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COMMENTARY

Dendritic cell vaccination in melanoma patients: From promising results to future perspectives

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ABSTRACT

Dendritic cells (DCs) play an important role in the induction of antitumor immunity. Therefore, they are used as anti-cancer vaccines in clinical studies in various types of cancer. DC vaccines are generally well tolerated and able to induce antigen-specific T cell responses in melanoma patients. After DC vaccinations, functional tumor-specific T cells are more frequently detected in stage III melanoma patients, as compared to patients with advanced melanoma, indicating that the tumor load influences immunological responses. Furthermore, long-lasting clinical responses were rarely seen in metastatic melanoma patients after DC vaccination. Since more potent treatment options are available, e.g. immune checkpoint inhibitors and targeted therapy, DC vaccination as monotherapy may not be preferred in the treatment of advanced melanoma. However, encouraging results of DC vaccines combined with ipilimumab have been reported in advanced melanoma patients with an objective response rate of 38%. DC vaccines show promising clinical results in stage III patients, although clinical efficacy still needs to be proven in a phase 3 trial. The clinical and immunological results of DC vaccination in stage III melanoma patients might be further improved by using naturally circulating DCs (myeloid DCs and plasmacytoid DCs) and neoantigens to load DCs.

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Introduction

Dendritic cells (DCs) were first discovered by Steinman and Cohn in 1973.[1] DCs are the most potent antigen-presenting cells of the immune system. Under steady state conditions, immature DCs sample peripheral tissues in search for pathogens or tissue injury, but when encountering danger signals, they quickly differentiate into activated (mature) DCs and migrate to lymphoid organs to induce an adaptive immune response. In lymphoid tissues, mature DCs initiate immune responses by presenting captured antigens to naïve T cells, in the form of peptide-major histocompatibility complex (MHC) molecule complexes. These T cells will proliferate and differentiate into effector cells that are able to kill target cells in an antigen-dependent manner.[2]

Under ideal circumstances, tumor growth would be controlled by an in vivo cancer-immunity cycle, in which DCs take up tumor material and induce tumor-specific T cells that infiltrate the tumor bed and kill their target cells by recognition of specific antigens, thereby releasing new tumor antigens that can be picked up by DCs again. However, in cancer patients, this cycle is hampered: tumor antigens may not be detected by DCs, DCs and T cells may treat antigens as self rather than foreign, T cells may not properly infiltrate tumors, or factors in the tumor microenvironment might suppress effector cells.[3] Therapeutic vaccination, like DC vaccination, can be used to overcome some of these problems and thus accelerate and expand the production of tumor-specific T cells. The first clinical study of a DC vaccine was reported in 1996 by Hsu and colleagues[4] in patients with B-cell lymphomas. Since then, multiple studies with DC vaccination have been reported in various tumor types, e.g., melanoma, prostate cancer, and glioma.[2] Here, we will focus on DC vaccination in melanoma patients.

Dendritic cell vaccines

The goal of DC vaccination is to induce tumor-specific T cell responses by injecting activated DCs loaded with tumor antigens.[5] Over the past years, different sources of DCs, maturation factors, and ways of tumor antigen loading have been used in clinical trials in melanoma patients.[6] Until recently, most DCs for immunotherapy were in vitro differentiated from precursors like monocytes, by culturing them in the presence of interleukin (IL) 4 and granulocyte-macrophage colony-stimulating factor (GM-CSF).[7] Additionally, these immature monocyte-derived DCs (moDCs) need to be matured, as mature DCs induce more potent anti-tumor immune responses than immature DCs in melanoma patients.[8,9] Different methods have been used to mature DCs, including cytokine cocktails

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consisting of monocyte-conditioned medium, tumor necrosis factor-α, prostaglandin E_2, IL1-β, and IL-6; prophylactic vaccines used as TLR ligands; and electroporation with mRNA encoding CD40L, CD70, and constitutively active TLR4.[10-12] Finally, the mature DCs must be loaded with relevant tumor-antigens, for which several methods have been applied, including short peptides, long peptides, tumor cell lysates, and mRNA transfection.[13]

