long peptides: contain both $CD4^+$ and $CD8^+$ T-cell epitopes, prolonged antigen presentation/need for antigen identification
- Tumor cell lysates: no antigen identification needed/tumor cells required, presentation of self-antigens
- Dendritic/tumor fusion cells: very long antigen presentation/tumor cells required
- RNA transfection: encodes specific antigens, can also encode maturation factors/poor $CD4^+$ T-cell induction
- DNA transfer: high levels of antigen presentation/integration into host genome, immune response against the viral vector
- Neoantigens: patient/tumor-specific antigens/difficult (32)

were primarily aimed at determining potential toxicity, the clinical results were promising, with a relatively larger number of patients surviving more than 2 years after DC vaccination (12, 14). A major advantage of these natural DCs is their rapid isolation procedure with antibody-coated magnetic beads (CliniMACS Prodigy). This method is highly standardized and is being explored for application in multicenter trials.

Safety

The safety of DC-based immunotherapy has been well documented in many phase I and II clinical trials. Side effects seen with the majority of DC vaccination protocols were minimal and self-limiting, mainly including flu-like symptoms, fever, and local reactions at the injection site. Grade 3 or 4 treatment-related toxicity is extremely uncommon when DC vaccination is given as monotherapy (15). These data are confirmed by the available data from phase III trials where DC vaccination is compared with placebo (16–19). Therefore, DC vaccination is considered safe and expected to preserve the quality of life in cancer patients.

Maturation of DCs

In the first clinical studies, immature or semimature monocyte-derived DCs have been used. Later trials demonstrated superiority of mature DCs over their immature counterparts in terms of the immunogenicity and clinical outcome (15, 20–22). Besides their enhanced migratory capacity (23), mature DCs also have a higher expression of MHC and costimulatory molecules. Together, these findings show the superiority of mature DCs in antigen presentation and therefore in inducing T-cell responses (21, 24). Immature DCs are potentially tolerogenic and might even promote antigen-specific tolerance when used in DC vaccines (25). DC maturation is a complex process in which the outcome depends on the type of signals the DCs receive. While these maturation signals primarily come from contact with pathogens or tissue injury *in vivo*, *ex vivo* maturation can be achieved by culturing DCs with a variety of stimuli (Box 1).

Tumor Antigens and Loading of DCs

To induce an immune response in cancer patients, the MHC molecules of a mature DC must be loaded with relevant tumor antigens, proteins that are overexpressed in tumor cells or cancer-testis antigens. Several methods of loading of DC with relevant tumor epitopes have been examined (Box 2). At present, the optimal method for antigen loading remains unknown but would ideally induce both $CD4^+$ and $CD8^+$ T-cell responses, as this is of crucial importance for the induction of a strong and sustained antitumor T-cell response (26, 27). Besides the induction of an immune response against an antigen loaded on the DC vaccine, antigen spreading, i.e., the induction of immune responses against antigens that were not in the vaccine, may occur, indicating that a secondary round of T-cell priming has occurred with antigens taken directly from tumor cells (28, 29).

Currently, one of the most innovative developments is the use of RNA sequencing technologies to determine somatic mutations within the tumor, which may provide neoantigens and could enable patient- and tumor-specific antigens for vaccines (30, 31). Vaccination with neoantigen-loaded DCs has recently been shown to be feasible and promoted neoantigen-specific T-cell responses; it even expanded the diversity of the antitumor response (32). However, the frequency of neoantigens is strongly dependent on tumor type (33, 34), which may serve to recommend the combined use of shared antigens (differentiation antigens, cancer-testis antigens) and neoantigens for DC loading.

Immune Responses upon DC Vaccination and Biomarkers

The primary goal of DC vaccination is to stimulate tumor antigen-specific T cells that can recognize and eliminate tumor cells, which are induced quite potently (30). Different vaccination protocols are immunogenic in terms of T-cell induction but lack

clear identification of a superior route of administration, dose, and vaccination schedule. In cancer patients, imaging DCs with scintigraphy and MRI has shown different migration efficacy of DCs injected intradermally or intranodally (35). After intradermal injection, a consistent but low percentage of DCs migrate to lymph nodes, while with intranodal vaccination higher percentages of DCs reach subsequent lymph nodes when injected correctly. Despite these differences, immune responses were induced via both routes (36, 37). Lymphocyte proliferation was detected after intranodal injection of DCs with [^{18}F]FLT PET scans. FLT uptake correlated with the presence of DCs in lymph nodes and with antigen-specific immune responses *ex vivo* (38).

