The use of dendritic cell vaccinations in melanoma: where are we now?

“...dendritic cell vaccination might be more effective in patients in earlier stages of disease...”

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Dendritic cells (DCs) act as the key initiators and mediators of adaptive immune responses. In a resting, immature state they reside in tissues close to the external environment in search of antigens. They are very efficient in antigen uptake and processing. Upon encountering danger signals, a complex maturation process occurs changing the phenotype and function of DCs. Furthermore, DCs can then migrate to lymphoid organs to induce an adaptive immune response. There they can activate T-helper cells, cytotoxic T cells and B cells. DCs can also interact with cells of the innate immune system, like natural killer cells and natural killer T cells. Classically, internal antigens, such as self peptides and viruses, are presented on MHC class I molecules while exogenous antigens, like

bacteria and also tumor antigens, are presented on MHC class II molecules. In addition, DCs have the exceptional ability to cross-present, thereby presenting antigens from exogenous sources on both MHC class I and II molecules. This is of importance for presentation of tumor antigens to cytotoxic CD8⁺ T cells. As such they have a vital role in the induction of antitumor responses, making them suitable for immunotherapy purposes [1,2].

Cancer DC-based vaccines use the unique capacities of DCs as professional antigen-presenting cells in order to elicit a strong antitumor T-cell response, aiming to elicit longstanding tumor regression and eradication. This is effectuated by administration of mature DCs that have been activated and loaded with tumor

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antigen *ex vivo*. In past years, different DCs and also many different methods for culturing, maturation and antigen loading have been employed.

As naturally circulating blood DCs constitute only about 1% of peripheral blood mononuclear cells; for over a decade, DCs have been generated *ex vivo* by culturing CD34⁺ progenitors or monocytes with cytokine combinations after blood apheresis. This has shown good results, however, is quite laborious and specific expertise and facilities are needed. Moreover, a shorter culture period might prevent exhaustion and improve the immunological results [3]. Recently, it became possible to isolate naturally circulating DCs from blood apheresis suitable for clinical application. This holds true for plasmacytoid DCs (pDCs) and BDCA1⁺ myeloid DCs (mDCs). Trials have proven the safety and immunological efficacy of these subtypes, while BDCA3⁺ mDCs, which constitute about 0.05% of peripheral blood mononuclear cells, can be isolated in adequate amounts from blood apheresis, but have not been used in trials thus far [4].

In the infancy of DC vaccination, immature DCs were used. However, mature DCs have proven their superiority in clinical trials concerning immunogenicity and clinical outcome. They have an enhanced migratory capacity, higher expression of MHC and costimulatory molecules and as such show better induction of T-cell responses. Immature DCs might even be contraproductive for tumor eradication by inducing tolerance [5,6]. Maturation is induced by several danger signals, such as contact with pathogenic antigens *in vivo*. *Ex vivo* maturation can be simulated by culturing DCs with a multitude of stimuli. The most widely used method is a cytokine cocktail that includes TNF- α with any of the following cytokines in any combination: IL-1 β , IL-6, prostaglandin E2 or the supernatant of activated autologous monocytes [5]. More recently, Toll-like receptor (TLR) ligands have been used to stimulate TLRs and mature DCs in a more physiological way. TLRs are part of the pattern recognition receptors by which DCs can detect pathogens. Many other methods have been exploited as well [4].

Besides DC maturation, loading of relevant tumor antigen in MHC receptors of DCs is necessary for an adequate antitumor response. Various methods of antigen loading have been explored and no superior method is determined.

The most used methods, each with its own advantages and disadvantages, are loading with short or long peptides, tumor cell lysates and mRNA transfer. Whatever the method, it is key to induce both CD4⁺ and CD8⁺ antitumor responses, as this allows a strong and long-lasting antitumor T-cell response [5]. Recently, a promising method of DC loading was described, which entails the determination of patient-specific somatic mutations within the tumor, in order to provide unique tumor neo-antigens. This allows personalized immunotherapy, targeting patient-specific somatic tumor alterations [7].

To determine the efficacy of DC vaccination, it is highly important to measure the induced immunological effects. Many assays are employed for this purpose. Commonly used are ELISpot assays and tetramer analyses of tumor-specific T-cell responses in peripheral blood, but their disadvantages are a low prevalence of cytotoxic T cells in peripheral blood. More recently, delayed-type hypersensitivity biopsies taken after a cycle of DC vaccination are used to evaluate the presence of antigen-specific CD8⁺ T cells and the occurrence of functional T-cell responses [8,9]. Although T-cell responses in immunomonitoring assays are correlated with clinical outcome, these assays remain laborious and are not validated in prospective trials [8,9].

