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Dendritic cells in cancer immunotherapy: vaccines and combination immunotherapies

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Abstract

Dendritic cells (DCs) are specialized immunostimulatory cells involved in the induction and regulation of immune responses. The feasibility of large-scale *ex vivo* generation of DCs from patients' monocytes allows for therapeutic application of *ex vivo*-cultured DCs to bypass the dysfunction of endogenous DCs, restore immune surveillance, induce cancer regression or stabilization or delay or prevent its recurrence. While the most common paradigm of the therapeutic application of DCs reflects their use as cancer 'vaccines', additional and potentially more effective possibilities include the use of patients' autologous DCs as parts of more comprehensive therapies involving *in vivo* or *ex vivo* induction of tumor-reactive T cells and the measures to counteract systemic and local immunosuppression in tumor-bearing hosts. *Ex vivo*-cultured DCs can be instructed to acquire distinct functions relevant for the induction of effective cancer immunity (DC polarization), such as the induction of different effector functions or different homing properties of tumor-specific T cells (delivery of 'signal 3' and 'signal 4'). These considerations highlight the importance of the application of optimized conditions for the *ex vivo* culture of DCs and the potential combination of DC therapies with additional immune interventions to facilitate the entry of DC-induced T cells to tumor tissues and their local antitumor functions.

Keywords

cancer; chemokines; chemokine receptors; cytokines; cytotoxic T lymphocyte; dendritic cells; immunotherapy; NK; vaccines

Dendritic cells & cancer immunotherapy

Many advances have been made in cancer therapy and prevention, which have led to a recent decrease in the rate of cancer-related deaths [1,2]. However, despite these improvements,

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cancer remains a leading cause of mortality in the developed world. For many cancer patients, especially with advanced disease where surgery is not an option, the available treatments have limited impact on survival. While many standard therapies are effective at eliminating tumor bulk, they are not curative as they lack the capacity to remove residual cancer cells. It is this deficit in particular that provides the rationale for the inclusion of immunotherapy strategies with standard treatment, so the antitumor functions of the immune system can be utilized to eliminate the rare ‘non-self’ tumor cells.

In the past century, a considerable effort has been made to develop an understanding of the basic paradigms of how the immune system recognizes and eliminates tumor cells and how to manipulate these functions for clinical use for cancer immunotherapies. Over the past 2 years, several novel immunotherapies have been approved by the US FDA, highlighting the advances that have been made and the different strategies for immunotherapy [3]. These include sipuleucel-T (Provenge®; Dendreon, WA, USA) — the first cellular therapy product composed of antigen-loaded dendritic cells (DCs) and T cells for the treatment of advanced prostate cancer [3–6].

Immunotherapy can be separated into two classes: nonspecific or antigen specific. Nonspecific immunotherapies target multiple cell types (such as the stroma, tumor cell or immune-cell subsets) and consist of cytokines (such as pegylated IFN- α , Sylatron® [Merck, NJ, USA]), adjuvants or checkpoint blockade (such as ipilimumab, Yervoy® [Bristol-Myers Squibb, NJ, USA]). Antigen-specific immunotherapies are therapeutic vaccines that attempt to instruct tumor-specific immune cells to kill cancer cells (such as sipuleucel-T) [7–9]. The selective targeting of a few cells for activation during therapeutic vaccination provides strong and unique advantages over less specific therapies in that the toxicity is low, but the effects are prolonged by the development of long-lived tumor-specific memory cells that provide immunosurveillance.

Since Steinman *et al*s discovery of DCs [10–13], these specialized antigen-presenting cells have been shown to play a pivotal role in the induction of immune responses by supporting the survival and effector functions of primed T cells, while also promoting crosstalk between other components of the immune system [14,15]. Many vaccines involving antigenic peptides, proteins or genetically modified tumor cells or viruses depend on antigen cross-presentation by the patients’ endogenous DCs, which show different levels of dysfunction in cancer-bearing hosts, triggered by immunosuppressive factors present in the tumor microenvironment, which can reduce their activity [16–18] or even redirect DC differentiation towards myeloid-derived suppressor cells (MDSCs) [19,20]. Therefore, much effort has focused on developing techniques for the *ex vivo* generation of DCs that can be manipulated to be superior inducers of immune responses. Human DCs can be generated in large quantities from blood or bone-marrow progenitors, which make these *ex vivo* techniques feasible for clinical applications [21–23]. In addition to developing outside of the tumor-associated suppressive conditions like endogenous DCs, *ex vivo*-generated DCs can be matured to acquire resistance to these suppressive factors [24–26].