An abundance of assays are used to measure tumor antigen-specific T-cell responses and their (multi)functionality, including ELISpot assays, tetramer staining on peripheral blood, and delayed-type hypersensitivity skin test biopsies (39). In a phase III trial in prostate cancer, DC vaccination induced antibody and T-cell responses in the majority of patients, and their presence correlated with survival (19, 40). Although correlation of T-cell responses with clinical outcome is shown more often (41–44), measuring T-cell responses remains notoriously difficult and laborious and assays are not validated in prospective clinical trials. Correlation with survival might become more accurate by broadening the immune cells analyzed, for example, with NK cell activation markers or tumor-specific antibodies (45–47). The ideal immunologic test might act as a surrogate endpoint in clinical trials, potentially providing insight on the immunogenicity of adjustments in the DC vaccine.

Besides validated immunomonitoring assays, a biomarker that could predict the response to DC-based immunotherapy, or immunotherapy in general, would move the field forward. The presence or absence of specific immune cells within the tumor microenvironment prior to treatment, either in a metastatic lesion or the primary tumor, is a promising biomarker currently being explored in multiple tumor types (39, 48).

Translation of Immunologic Efficacy into Clinical Benefit

Immune responses measured in patients who received DC vaccines support the concept of using DC-based immunotherapy to treat cancer. The first proof-of-principle studies exploring DC vaccination were performed in the 1990s, a and the first clinical study of a DC vaccine was reported in *Nature Medicine* in 1996 (49). Currently, over 200 clinical studies have been or are being carried out in cancer patients, mostly small exploratory studies with monocyte-derived DCs aimed at optimizing vaccines and measuring immune responses.

Although immune responses are frequently reported using diverse immune monitoring methods and different culture protocols, objective clinical responses remain low, with classic objective tumor response rates rarely exceeding 15%. A recent meta-analysis including >100 phase I–III clinical trials showed objective response rates of 7.1% in prostate cancer, 8.5% in melanoma, 11.5% in renal cell carcinoma, and 15.6% in glioma (50). Interestingly, however, in cases where clinical responses were induced, these were often long lasting.

In theory, immunotherapy, including DC vaccination, should be applicable to all cancer types, although it might be more effective in more immunogenic tumors, as mutational load might correlate with benefit from immunotherapy (51). So far, only specific types of tumors have been studied, mainly due to practical limitations. These include the lack of appropriate tumor antigens or the absence of sufficient tumor material when tumor lysates are used for antigen loading of DCs.

To date, very few phase III trials have been performed with DC-based immunotherapy, perhaps because DC vaccines had not yet reached their full potential but mainly because financial support is hard to obtain as most pharmaceutical companies are not interested in producing laborious patient-specific vaccines. However, shortly after the first report on DC-based therapy was published, a prospective phase III trial was initiated in 2000 that compared standard dacarbazine chemotherapy with a DC vaccine as first-line treatment in patients with melanoma (16). This trial was prematurely stopped due to lack of any sign of efficacy in the treatment arm (objective response rate <6% in both arms). Possible negative contributing factors included a variable quality of the DC vaccine among participating centers and a suboptimal maturation status of the DCs. In retrospect, this trial was carried out too early and was performed at a time when DC vaccination was still fully in development. Furthermore, DC vaccination might come too late for end-stage cancer patients and could be more effective in earlier stages of disease.

More recently, in 2010, a DC-based vaccine used in men with metastatic castration-resistant prostate cancer has been brought to the market by a private company (Sipuleucel-T; Dendreon). This cell-based vaccine consists of autologous PBMCs obtained by leukapheresis, which include DCs activated with a fusion protein of a prostate antigen and GM-CSF. Sipuleucel-T was approved on the basis of results from three placebo-controlled phase III trials. No significant difference in time to biochemical failure (17), or improvement in progression-free survival could be shown; however, median overall survival was prolonged by approximately 4 months compared with the placebo group (18, 19). Despite the discussion about the DC characteristics of this vaccine, as the vaccine consisted of less than 20% antigen-presenting cells of which the majority were CD14$^+$ (40), the impact of this first FDA-approved cancer vaccine has been significant and certainly boosted the field. More phase III trials testing DC vaccination with survival as the primary endpoint are currently ongoing (Table 1).

After melanoma and prostate cancer, DCs are mostly studied in glioma. A large phase III trial is ongoing after numerous smaller trials showed increased progression-free and overall survival in both recurrent and newly diagnosed glioma (50, 52). The phase III trial uses tumor lysate–loaded DCs.