The first clinical study of DC vaccination was performed in patients with B-cell lymphoma and published in 1996 in *Nature Medicine* [10]. Ever since, a multitude of clinical trials have been performed in several tumor types, including melanoma. Most DC vaccination studies in melanoma are executed in patients with advanced disease using monocyte-derived DCs [8–9,11]. In this patient group, long-lasting clinical responses are limited, despite adequate immunological responses. A recent meta-analysis showed an objective clinical response rate (achieving either a complete or partial response as defined by WHO criteria or by the Response Evaluation Criteria In Solid Tumors) in 8.5% of in-total 1205 melanoma patients [11]. The only prospective Phase III trial to date compared standard dacarbazine chemotherapy with monocyte-derived DC vaccination as first-line treatment. This trial was prematurely stopped because of lack of clinical response in both treatment arms (<6%) [12]. In retrospect, the DC vaccines used had a variable quality and suboptimal maturation of DCs. Furthermore, DC vaccination might be more effective in patients in earlier stages of disease,

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that is, with lower tumor load. The rationale is that tumor-specific T cells, once induced in the lymph node, should be able to exert their cytotoxic capacities within the tumor. However, this is hampered by a broad spectrum of tumor-induced immunosuppressive factors. Indeed clinical studies applying DC vaccination in the adjuvant setting, when there is a low tumor burden, have shown tumor-specific immunological responses that are two- to three-times higher as compared with the metastatic setting [13].

Trials using naturally circulating DCs are limited thus far, but have shown favorable immune responses after injection of pDCs [14] and correlation of immune responses with improved progression-free survival after vaccination with BDCA1⁺ mDCs [3].

Recently, CTLA-4- and PD-1-blocking antibodies have been approved by the US FDA for the treatment of advanced melanoma. These monoclonal antibodies block immune checkpoint molecules that downregulate pathways of T-cell activation, and as such are called immune checkpoint inhibitors. They have shown long-term benefit and improved survival rates in 10–40% of metastatic melanoma patients, but this comes at high cost and with severe toxicity [15]. It is hypothesized that adding DC vaccination to immune checkpoint therapy can lead to a higher response rate. This hypothesis is supported by data of two small trials which showed that the combination of DC vaccination and anti-CTLA-4 antibodies seems to be more effective than the use of these single agents [16,17]. Expectations are high for trials combining anti-PD-1 antibodies and DC vaccination, but to date no data are available.

Despite adequate immune responses, clinical responses are present in only a small fraction of patients. Future trials might be able to improve clinical responses by optimizing vaccines. In our group, there is an ongoing trial (NCT02574377) combining adjuvant vaccination of mDCs and pDCs compared with adjuvant mDCs or pDCs alone. Indeed, naturally circulating DCs have shown complementary and augmented functions *ex vivo* and in animal models [18]. Developing better DC vaccines can also be advanced by optimal antigen loading. The previously mentioned neo-antigens providing personalized DC vaccination might be a step in the right direction [7]. An alternative approach for *ex vivo* DC generation is targeting DCs *in vivo* by antibodies with activating

agents and tumor antigens. A Phase I trial was executed in 45 patients with advanced malignancies, using an antibody expressed on DCs (DEC205), fused with a tumor antigen (NY-ESO-1) and activating agents (different TLR agonists). Treatment with this synthetic vaccine induced tumor antigen-specific immune responses and led to tumor regression in two patients [19]. A different, and also exiting approach is using intravenously administered RNA lipoplexes (encoding tumor neo-antigens) to induce strong effector and memory T-cell responses against tumors [20]. A big advantage of these methods would be the development of an off-the-shelf product. Further investigations are however needed. Furthermore, there is a strong need for biomarkers that can predict whether a patient will or will not benefit from DC vaccination therapy. Our recent findings suggest that the ratio of intratumoral over peritumoral CD3⁺ lymphocytes in the primary melanoma is a potential predictive biomarker for treatment selection [21].

Last, besides clinical efficacy, safety of cancer therapy is of high importance. Luckily, DC vaccination-related adverse effects, both local reactions at the injection sites as well as systemic effects, most commonly flu-like symptoms, are generally mild and self-limiting. Severe systemic toxicity is very uncommon if DC vaccination is given as monotherapy. Furthermore, the presence of immune-related side effects correlates with immunologic and clinical outcome [11,22].

In conclusion, multiple trials have proven the safety and feasibility of DC vaccination with established immunological and clinical results. Already major steps have been taken in DC vaccination optimization. Improvement of DC vaccines, adjuvant administration and combination therapy with immune checkpoint inhibitors looks promising. Prospective (Phase III) clinical trials will have to prove the value of these new methods.

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