Vaccination in the therapeutic setting

Protective and therapeutic vaccines share a common goal: to induce a high number of antigen-specific T cells. However, the obstacles to achieve this goal during the priming and recall response phases are dramatically different. In the protective setting, the vaccine is administered prior to disease, so the initial priming occurs in a naive environment. During the recall response, the tissue-invading microorganism induces proinflammatory signals in the infected tissue and activates innate immune cells, which promote the development of effector functions in the vaccination-induced memory cells. This is different in the case of therapeutic vaccines where the vaccine is administered during an ongoing, though dysfunctional, immune response which can limit vaccine effectiveness [18] or eliminate the vaccine-carrying antigen-presenting cells [27,28]. In addition, the proinflammatory signals that are present during bacterial or viral infections are absent in cancer patients, typically replaced by tumor-induced immunosuppressive/anti-inflammatory signals. Thus, therapeutic vaccines not only have to provide the antigenic stimulus to induce the specific T-cell response, but also the inflammatory signal to drive effector cell functions [18,29–31].

When microorganisms invade tissues, they induce chemokines that recruit effector cells (such as cytotoxic T cells, Th1 cells or NK cells) to the site of pathogen entry [30,32]. However, while tumors do express chemokines that can support their growth, metastatic spread and survival, they tend to downregulate the effector cell-attracting chemokines [33,34] and enhance the production of the chemokines that attract suppressor cells, such as Tregs [35–37], suppressive plasmacytoid DCs [38] and MDSCs [39,40]. Therefore, the therapeutic vaccine either needs to be capable of inducing T cells that are responsive to the spontaneously expressed tumor-associated chemokines or be administered in conjunction with additional factors to modulate the chemokine profile of the tumor microenvironment [37,39].

Once the vaccine-induced tumor-specific T cells arrive at the tumor site, they are faced with an additional set of challenges that are not a concern in the protective vaccine setting. Most types of cancer (including melanoma, ovarian, breast, renal, prostate, lung, head and neck cancer) produce many factors that contribute to immune dysfunction, such as IL-10, TGF- β , VEGF, IL-6 and COX2 products (such as PGE₂), by suppressing the functions of endogenous or adoptively transferred DCs and T cells [17,41–43]. In addition to directly suppressing DC and T-cell functions, these factors can enhance the recruitment, expansion and activation of Tregs and MDSCs [35,36,44,45]. Notably, some of the DC vaccine strategies that have been used can further expand Tregs [44,46], the phenomenon likely to be promoted by any effective vaccine capable of inducing high-IL-2-producing effector cells [47], but may be differentially regulated by the conditions of DC maturation (see below) [48].

Unlike traditional protective vaccines, which typically use mutated or killed pathogens with adjuvants designed to elicit a proinflammatory environment where the endogenous antigen-presenting cells take up the pathogen and become activated *in vivo*, DC-based therapeutic vaccines are administering a live cell that must induce the immune response, based on the activation signals that they received *ex vivo*, prior to their injection. While this allows for the

desirable manipulation of DCs during their *ex vivo* generation to elicit the appropriate form of immune response against tumor antigens, the optimal application of this approach involves unique challenges associated with the unique biology of DCs and their limited lifespan.

Building a DC vaccine

During the *ex vivo* generation of DCs for therapeutic vaccines, there are many stages where the nature of the vaccine-induced immune response can be influenced (Figure 1). *Ex vivo* antigen loading and maturation of the DCs assure the efficient delivery of ‘signal 1’ and ‘signal 2’ (antigen and costimulation, respectively) to tumor-specific T cells [7,41,49]. The maturation process instructs the DCs to induce the desirable type-1 effector mechanisms required for effective cancer immunity (‘signal 3’) and to preferentially interact with specific immune-cell subsets (such as Th1, CD8 and NK cells rather than regulatory cells) [15,29,30]. Last, the DCs can be instructed to induce desirable homing properties to the activated T cells (‘signal 4’) [30,50,51].

