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Personalized dendritic cell-based tumor immunotherapy

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Abstract

Advances in the understanding of the immunoregulatory functions of dendritic cells (DCs) in animal models and humans have led to their exploitation as anticancer vaccines. Although DC-based immunotherapy has proven clinically safe and efficient to induce tumor-specific immune responses, only a limited number of objective clinical responses have been reported in cancer patients. These relatively disappointing results have prompted the evaluation of multiple approaches to improve the efficacy of DC vaccines. The topic of this review focuses on personalized DC-based anticancer vaccines, which in theory have the potential to present to the host immune system the entire repertoire of antigens harbored by autologous tumor cells. We also discuss the implementation of these vaccines in cancer therapeutic strategies, their limitations and the future challenges for effective immunotherapy against cancer.

Keywords

anticancer immune response; chaperone protein; dendritic cell; idiotypic; personalized tumor vaccine; tumor lysate

The concept of cancer immunotherapy is based on the manipulation of the host's immune system to fight cancer. The primary advantages of active immunotherapy are its relative lack of side effects, its specificity against target tumor cells and the generation a long-lasting memory response against tumor-specific antigens [1-3].

Dendritic cells (DCs) play a central role in the initiation and regulation of tumor-specific immune responses, as they are endowed with the unique potential to activate anti-tumor effector T and B lymphocytes, and are capable of promoting natural killer (NK) T cells or NK cell activation [1,2]. These capacities have been extensively exploited during the last decade, leading to the development of DC-based cancer immuno-therapy. Evidence that anti-tumor immunity can be generated by vaccination with tumor antigen-loaded DCs has been demonstrated in numerous studies [4-9]. However, effective clinical responses in cancer

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patients have been limited [10] and do not necessarily correlate with the induction of tumor-specific T cells [11,12].

The identification of specific tumor antigens has significantly advanced the field of tumor immunotherapy. Improved understanding of the molecular bases of antigen recognition has resulted in the development of rationally designed tumor antigen-specific vaccines based on motifs predicted to bind to MHC class I and II molecules. Tumor antigen-specific peptide-based vaccines have thus advanced from preclinical to clinical studies over the last decade and several important issues have been addressed during this process [13-15]. The use of synthetic peptides in cancer vaccines offers practical advantages, such as streamlined construction and production. However, it confines the immune response to a restricted repertoire of tumor-specific T lymphocytes recognizing a limited number of epitopes present in the peptides [15, 16]. Although some antigens are unique to their tumors (such as idiotypes produced by malignant B cells or viral antigens from virally induced malignancies), most are 'self' antigens either overexpressed or mutated in the tumor [17,18]. Personalized cancer vaccines utilizing antigens derived from total tumor cell lysates, tumor-derived chaperone proteins, or apoptotic or necrotic tumor cells contain a pool of multiple undefined antigens. They allow immunization against a wider array of tumor antigens and do not require identification of cancer-specific antigens [19]. This concept is especially important for patients with advanced disease, for which immune escape mechanisms are exacerbated [12,20-24].

In this review we focus on the recent advances in the field of personalized DC-based vaccines for cancer immunotherapy, and discuss their limitations and possible improvements for future clinical trials.

DC-based tumor vaccines

Dendritic cells represent a major focus in cancer immunotherapy as their primary functions are the initiation and regulation of immune responses. DCs generated *ex vivo* and incubated with a source of tumor-specific antigen(s) have been used both in animal models and in clinical trials [4,5,25-28]. Multiple techniques have been evaluated to produce, expand, load and activate DCs *in vitro* (FIGURE 1) (for an extensive review, see Aarntzen *et al.* [28] and Tuyaerts *et al.* [29]). The primary source of DCs currently used in clinical trials and in most animal studies consists of monocyte-derived DCs (myeloid DCs). Sequential density centrifugation of apheresis peripheral blood mononuclear cells represents a main approach to enrich for blood DCs for clinical use [29]. Cultured in the presence of GM-CSF and IL-4, monocytes isolated from the blood of patients or from the bone marrow (primarily in experimental animal models), differentiate into immature DCs after a period of 4 to 5 days. Flt3L has also been used to obtain DC expansion. Further activation with TNF- α , IFN- γ , LPS, CpG, IL-1 or CD40L leads to the generation of mature DCs. CMRF-44 antigen, as well as CD1c, BDCA-4 and other DC-specific markers, have also been used to promote DC maturation [29]. In addition, unselected bone marrow cells [5,11,30-32], or CD34⁺ precursors from blood or bone marrow [33-35] have been utilized to generate DCs. CD34⁺ cells can be mobilized by GM-CSF administration, which may adversely skew the immune response toward T helper (Th)2 [29]. The purity and the functional grade of the subpopulations of DCs that would ensure highly qualified anti-cancer immune responses [28,29], however, require further standardization.