Recently, naturally circulating DCs have been used to vaccinate advanced melanoma patients. Different subsets of naturally circulating DCs can be distinguished in the human peripheral blood by the expression of surface molecules: BDCA2+ plasmacytoid DCs (pDCs) and 2 subsets of myeloid DCs (mDCs): CD1c+ (also known as BDCA1+) mDCs and CD141+ mDCs, which differ in function and localization. Activated pDCs secrete large amounts of interferon-α (IFN-α) in response to viral products and they can induce the maturation of B cells into plasma cells, while mDCs are specialized in immunity against bacteria and fungi.[2,6,14] Until now, pDCs and CD1c+ mDCs have been used in clinical trials with advanced melanoma patients.[15,16] GM-CSF was used to activate CD1c+ mDCs and Frühsommer-meningoencephalitis vaccine to activate pDCs. Both mDCs as well as pDCs can be loaded with melanoma-associated peptides.[15,16] The most important advantages of using naturally circulating DCs over moDCs are a highly standardized rapid isolation procedure with antibody-coated magnetic beads (CliniMACS Prodigy), resulting in clinically applicable purified DCs, and the absence of an extensive culture period (overnight versus 8–9 d in moDCs), which may have a positive effect on the immunological capacity.[15,16] This makes naturally circulating DCs more suitable for large scale multicenter application.

Immune monitoring of DC vaccination

Immunologic monitoring is of great importance in clinical trials to determine the efficacy of DC vaccination. Enzyme-linked immunosorbent spot assays and tetramer analyses of tumor-specific T cell responses in peripheral blood are commonly used, but a low prevalence of tumor-specific T cells in peripheral blood makes these procedures less suitable for routine immunomonitoring. Besides peripheral blood samples, we evaluate skin-test infiltrating lymphocyte cultures from delayed-type hypersensitivity (DTH) biopsies taken within 2 weeks after each cycle of DC vaccinations.[17,18] These biopsies are analyzed for the presence of antigen-specific $CD8^+$ T cells by tetrameric MHC-peptide complexes and for the occurrence of functional T cell responses, by measuring specific production of cytokines in response to different target cells. Since this assay takes multiple parameters of T cells into account, including their capacity to migrate into the skin and to produce cytokines upon antigen encounter, it is probably more suitable to monitor T cell responses.

Safety

DC vaccination has proven to be a safe treatment in melanoma patients. Most common adverse events are grade 1–2 flu-like symptoms and local injection site reactions. Flu-like symptoms usually last up to 48 hours and consist mostly of fever, fatigue and chills. Injection site reactions, are usually small and self-limiting within 2–7 d.[19] DC vaccination is rarely associated with severe immune-related toxicity, which is in sharp contrast to immune checkpoint inhibitors and cytokines, that frequently show grade 3–4 immune-related adverse events.[20] This can be explained by nonspecific activation of the immune system by these immunotherapeutic agents as compared with an antigen-specific activation by DC vaccination. Therapy-related grade 3 adverse events, including hepatitis and pneumonitis, occurred only in patients treated in protocols using prophylactic vaccines as TLR ligands to mature DCs. These adverse events were attributed to the BCG vaccine used in this DC maturation cocktail.[11] No grade 4 or 5 therapy-related adverse events were observed in our DC vaccination trials. Thus, DC vaccinations are generally well tolerated in melanoma patients.

Position of DC vaccination in the treatment of advanced melanoma

Most clinical studies in advanced melanoma patients were performed with moDCs.[21-25] Although antigen-specific immune responses were found, long-lasting clinical responses were limited in advanced melanoma patients. A recent meta-analysis showed objective response rates of 8.5% in 1205 advanced melanoma patients treated with DC vaccination.[26] The only phase 3 trial comparing moDCs monotherapy with dacarbazine in advanced melanoma patients was stopped prematurely due to low response rates (< 10%) in both treatment arms.[25] In retrospect, this trial was probably performed too early, since DC vaccination was still in development, leading to a variable quality of the DC vaccines and suboptimal maturation of DCs.