Delivery of antigen (signal 1)

One of the important characteristics of DCs in their role as professional antigen-presenting cells is their ability to take up, process and cross-present proteins from their environment [41]. Because of this cross-presentation ability, the source of antigen for loading onto DC vaccines is not limited to peptides [52–54]. Rather, recombinant proteins, tumor lysates or even whole tumor cells from autologous or allogeneic sources can be used, reducing the reliance on known tumor antigens and increasing the ability of an immune response to be made against undefined, patient-specific antigens. However, the ability to uptake and process antigen differs in the various DC subsets and is controlled by the developmental stage of the DC, as well as the conditions in which the DC is activated and matured [52,54]. While the optimal window for uptake and cross-presentation is early in the maturation process [52,54], the effectiveness of this process can be affected by the factors that are used to induce maturation, as shown by the studies demonstrating the opposite effects of PGE₂ versus interferons upon the expression of TAP transporters and other molecules involved in antigen processing and loading, resulting in different activation of MHC class I-restricted cytotoxic T lymphocytes (CTLs) [55].

DC maturation stage: expression of costimulatory molecules (signal 2) & lymph node homing ability

When DCs mature, the ability to uptake and process antigens decreases, while molecules that costimulate T cells (such as the B7 family members CD80 and CD86) are upregulated [56]. In addition, the DC gains the ability to respond to the lymph nodehoming chemokines CCL19 and CCL21 by upregulating CCR7 [57,58]. The first generation of DC vaccines used immature or partially mature DCs that maintained the ability to cross-present tumor antigens, but lacked the costimulatory ability or lymph node homing capacity (‘signal 2’), which prompted the development of new protocols to fully mature the DCs for clinical use (the second-generation vaccines) (Figure 2). Two of these protocols, involving either the use of monocyte-conditioned medium [59] or a cytokine cocktail including IL-1 β , TNF- α , IL-6

and PGE, [60], were shown to induce high expression of costimulatory molecules and CCR7, which mediated high migratory capacity to CCL19 and CCL21 [61,62]. Based on their enhanced immunogenicity *in vitro* and *in vivo* in healthy volunteers, numerous clinical trials were started [57,63]. However, the promise of these vaccines diminished after a randomized comparison with dacarbazine in a multicenter Phase III trial for advanced melanoma [64]. Less than 5% of the patients receiving the vaccine experienced clinical response and there was no detectable influence on patient survival [64]. Several factors could have had a negative impact on the trial results, such as variations in the quality of the DCs generated by the various facilities in the trial. Also, both protocols used for the maturation of second-generation DC vaccines contained PGE₂. PGE₂ is known to negatively regulate the ability of DC vaccines to produce IL-12p70 [15,65], a proinflammatory cytokine shown to be associated with the effectiveness of DC vaccines *in vitro* [66,67] and *in vivo* [68]. Thus, while second-generation or 'PGE₂-matured DCs' have high costimulatory molecule expression and lymph node-homing properties, these DCs lack the factors necessary to induce antitumor effector functions in T and NK cells (see the discussion of signal 3' below).

Inducing efficient antitumor effector functions (signal 3)

During typical pathogen infection, various DC-activating signals are provided by infected cells, innate immune cells and by the pathogen itself. These signals determine the cytokine profile that is produced by the mature DC when it interacts with naive T cells and induces the appropriate effector functions (signal 3) [15]. A prototypical example of signal 3 is IL-12p70, an inducer of type-1 immunity which is upregulated in response to IFN- γ , a cytokine produced by activated NK cells at the site of infection. The second-generation DC vaccines lacked IL-12p70 expression and thus, while eliciting expansion of tumor-specific T cells, these DCs do not induce the desirable effector mechanisms in T and NK cells [51,69,70]. Many studies in mice have shown that the character (Th1 dominance and avoidance of Treg activation) [71], rather than the overall magnitude of the response, is more predictive of the therapeutic activity of the vaccine to promote tumor rejection [72–75]. Human preclinical and clinical studies further support the importance of IL-12p70 production by antigen-loaded DCs as a predictive indicator of the ability to induce tumor-specific CTLs *in vitro* and translate to clinical benefit *in vivo* [66–68].