Choice of a source of tumor antigens to be loaded onto DCs

The activation of specific anti-tumor CD8⁺ or CD4⁺ T-cell clones requires the presentation of defined tumor antigens on MHC class I and class II molecules expressed by DCs. The choice of the nature of the tumor antigens used to pulse DCs impacts the specificity and quality of the immune response that will be generated by the vaccine [36-44]. Frequently used in clinical trials, the loading of DCs with peptides derived from defined tumor antigens, or with DNA or

RNA encoding one or a few characterized tumor antigens, has the obvious limitation of the requirement to identify specific antigens expressed by tumors. In addition, such vaccines usually lead to the selective expansion and activation of a restricted repertoire of T-cell clones. A microarray analysis of normal and malignant tissues involving the isolation and sequencing of HLA-class I binding peptides from the surface of malignant cells has been described, and allows the identification of patient-specific overexpressed tumor-associated antigens (TAAs) and corresponding HLA-class I peptides [45]. Immunogenic peptides have been identified for numerous known TAAs (including telomerase, tyrosinase, MAGE, Melan-A/MART, MUC1, CEA, p53, HER-2/neu, survivin, etc.) and are usually restricted to a certain HLA type [45-47]. Alternatively, a full-length protein can be presented to DCs, resulting in both CD4⁺ and CD8⁺ T-cell responses [29,46]. The use of autologous or allogeneic tumor cells, apoptotic bodies, tumor lysates, tumor RNA or DNA to pulse DCs leads to a considerably more diverse immune response involving numerous clones of CD4⁺ T cells and CTLs. Additional procedures to pulse DCs, including DC–tumor cell fusion, have been described [39,48]. Enhanced vaccine efficiency of fused DC–tumor cells compared with lysate-pulsed DCs has been reported in different tumor models [49-51]. Other genetic approaches, such as various viral and nonviral gene transfer systems, have also been developed, and are reviewed more extensively by Ribas [52] and Breckpot *et al.* [53].

This multiplicity of DC-loading techniques warrants further comparative studies. Thus, an optimal strategy that most efficiently stimulates DC-antigen processing and presentation has not yet been widely accepted. However, complex antigenic mixtures derived from autologous tumor cells may prove superior to other approaches as true tumor-rejection antigens may be specific for each individual patient. Supporting this concept, reports in melanoma suggest that T-cell reactivity against autologous tumor cells is largely patient-specific, and most of these T cells do not recognize epitopes from allogeneic tumors [19,54].

Activation & *in vivo* delivery of tumor antigen-loaded DCs

The efficient stimulation of tumor-specific T lymphocytes by DCs requires the presentation of tumor-derived epitopes on MHC class I and II molecules in the context of a second signal displayed by DC costimulatory molecules (e.g., CD80, CD86 and CD40) and supported by proinflammatory cytokines (IL-12 and TNF- α) secreted by activated DCs. A wide variety of DC-activation agents have been used including cytokines (e.g., interferons, TNF- α , GM-CSF or IL-1 β), ligands of the TNF receptor family (e.g., CD40L), or adjuvants such as TLR ligands (LPS, CpG and poly-I:C) [1,5,11].