We have conducted 2 small proof of principle clinical studies exploiting naturally circulating DCs in advanced melanoma patients, the first study using pDCs and the second study using CD1c+ mDCs. Tumor-specific T cell responses were found after vaccination with both DC populations. Objective responses were found in a limited number of patients treated with either subset. However, the study with pDCs did show an improved overall survival (OS) for patients treated with pDCs as compared with matched historical controls (median OS 22.0 months vs. 7.6 months), and the objective responses (14%) and prolonged progression-free survival in the mDC trial were seen in patients with functional antigen-specific T cells in blood and DTH.[15,16]

In the last years, multiple new therapies have been approved for the treatment of advanced melanoma. The clinical outcome after DC vaccination needs to be compared with the results of these approved therapeutic options, including immune checkpoint inhibitors and targeted therapies. Immune checkpoint molecules that down-regulate pathways of T cell activation, like cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1), can be blocked with monoclonal antibodies (mAbs; Fig. 1).[27] A pooled analysis of phase 2–3 trials with anti-CTLA-4 mAb ipilimumab in advanced melanoma patients showed a median OS of 9.5 months and a plateau at 21% in the survival curve around 3 y after start of ipilimumab.[28] Trials with anti-PD-1 mAbs nivolumab and pembrolizumab in advanced melanoma patients showed approximately

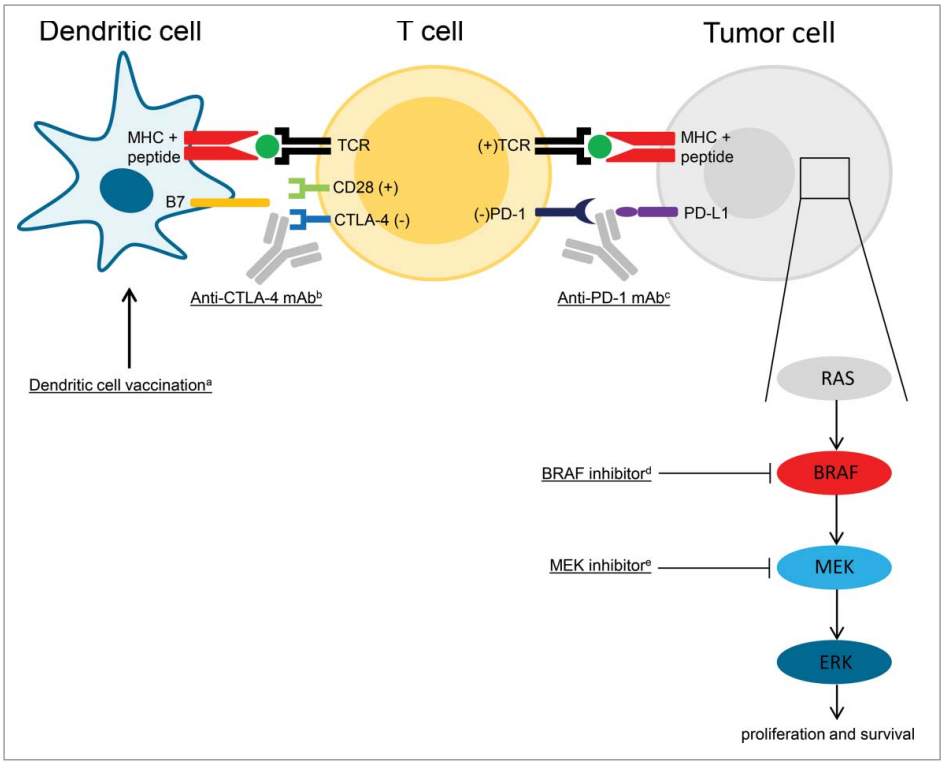

Figure 1. Immune therapy and targeted therapy of melanoma. [a]By injecting activated dendritic cells (DCs) loaded with tumor antigens, DC vaccination aims to induce tumor associated antigen-specific T cells. Antigen presentation by DCs and co-stimulation signals (B7-CD28) result in T cell activation and proliferation. [b]To keep an immune response in control, CTLA-4 is then up regulated on the surface of T cells, which binds stronger to B7 than CD28 and causes an inhibitory signal. Blocking CTLA-4 with monoclonal antibodies (ipilimumab) enhances T cell activation.[49] [c]Binding of PD-1 on the T cells to PD-L1 on the tumor results in downregulation of effector functions of T cells, which inhibits the killing of tumor cells. Blockade of this ligation by anti-PD-1 antibodies (nivolumab, pembrolizumab) makes it possible for T cells to maintain their antitumor functions, which allow them to kill tumor cells.[50] [d]BRAF is a kinase that is part of the RAS-BRAF-MEK-ERK mitogen-activated protein kinase (MAPK) pathway of cell proliferation. The tumors of approximately 40–60% of advanced melanoma patients harbor activating BRAF V600 mutations. The mutated kinase is constitutively active, which results in unregulated cell proliferation. This process can be blocked by selective BRAF inhibitors (vemurafenib, dabrafenib).[33] [e]Single-agent BRAF inhibition results commonly in progressive disease due to acquired resistance, which is commonly caused by genetic escape mechanisms resulting in MAPK pathway independant signaling. Upfront inhibition of both MEK (cobimetinib, trametinib) and the mutated BRAF kinases might counteract this form of resistance.[34] (+) indicates a stimulatory effect; (−) indicates an inhibitory effect. Abbrevations: CTLA-4, cytotoxic T-lymphocyte-associated antigen 4, MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, programmed death ligand-1; TCR, T cell receptor.