The decreased IL-12-producing function and disappointing clinical activity of PGE₂-matured DCs (no different than the control arm of dacarbazine-treated patients) [64] prompted the efforts of many groups, including ourselves, to develop protocols to induce 'nonexhausted' DCs, or at least DCs that could transiently produce high levels of IL-12p70. To this end, DCs were matured in conditions that mimic viral infection, either by coculture with the infection-responding immune cells, such as IL-18-activated NK cells or memory-type CD8⁺ T cells [28,76–80], or a combination of type-1 and type-2 interferons with Toll-like receptor (TLR) ligands [26,70,78,79,81–87]. Maturation in these conditions induces 'type-1-polarized' DCs (DC1) that have superior capacity for producing IL-12p70 upon interaction with CD40L-expressing CD4⁺ Th cells within 24–48 h after removal from the maturation cultures [26,78]. This elevated level of IL-12p70 translates into an increased ability to induce long-lived tumor-specific effector cells in both human *in vitro* [78,79,81–

85] and mouse *in vivo* studies [86,87], and to induce NK cells with enhanced tumoricidal functions [69]. Such ‘non-exhausted’ or type-1-polarized DCs can be induced by the combination of TNF- α and IL-1 β with different TLR3, TLR4 or TLR9 ligands (such as poly-I:C, lipopolysaccharide, monophosphoryl lipid A or CpGs) and IFN- γ preferentially used by different groups [26,78,79,81–85]. Addition of IFN- α to the maturation-inducing cocktail (such as used in α -type 1-polarized DCs [α DC1]), further enhances the expression of the lymph node-homing receptor CCR7 [78], and the production of chemokines that preferentially drive DC interaction with naive, memory and effector T cells and NK cells, while limiting the production of CCL22 and the resulting interaction of the DCs with undesirable Tregs [48,69,88].

Importantly, for the possibility to treat patients with uncommon MHC types or with cancers without identified tumor rejection antigens, α DC1s express high levels of multiple components of the antigen-processing machinery and effectively take up and process autologous or allogeneic apoptotic tumor cells [55], conferring α DC1s the ability to cross-present the tumor antigens in the context of MHC class I and effectively induce MHC class I-restricted CTL responses [68,78,81,82]. Moreover, it has also been shown that α DC1s are particularly effective in enhancing antitumor activities of NK cells [69]. In further support of the feasibility of clinical application, α DC1s (and several other types of type-1-polarized DCs) can be generated from patients with all forms of advanced cancer tested so far, including melanoma [48], prostate [82], chronic lymphocytic leukemia [81], gliomas [68], colorectal, breast, ovarian and endometrial cancers and multiple hematologic malignancies [Wieckowski E, Urban J, Kalinski P *et al.*, Unpublished Data].

Both the second-generation (PGE₂-matured) DCs as well as polarized α DC1s are similarly capable of inducing efficient expansion of naive CD8⁺ T cells and the conversion from CD45RO⁺ (naive) to CD45RO⁺ (effector) cells [51]. However, when the high IL-12p70-producing α DC1s are used for the priming of CD8⁺ T cells, they have a superior ability compared with PGE₂-matured DCs in inducing T-cell expression of granzyme B and perforin, as well as an increase in cytolytic effector functions against tumor targets [51]. This superior induction of effector functions in α DC1-primed CD8⁺ T cells can be observed both in polyclonally activated naive T cells and in recall responses to tumor-specific antigens such as MART-1.