Sipuleucel-T clinical development has taught us some important lessons. A cellular product from cancer patients, cultured for at least 5 days at a production site distinct from the hospital, is a risk. The autologous cells, the starting material for

Table 1. Phase III trials with DCs

ClinicalTrials.gov identifier	Tumor type	Intervention
Recruiting		
NCT00045968	Glioma	DC only
NCT01582672	Renal cell carcinoma	DC + sunitinib
NCT01875653	Melanoma	DC + irradiated autologous tumor cells
NCT01983748	Uveal melanoma	DC only (adjuvant)
NCT0211157	Prostate cancer	DC + chemotherapy
Not yet recruiting		
NCT02503150	Colorectal cancer	DC + chemotherapy
NCT02546102	Glioma	DC + radiochemotherapy

Sipuleucel-T, are collected at the hospital but produced after shipping with the risk that this fresh product does not qualify once it arrives at the production site. Likewise, shipping the living Sipuleucel-T vaccine might result in an unacceptable product due to delayed arrival at the hospital. These risks resulted in high production costs for Sipuleucel-T (>$80,000). Although marketing authorization was received from both the FDA and European Medicines Agency, the product is not readily available for various reasons, including financial problems.

For future success of DC vaccines, besides showing efficacy in phase III trials, a well-established and reproducible product needs to be obtained. Currently, cost–benefit ratios for DC vaccines and other cellular therapies are poor, but recent developments may turn the tide. For example, natural DC vaccine products are isolated in a closed system according to a highly standardized protocol whereby they are cultured for only 48 hours and can be frozen (14). This practice could result in lower production costs of future DC vaccines.

In Vivo Targeting of DCs

Another recent approach to exploit natural DCs for cancer immunotherapy is to target DC subsets *in vivo*, by antibodies with activating agents and antigens. In a way, various vaccination strategies (e.g., with DNA, peptides) can also be characterized as *in vivo* DC loading; however, this will not be discussed here. Early studies have shown that when antigen is bound to antibodies directed against surface receptors of DCs that are implicated in endocytosis, this leads to uptake of antigen. The DCS are then efficiently channeled into endocytic compartments for loading of MHC class I and II molecules and subsequent induction of immune responses (53). However, if these antibody–antigen conjugates are not accompanied by adjuvant to stimulate the immune system, tolerance rather than immunity might occur (53). Therefore, in a phase I trial with an antibody against DEC205, a molecule expressed on DCs, fused with the tumor antigen NY-ESO-1 (Fig. 2), this vaccine was combined with a topical or subcutaneously administered adjuvant consisting of different TLR agonists (54). This combination appeared safe, induced NY-ESO-1–specific immune responses, and led to two objective responses. Likewise, other investigators have developed (nano)particles loaded with both antigen and adjuvant and coated with antibodies to target natural DC subsets (Fig. 2; ref.55). The advantage of this approach is that adjuvants only activate those DCs that are targeted by the antibodies, thereby preventing systemic activation and toxicity, and conversely, that DCs loaded with antigens are also stimulated and matured with adjuvant, so that no immature DCs are loaded with tumor antigens (56). However, the specificity of *in vivo* targeting techniques, the ideal antibody to target, and whether enough DCs will be activated are under investigation. The main advantage of *in vivo* targeting strategies is the development of an off-the-shelf product. Several clinical trials are expected to start in the next few years.