2-times higher response rates than ipilimumab and significantly longer progression-free survival.[29,30] However, long-term survival rates of these anti-PD-1 mAbs are still pending. The combination of ipilimumab and nivolumab resulted even in an objective response rate of 58%, however, at the expense of significant toxicity.[30] Besides the immune checkpoint inhibitors, BRAF inhibitors (vemurafenib, dabrafenib) have shown significant improvement of OS in patients with an activating BRAF mutation (Fig. 1).[31,32] However, responses are short-lived in many patients due to various resistant mechanisms.[33] Recently, it became apparent that adding a MEK inhibitor (cobimetinib, trametinib) to a BRAF inhibitor was associated with a further improvement of survival as compared to BRAF inhibition alone (Fig. 1), with overall response rates of 64–68% and median progression-free survival of 9.3–11.4 months.[34-36]

Altogether, these results suggest that DC vaccination monotherapy is not preferred in the treatment of advanced melanoma patients, despite the capability of inducing tumor-specific T cells. This may be explained by several immunosuppressive counter mechanisms against these T cells induced by the tumor (microenvironment), e.g. release of immunosuppressive cytokines, induction of regulatory T cells (Tregs) and myeloid derived suppressor cells, and expression of immune checkpoint molecules, like CTLA-4 and PD-1.[27,37] Therefore, the clinical efficacy of DC vaccination in advanced melanoma patients might be improved by combining it with other therapies that neutralize these immunosuppressive counter mechanisms. The mild toxicity profile of DC vaccination makes it an ideal candidate for combination-treatment. Unfortunately, combination of DC vaccination with the anti-CD25 mAb daclizumab did not show an enhancement of the efficacy of the DC vaccine, despite inducing a depletion of Tregs in the peripheral blood. This might be explained by simultaneous depletion of CD25+ effector T cells by daclizumab.[38] Combining DC vaccination with immune checkpoint inhibitors blocking CTLA-4 or PD-1 might be more effective. A phase 2 trial of moDCs in combination with ipilimumab conducted by Wilgenhof and colleagues showed tolerability and an objective response rate of 38% in 39 heavily pre-treated advanced melanoma patients, which supports further investigation of this combination.[39] Clinical studies with the combination of DC vaccination and anti-PD-1 mAbs in melanoma patients have not been described yet, but preclinical data support a potential synergistic effect.[40]

Dendritic cell vaccination in stage III melanoma

Stage III melanoma patients have a high risk of recurrent disease, even after a radical lymph node dissection (RLND) with

curative intent.[41] Therefore, adjuvant treatments that will improve survival rates are warranted. Stage III melanoma patients may have a more potent immune system than patients with advanced disease due to a lower tumor burden.[37] Therefore, DC vaccination might be more effective in stage III melanoma patients than in advanced melanoma patients. A retrospective analysis of 78 stage III patients treated with moDCs showed functional tumor-specific T cells in DTH skin-test biopsies of 71% of patients, which is substantially higher than in patients with distant metastasis (23%).[17,19] Furthermore, OS was significantly higher in this population, when compared to 209 matched controls who underwent RLND without adjuvant DC vaccination (median OS 63.6 months versus 31.0 months; $p = 0.018$).[19] However, these promising results have to be confirmed in a prospective randomized phase 3 clinical trial and should be compared to other (potential) adjuvant treatments, like IFN-α, ipilimumab and anti-PD-1 mAbs. IFN-α is an unattractive adjuvant treatment in stage III melanoma patients, since it only minimally improves survival and comes with substantial toxicity.[42] Ipilimumab has recently been approved by the Food and Drug Administration based on a significant improvement of recurrence-free survival compared to placebo, but OS data are still awaited.[43] However, adjuvant ipilimumab induced significant grade 3–4 adverse events and 49% of patients did not complete the treatment schedule due to drug-related adverse events.[43] Furthermore, the dosage of 10 mg/kg used in this trial could be debated, because a dosage of 3 mg/kg is commonly used in advanced melanoma patients. Clinical trials with adjuvant anti-PD-1 mAbs are currently ongoing (NCT02388906, NCT02362594). The mild toxicity profile of DC vaccination gives this therapy an advantage over immune checkpoint inhibitors when a phase 3 trial shows comparable clinical results, despite the fact that manufacturing a cellular product is more labor-intensive.