Taken together, the data accumulated from these preclinical studies suggest that the ability of DCs to induce effective antitumor immunity can be modulated by the factors regulating their level of IL-12p70 production and potentially other Th1-, CTL- and NK-cell activating cytokines. Currently, the authors are evaluating the use of α DC1s in Phase I/II trials in patients with chronic lymphocytic leukemia, glioma, colon and prostate cancers or melanoma. In the recently completed Phase I/II trial in patients with recurrent high-grade malignant glioma (NCT00766753 [202]), where progression-free survival (PFS) is typically 2–4 months, the use of α DC1 vaccines combined with poly-ICLC prolonged the PFS to at least 12 months in nine of 22 patients [68]. Four patients whose disease initially stabilized proceeded to demonstrate delayed-onset objective clinical responses (two complete

²⁰²ClinicalTrials.gov. <http://clinicaltrials.gov>

responses, two partial responses), three of which were observed after the publication of the initial report [68,89]. Importantly, the best predictive marker for the prolonged PFS was the ability of the patient's individual α DC1 vaccine to produce IL-12p70, rather than the numbers of circulating tumor-specific T cells induced by the vaccines [68], consistent with the possibility that it is the IL-12-dependent ability of DCs to induce the effector functions and tumor-homing potential of tumor-specific T cells (rather than their overall stimulatory potential, which is mostly regulated by the expression of costimulatory molecules), that determines their ability to mediate the antitumor effects [51]. A recent study in neoadjuvant settings by Sharma *et al.* from the University of Pennsylvania (PA, USA) [90] have demonstrated the ability of peptide-loaded alternatively polarized DCs (TLR4 ligand combined with IFN- γ) to induce systemic and local (intratumoral) immunity to Her-2 in patients with ductal carcinoma *in situ*. Interestingly, these immunologic changes were associated with the elimination of malignant cells in a significant proportion of subsequently resected patients and with long-term lack of disease recurrence [90,91].

Inducing tumor-homing properties (signal 4)

When naive T cells become activated, there is a concomitant change in the expression profile of chemokine receptors so that the lymph node-homing receptors important for maintaining naive T cells in the lymph node are downregulated, while the peripheral homing receptors are upregulated. Recent work in mouse models has demonstrated that different subsets of DCs, isolated from various tissues, affect the peripheral homing chemokine expression profile on the T cells they activate, thus directing the T cells to distinct tissues [92–95]. The authors have demonstrated that differential maturation of human *ex vivo* generated DCs also affect the pattern of chemokine expression on the activated cells [51]. Specifically, tumor-specific CD8⁺ T cells from HLA-A2⁺ melanoma patients sensitized *ex vivo* with α DC1s (but not by PGE₂-matured DCs) showed strongly elevated expression of CCR5 (the receptor for CCL2 and CCL5) and CXCR3 (the receptor for CXCL9, CXCL10 and CXCL11) [51], two peripheral homing chemokine receptors involved in T-cell entry into melanoma and other tumors [96,97].

Promoting the interaction of DCs with desirable immune cells

Several recent trials using the PGE₂-matured DC vaccines have demonstrated an increase in the expansion of undesirable Treg cells in the cancer patients [44,46,96–98], prompting investigation into how the pattern of DC interaction with Tregs can be altered to preferentially allow the DC to interact with naive, effector and central memory cells, while avoiding Treg activation. The authors have recently demonstrated that the factors inducing DC maturation also program the DC for which chemokines will be expressed. By replacing PGE₂ with IFN- α in the DC-maturation inducing cocktail, the expression of CXCL9, CXCL10, CXCL11 and CCL5 (effector-attracting chemokines) are enhanced, while the expression of CCL22 (Treg-attracting chemokine) is diminished, thus promoting the DC interactions with the more desirable CXCR3- and CCR5-expressing immune cell subsets (CTLs, Th1 and NK cells) and reducing the interaction with Tregs and other suppressor cells that express CCR4 (receptor for CCL22) [48,69,88].

Limits of the ‘vaccine’ paradigm: combinatorial immunotherapy involving DCs

Traditional consideration of DC-based immunotherapy as ‘therapeutic vaccination’ led to the first successful commercialization of DC-related therapies in sipuleucel-T, as a life-prolonging cellular product for patients with hormone-refractory prostate cancer [99,201]. While each batch of Provenge is made specifically for each patient, representing an ultimate personalized medicine approach, each cellular product is prepared in a centralized cell production facility. Since many aspects of the production and administration of DC vaccines more closely resemble the process involved in bone marrow or pancreatic islets transplantation or blood transfusion [89], it remains to be determined if the wide application of DC therapies can be best advanced by their production and distribution as a vaccine product or, perhaps, as a medical procedure performed in individual, specialized cancer centers.