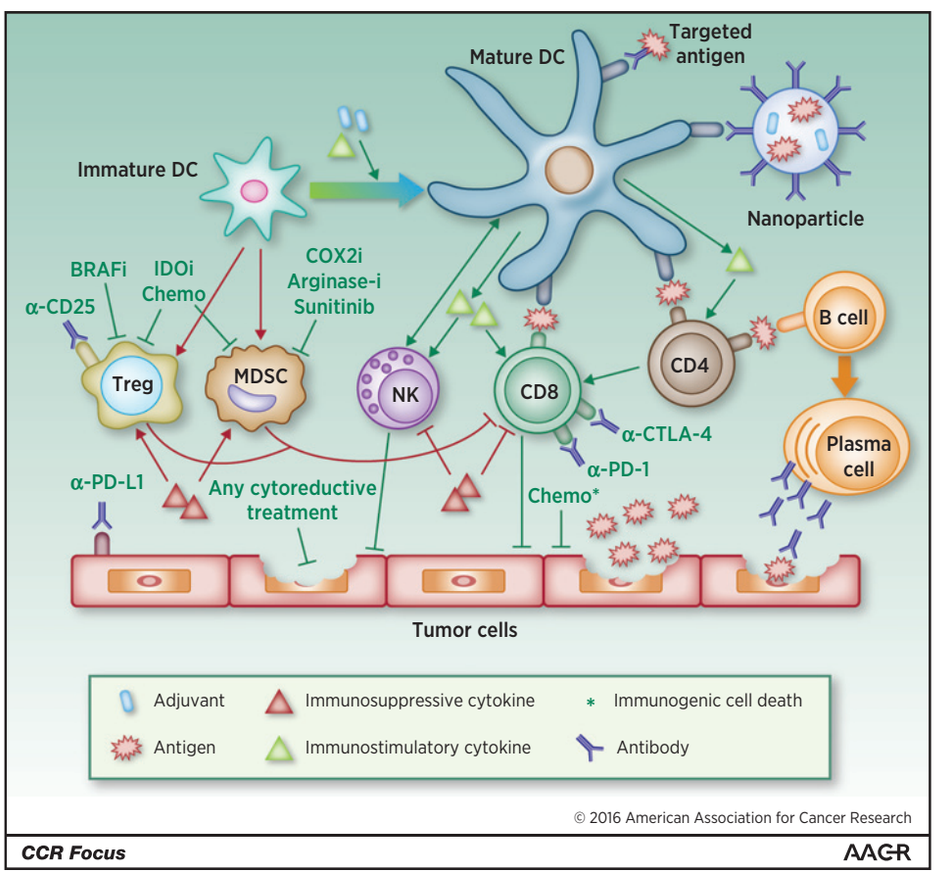

Figure 2.
Interplay between dendritic cells, other immune cells, tumor cells, and therapies. Arrows indicate a stimulatory effect on cell function or a process, and blockers indicate an inhibitory effect on cell function or a process. The arrows/blockers shown in red indicate interactions that favor tumor growth. Arrows/blockers shown in green indicate interactions that favor tumor killing, for example, sunitinib inhibits (blocker) MDSCs and by blocking MDSCs this favors tumor killing (green). To promote clarity, not all known interactions are depicted in this figure. αCD25, anti-CD25 antibody; αCTLA-4, anti-CTLA-4 antibody; αPD-1, anti-PD-1 antibody; αPD-L1, anti-PD-L1 antibody; Arginase-i, arginase inhibitor; BRAFi, BRAF inihibitor; CD4, $CD4^+$ T-helper cell; CD8, cytotoxic $CD8^+$ T cell; Chemo, chemotherapy; IDOi, indoleamine 2,3-dioxygenase inhibitor; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell.

DC Vaccination Early in the Course of Disease

To translate DC-induced immunologic responses into clinical benefit, tumor-specific T cells should be able to exert their function within the tumor. As tumors have evolved various mechanisms to evade immunologic surveillance or to counterattack the immune response to facilitate their own progression (57), it is essential to overcome this immunosuppressive barrier that may hamper the success of DC-based immunotherapy. Tumor-induced immunosuppression is dependent on the amount of tumor, making immunotherapy less effective in patients with a large tumor burden (58). In the clinical setting, we have shown that tumor-specific immunologic response rates obtained after DC vaccination in the adjuvant setting are about two to three times as high as compared with the metastatic setting (59). These findings provide a rationale for the use of DC-based immunotherapy earlier in the course of disease, when tumor burden is still minimal, for example, in the adjuvant setting in patients at high risk of recurrence or in patients with minimal metastatic disease, but so far, most trials have been performed in end-stage cancer patients with high tumor loads.

Boosting DC Vaccines with Adjuvants or Cytokines

Tumor cells produce a variety of cytokines and small molecules to promote tumor progression, mainly by increasing tumor invasiveness and angiogenesis, but also by impairing DC and T-cell function, for example, via TGFβ, VEGF, IL10, and prostaglandin E2. To counteract these immunosuppressive cytokines it is possible to use immunostimulatory cytokines or TLR ligands as DC vaccine adjuvants, either incorporated in the vaccine itself or applied concomitantly.

IL2 is the most extensively studied cytokine in combination with DC vaccination, as IL2 is considered to protect effector $CD8^+$ T cells from tumor-mediated dysfunction or death. Despite strong preclinical evidence (60), clinical trials combining DC vaccination with IL2 have not shown superior immune or clinical responses (61, 62). Also, the combination with IFNα has not lived up to its expectations, with only limited immune responses induced (63). Other cytokines that are under investigation in combination with DC vaccination include GM-CSF, IFNγ, and IL12. Thus far, clinical successes of DC vaccination combined with cytokines or TLR ligands are limited, partly due to the restricted systemic administration because of toxic effects, but occasionally promising results without substantial toxicity are shown (64).