Future perspectives of DC vaccination in melanoma patients

Until now, all trials in stage III melanoma patients have been performed with moDCs, but currently we are conducting a trial (NCT02574377) in which immunogenicity of combined adjuvant mDC and pDC vaccination vs. adjuvant mDC or pDC vaccination alone is tested in stage III melanoma patients. Naturally circulating DCs have complementary functions and they can activate each other. Co-culture of mDCs and pDCs during activation augments the expression of co-stimulatory molecules and secretion of proinflammatory cytokines, providing the rationale for combining mDCs and pDCs in a DC vaccine.[44] In a murine tumor model, immunization with a mixture of activated pDC and mDC resulted in increased levels of antigen-specific $CD8^+$ T cells and an enhanced antitumor response compared with immunization with either DC subset alone.[45]

Besides the source of DCs, the antigens used in DC vaccination might influence the clinical effectiveness. Melanoma differentiation antigens, e.g., tyrosinase and gp100, are frequently used, as they are commonly expressed on melanoma cells and DCs with these antigens have shown to induce antigen-specific T cell responses.[22] Furthermore, cancer-testis antigens (e.g. MAGE-A3) have been used in melanoma patients to load DCs.[23] However, melanoma is the tumor with the highest prevalence of somatic mutations, resulting in the formation of many neoantigens, which have proven to play an important role in antitumor immunity.[46] These results formed the basis for a proof of concept study with a DC vaccine loaded with patient-specific neoantigens, which resulted in an enhanced $CD8^+$ T cell response against some of these tumor neoantigens.[47] Although these results are promising, the biggest challenge of such a personalized vaccination strategy will be the identification of the optimal immunogenic neoantigens.[48] To induce a broad immune response, it might be best to combine melanoma differentiation antigens, cancer-testis antigens, and neoantigens in DC vaccines.

Finally, combinations of DC vaccination and immune checkpoint inhibitors deserve further investigation in melanoma patients, since the first results of combination-treatment look promising.

Conclusions

In conclusion, DC vaccination is safe and adjuvant DC vaccination monotherapy shows promising results in stage III melanoma patients after RLND, while in advanced melanoma patients DC vaccination might be more suited as combination-treatment with immune checkpoint inhibitors. However, clinical effectiveness of DC vaccination monotherapy in stage III melanoma still has to be proven in a prospective randomized phase 3 clinical trial. Furthermore, the efficacy of DC vaccines might be further improved by using naturally circulating DCs as a source for vaccination and neoantigens to load the DCs.

Abbreviations

CTLA-4	cytotoxic T-lymphocyte-associated antigen 4
DC(s)	dendritic cells
DTH	delayed-type hypersensitivity
GM-CSF	granulocyte-macrophage colony-stimulating factor
IL	interleukin
IFN-α	interferon-α
OS	overall survival
mAb	monoclonal antibody
mDCs	myeloid dendritic cells
MHC	major histocompatibility complex
moDCs	monocyte-derived dendritic cells
PD-1	programmed death-1
PD-L1	programmed death ligand-1
pDCs	plasmacytoid dendritic cells
RLND	radical lymph node dissection
TCR	T cell receptor
Tregs	regulatory T cells

Disclosure of potential conflicts of interest

WRG received speakers fees from Astellas, Bayer, Bavarian Nordic, Bristol-Myers Squibb, Janssen-Cilag and ESMO; WRG participated in advisory boards of Amgen, Astellas, Bayer, Bristol-Myers Squibb, Dendreon, Janssen-Cilag, Morphosys, Sanofi and Transgene; WRG participated in ad hoc consultancy for Psioxus Therapeutics, Sotio and Transgene; WRG is founder of Carcinos (global oncology education: immunotherapy of cancer).

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