Alternative approaches such as those involving the *in vivo* targeting of antigen to different subsets of DCs [18] or inducing DCs with desirable features within the patients’ bodies would eliminate the need for cell manipulation *ex vivo*. Since the levels of immune deficit and dysfunction of endogenous DCs are likely to be different in patients with different tumor types and with different tumor burden, all of these approaches are likely to be applied in parallel to different groups of patients in order to maximize the clinical benefit and the feasibility of their applications.

Independent of the mode of induction of DCs, their therapeutic potential is likely to benefit from their concomitant application with additional interventions aimed at promotion of the effective homing of the vaccination-induced T cells into tumors and supporting their local antitumor functions and prolonging the duration of the effector phase of immunity [100].

The desirable effector cells (CTLs, Th1 and NK cells), including the effector induced by DC vaccines, all express typical type-1 chemokine receptors such as CCR5 and CXCR3. One of the challenges for the *in vivo* effectiveness of such spontaneously arising or treatment-induced effector cells is the insufficient expression of the effector cell-recruiting chemokines in tumor tissues, often associated with local overexpression of Treg-attracting chemokines [33,34,37] and the chemokines preferentially attracting other undesirable types of immune cells such as CXCL12/stroma-derived factor-1 known to attract suppressive plasmacytoid DCs and MDSCs [38–40]. Furthermore, different tumors, even within the same patient, can express different patterns of effector cell-recruiting chemokines [37]. These observations suggest that the antitumor effectiveness of the CCR5- and CXCR3-expressing CTLs (occurring spontaneously or induced by αDC1s or other effector T-cell-inducing vaccines) may be enhanced by the increasing production of the relevant chemokines in tumor lesions. In an attempt to achieve this goal, the authors have recently demonstrated that exposure of the tumor lesions with type 1 interferons, TLR ligands and COX2 inhibitors (to reduce the level of PGE₂) allows for the selective induction of effector-attracting chemokines and

²⁰¹Corbett Dooren J. Novel Cancer Treatment Cleared: Dendreon’s ‘Therapeutic Vaccine’ Prompts Body to Attack Prostate Tumors; High Cost Is a Hurdle. 29 April 2010. <http://online.wsj.com/article/SB10001424052748704302304575214302390884556.html?K>

downregulation of Treg-attracting chemokines [37]. Interestingly, this effect was observed selectively or at least preferentially within tumor lesions (rather than marginal tissues), as a result of the tumor-associated dysregulation of the NF- κ B system, essential for chemokine induction [37]. The additional beneficial effect of COX blockade is the suppression of CXCL12/stromal cell-derived factor-1 production in the tumor microenvironment and downregulation of its receptor, CXCR4, on MDSCs [39].

An additional area of opportunity is the combination of DC therapies with the strategies to counteract tumor-associated immune suppression and prolong the antitumor activity of DC-induced effector T cells [100,101]. The mechanisms relevant in this regard involve the inhibition of soluble mediators of tumor-associated immune suppression such as VEGF [102], TGF- β [103], IL-10 [104], PGE₂ [19,20,65], nitric oxide synthase [105] or indoleamine 2,3-dioxygenase [106–108], and checkpoint blockade (blockade of the suppressive interactions at later stages of immune responses mediated by the interactions of CTLA4 with B7.1/2 and the interactions between PD1 with PD-L1 [B7-H1] and PD-L2 [B7-DC] [109,110]) that have either been FDA approved for use as individual therapies (CTLA4 blockade) [111,112] or proven effective in Phase II clinical trials (blockade of PD1 or PDL-1 [111,113–115]). While the clinical benefit of combining CTLA4 blockade with peptide vaccination could not be demonstrated [112], the effectiveness of combining this and similar antippressive approaches with DC therapies (believed to be stronger T-cell activators than peptide vaccines) has been suggested by preclinical mouse studies and early stage clinical trials [116–121].