Combinations to Combat Tolerance Induced by Immunosuppressive Cells

DC vaccination might be more efficient in combination with therapies that break the suppressive tumor microenvironment (Fig. 2). Tumor escape mechanisms include the evasion of immune recognition by loss of tumor antigen expression or downregulation of MHC class I expression, the secretion of immunosuppressive cytokines, the expansion and recruitment of regulatory T cells (Treg), and myeloid-derived suppressor cells (MDSC), and the activation of negative regulatory pathways (57). Furthermore, the tumor vasculature itself can form an important barrier for T cells to reach the tumor (65). Potential approaches to counteract the tumor escape mechanisms and to tilt the balance toward more effective DC-based immunotherapy are numerous, but thus far only a few are being investigated in clinical trials.

Breaking peripheral tolerance by depletion of Tregs with anti-CD25 antibodies, thereby targeting the α-chain of the IL2 receptor, has demonstrated an improved immune-mediated tumor rejection in murine models (66). Unfortunately, in humans, anti-CD25 antibodies did not enhance the efficacy of DC vaccination in multiple tumor types, despite efficient depletion of Tregs from the peripheral circulation and increased tumor-specific T-cell frequencies (67, 68). In contrast, Treg-depleting drugs appeared to have paradoxical immunologic effects that could impair the activity of DC vaccination (e.g., depletion of NK cells, induction of tolerogenic DCs, and promotion of nonactivated Treg survival; ref.69).

Along with Tregs, MDSCs, a variety of cells including macrophages and granulocytes, directly suppress $CD8^+$ T-cell responses at the tumor site, hampering the immune response and supporting tumor growth. As with Tregs, it is hypothesized that depletion or inhibition of MDSCs leads to recovery of $CD8^+$ T-cell antitumor activity. To target MDSCs, several interventions are under investigation, including COX-2 inhibitors and arginase inhibitors (70). In addition, inhibitors of the indoleamine 2,3-dioxygenase (IDO) pathway form a novel class of immunomodulators that inactivate Tregs and MDSCs without known substantial immunologic effects that impair an effective antitumor response (71). The combination of an IDO inhibitor and DC vaccination is currently being tested in phase II trials in prostate and breast cancer (NCT01560923; NCT01042535-phase II part), and has been shown to be tolerated, but no objective responses were seen thus far (NCT01042535-phase I part).

Combination with Chemotherapy: An Unexpected Synergistic Effect

The combination of DCs with chemotherapy seems counterintuitive, as chemotherapy is known to have immunosuppressive effects, for example, chemotherapy-induced depletion of leukocytes. However, besides lowering tumor burden, this strategy has several other immune-potentiating effects, for example, depletion of MDSCs and Tregs and increasing tumor cell permeability to $CD8^+$ T-cell–derived cytolytic factors. Furthermore, by depleting immune cells, chemotherapy creates a cytokine milieu for optimal expansion of antitumor effector cells, and is for this reason often combined with adoptive T-cell transfer (72, 73). However, compared with adoptive T-cell therapy, DC vaccination has the advantage that it is able to induce immunologic memory. The combination of DC vaccination and adoptive T-cell transfer is being tested in multiple trials. These pilot trials have shown the feasibility of the approach, although without full conditioning for adoptive T-cell transfer with lymphocyte-depleting chemotherapy and IL2 infusion in most trials (74–76). Besides, a variety of chemotherapeutic agents seem to be able to induce immunogenic cell death, making these cells more susceptible for antitumor immunity elicited by DC vaccination (77, 78). Thus far, no clinical trials have been reported that exclusively tested the combination of chemotherapy and DC vaccination, but numerous trials are ongoing in which the combination is being tested

with or without additional therapies. Chemotherapy and DC vaccination were tested with the addition of a COX-2 inhibitor in melanoma in a phase III trial showing encouraging data, and with the addition of autologous T cells in lung cancer in two randomized trials showing longer overall survival compared with outcomes from chemotherapy alone (79–81).

Combination with Targeted Therapy: More than Tumor Reduction

Monoclonal antibodies and small molecules targeting receptors on the cell surface or intracellular enzymatic proteins of specific pathways involved in tumor growth are numerous and still expanding. Preclinical evidence exists to confirm beneficial immunomodulatory effects for many targeted drugs. For example, vemurafenib, a BRAF inhibitor, inhibits Treg function, and trastuzumab, an anti-Her2 antibody, can restore MHC class I expression on cancer cells (82, 83). Sunitinib, a tyrosinase kinase inhibitor targeting multiple receptors, decreased MDSCs in the tumor microenvironment, downregulated PD-1 expression on DCs, and decreased the secretion of immunosuppressive cytokines in preclinical studies (84, 85). A phase II clinical trial of sunitinib in combination with DC vaccination in advanced renal cell carcinoma patients showed expansion of $CD8^+$ T cells and promising survival data (86). The combination is currently being evaluated in a phase III trial (NCT01582672).

Combination with Immune Checkpoint Inhibitors: The Ideal Combination?

The success of antibodies that counteract the activation of negative regulatory pathways by tumors, thereby recovering T-cell function, has recently been a major breakthrough in cancer immunotherapy. CTLA-4– and PD-1–blocking antibodies have been approved by the FDA, and more immune checkpoint inhibitors are currently being tested in clinical trials, such as, anti–PD-L1 antibodies and IDO inhibitors (87). As treatment with immune checkpoint inhibitors is antigen nonspecific, although it can broaden the antitumor response, the combination with a vaccine could potentially direct the T-cell response in a more specific manner. In theory, this might lead to a higher response rate to immune checkpoint inhibition. Furthermore, the unique capability of DCs to cross-present antigens helps to induce an immune response to a wide variety of tumor antigens when applied in conjunction with immune checkpoint inhibitors (88). The timing of the combination may be of crucial importance for its efficacy. In theory, one should start with DC vaccination to enhance tumor-specific immune responses, and boosting this effect could result in higher numbers of circulating T cells by subsequent immune checkpoint inhibitors. The first anecdotal data that anti–CTLA-4 treatment after DC vaccination may indeed enhance DC vaccine–induced T-cell responses was published in 2005 (89), and there is some evidence that anti–CTLA-4 antibodies might be more effective after DC vaccination (90). In addition, two small trials have shown that DC-based immunotherapy in combination with anti–CTLA-4 antibodies seems to be more effective than the use of these agents alone, showing a best overall response rate of 38% in melanoma patients (91, 92), as is suggested in results from multiple small trials with other forms of antigen-specific immunotherapy (93, 94). Currently, no clinical data on the combination of DC vacciation with anti–PD-1 antibodies are available, but anti–PD-1 antibodies are being investigated in combination with DC vaccination and the first results are expected in 2016 (Table 2).

Table 2. Phase II trials testing anti–PD-1 antibodies in combination with DCs

ClinicalTrials.gov identifier	Tumor type	Intervention
Inclusion completed		
NCT01067287	Myeloma	DC–tumor fusion vaccine
NCT01441765	Renal cell carcinoma	DC–tumor fusion vaccine
Recruiting		
NCT01420965	Prostate cancer	With or without chemotherapy
NCT01096602	Acute myeloid leukemia	DC–tumor fusion vaccine
Not yet recruiting		
NCT02529072	Glioma	Phase I trial

Conclusions

In conclusion, DC vaccination has proven to be feasible and safe in multiple clinical trials. The DC-based vaccine Sipuleucel-T was tested in phase III trials in which it showed proof of concept and induced a survival benefit. Significant advances over the past two decades in the field of DC-based immunotherapy have been made and DC vaccines are continuously being optimized. However, further progress in the field is needed to improve clinical outcomes and to exploit the full potential of DC-based immunotherapy. We believe that DC vaccination earlier in the course of disease is acceptable because of its low toxicity profile and is more beneficial as it more frequently induces tumor-specific immune responses, most likely due to less tumor-induced immunosuppression. Therefore, DC-based immunotherapy might be most suitable in the adjuvant setting. Furthermore, two recent developments that might improve DC-based immunotherapy are (i) the use of neoantigens to load DCs to induce stronger immune response and (ii) the rapid and highly standardized, automated production of DC vaccines consisting of natural DC subsets which can improve the quality of the DC vaccines and most importantly enable multicenter trials.

Still, DC-based immunotherapy may have limitations as a monotherapy because of the immunosuppressive mechanism active in the tumor microenvironment. Combination therapy could play an important role in initiating DC vaccination, boosting antigen-specific antitumor immunity by subsequent treatment with immunomodulating agents. While the potential impact of such regimens is recognized, an optimal combination treatment is yet to be established.

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