Finally, in addition to the current prevalent use of DCs as *in vivo* inducers of tumor-specific CTLs (cancer vaccines), antigen-loaded DCs can also be used as *ex vivo* inducers of tumor-specific T cells for adoptive immunotherapy, which have traditionally involved expanded tumor-isolated T cells or genetically manipulated T cells from peripheral blood [122–130], in order to alleviate the logistical issues associated with genetic manipulation of immune cells or the need to obtain high numbers of sterile tumor-infiltrating lymphocytes from different forms of cancer. Also, in such forms of DC immunotherapy, its combination with systemic and local treatments aimed at enhanced T-cell homing to tumor tissues and enhanced/prolonged killer function in cancer microenvironments are likely to result in therapeutic synergy.

Expert commentary

The development of effective modes of cancer immunotherapy involving DCs has been delayed both by the complexity of DC functions within the immune system and by logistical challenges of preparation of autologous cellular products. In addition, similar to other modes of immune therapies, which, in contrast to chemotherapy, target the tumor cells indirectly (by modulating the pattern of molecular and cellular interactions within the immune system), the progress of this form of treatment is likely to be accelerated by recent identification of the specialized ways of evaluating the response to immunotherapies, reflecting the existence of the lag period between the onset of treatment and the clinical benefit [3,7,131]. Recent developments in the area of DC biology and the biology of tumor

microenvironment are likely to result in a strong improvement of the effectiveness of cell-based therapies involving DCs.

Five-year view

Taking into account recent activity in the field of DC-based cancer immunotherapy, it is reasonable to expect that the next 5 years will result in regulatory approval for additional DC products. Recent FDA approval of ipilimumab and positive results of clinical trials of PD1/PD-L1 blockade are likely to facilitate the evaluation of combination therapies involving these factors. Additional interesting combinations include interferons, TLR ligands and the blockers of soluble mediators of cancer-associated immunosuppression, including the inhibitors of indoleamine 2,3-dioxygenase, arginase, VEGF and COX2 (and selective blockers of PGE₂ receptors, EP2 and EP4).

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Key issues

- The ability of dendritic cells (DCs) to interact with and activate different types of immune cells depends on the mode of production, preservation, distribution and delivery of *ex vivo*-generated DCs.
- Modulation of the conditions of DC generation can be used to enhance the ability of DCs to induce the desirable forms of immunity (effector and memory responses), but to reduce their ability to amplify the pre-existing immunosuppression.
- Combination of DC therapies with the modulation of tumor microenvironment can be used to promote the entry of DC-induced effector cells (cytotoxic T lymphocytes, Th1 and NK cells) into tumors and to limit the local attraction and function of suppressive cells (Tregs and myeloid-derived suppressor cells).
- Progress in the area of DC and other immune therapies can be accelerated by the identification of the most relevant end points of efficacy of immune therapies, as well as the identification of the most effective logistic and regulatory pathways (drugs/vaccines vs medical procedures/transplants or blood products).

Figure 1. Building a dendritic cell vaccine regimen.

Ex vivo-generated DCs need to provide T cells with the antigenic and costimulatory signals required for activation and expansion (signals 1 and 2), the polarizing signal to drive effector cell differentiation (signal 3) and signals to imprint the tumor-specific homing properties (signal 4). Additional desirable features of DC vaccines involve their functional stability in the lymph nodes and preferential interaction with naive, effector and memory cells, while avoiding interaction with suppressive/regulatory cells. Once activated, the vaccination-induced effector cells need to be able to leave the lymph nodes and respond to tumor-produced chemokines in order to migrate to tumor sites. Combination of vaccines with systemic treatments able to promote the secretion of effector cell-attracting chemokines and suppress regulatory cell-attracting chemokines within tumor lesions may help the effector cells to enter all tumor lesions, resulting in systemic control of the visible tumor masses and long-term control of existing micrometastases.

CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; TLR: Toll-like receptor.

Figure 2. Evolution of dendritic cell vaccines.

First-generation DCs were immature or partially matured cells generated in the presence of GM-CSF (with or without IL-4, but without an additional DC maturation step). Second-generation DCs were matured with PGE₂-containing cocktails that induced high levels of expansion of both effector and suppressor cells. Third-generation DCs, matured with inflammatory cytokines, interferons and TLR ligands, have been developed in order to selectively enhance the activation of the desirable type-1 immunity (CTLs, Th1 and NK cells) and limit the activation of regulatory cells.

CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; TLR: Toll-like receptor.