Considering the expensive and fastidious manipulations inherent to the *ex vivo* generation of DCs, the ability to load and activate DCs directly *in situ* would facilitate the applicability of this type of vaccine. Numerous techniques have been explored to deliver to DCs *in vivo* both tumor antigens to MHC class I and class II molecules, and also activation signals [55]. Some examples include the administration of tumor antigens coupled with antibodies that recognize DC-specific markers such as DEC205, or tumor antigens conjugated to molecules that specifically bind to DC receptors. Simultaneous delivery of proinflammatory signals such as CD40-activating antibodies or TLR ligands, ensure full DC maturation [5,11,55,56]. DC maturation is accompanied by their migration to the draining lymph node, where they subsequently prime immune T cells. DCs that do not undergo maturation and activation are maintained in an immature/tolerogenic status, which may result in immune tolerance rather than immunity [56,57]. There is however, no consensus as to whether DC maturation should be induced *in vitro* or *in vivo* following their administration. In addition, if immature DCs are considered to be less potent at inducing T-cell activation compared with mature DCs, there is concern that activated DCs may become exhausted during the culture stage. Alternative

strategies include DCs genetically engineered to express costimulatory molecules or to secrete proinflammatory cytokines [58].

The site of DC inoculation is of fundamental importance since these cells should migrate to the secondary lymphoid organs where tumor antigen presentation to T cells takes place. In most vaccination protocols, DCs are delivered intradermally or subcutaneously [11], which may substantially limit the vaccine efficiency since only 5–10% of the cells reach the draining lymph nodes. Enhancement of DC migratory capacities could be obtained by preadministration of TLR ligands (that additionally support DC activation *in vivo*) or proinflammatory cytokines that increase CCL21, the ligand of CCR7 expressed by activated DCs and that direct their homing to the draining lymph nodes [11,59]. A benefit of direct intranodal DC delivery over intradermal or intravenous administration has also been reported [60]. The tumor itself may also be considered as a potential injection site [61].

The promotion of DC survival, obtained by DC transfection with members of the anti-apoptotic subfamily BCL-2 or BCL-xL [62], is of particular importance insofar as it increases the lifetime of these cells *in vivo* and, thus, fosters their ability to encounter, prime and sustain the activation of tumor-specific T cells [62,63].

Therefore, the choice of the most appropriate subset of DCs and the nature of the signal(s) used to trigger DC activation are important for the induction of an efficient anti-tumoral immune response. However, the nature of the tumor antigens to be loaded into the DCs remains critical for the generation of clinically effective anticancer vaccines.

Current personalized DC-based vaccines

Tumor cell-based vaccines

As previously underlined, an important limitation inherent to the use of defined tumor peptides to pulse DCs is their restriction to a relatively small number of HLA-binding epitopes that can be presented to T cells. This results in the induction of a limited repertoire of tumor-specific T-cell clones and therefore may lead to the emergence of tumor escape variants. Personalized tumor-derived vaccines, such as whole-tumor preparations (apoptotic or necrotic tumor cells or tumor lysates) may therefore be of considerable interest to overcome this issue since they contain a large number of antigens, known and unknown, allowing the stimulation of a polyclonal immune response [37,64,65]. Thus, DC loading with whole dead tumor cells has the theoretical advantage that essentially all of the antigenic components of the tumor could be presented to the immune system. This approach has the additional advantage to be more specific to a given patient's tumor. However, there is still debate about the most advantageous format of dead tumor cells (apoptotic vs necrotic vs whole lysates) to be used to pulse DCs [37,40,66-73]. Some concerns have also been raised about the lack of immunogenicity of lysates that may potentially contain immunosuppressive factors [74,75]. The balance between anti-tumor immunity and autoimmunity after vaccination with whole-tumor materials should also be considered [76,77]. Indeed, since most TAAs contained in the vaccine preparations are also self-antigens, the risk of inducing autoimmunity has been a concern, especially when whole-tumor cell preparations are used as the source of antigen [78]. For example, following vaccination against melanoma, vitiligo has been observed in some trials. Importantly, this correlates with clinical responses and better outcome [76]. The main limitation of using autologous tumor cells as a source of antigens to pulse DCs is their relative difficulty of production. The small size of many tumor specimens obtained by biopsy makes it difficult to obtain enough material for therapy, especially when multiple immunizations are required (an obstacle that may be overcome when using amplified tumor-derived RNA [79,80]).

In various clinical trials using whole-tumor cell preparations, an efficient anti-tumor immunity, as well as clinical responses to DC vaccination, has been reported. O'Rourke *et al.* demonstrated that durable complete responses can be achieved in patients with advanced melanoma after autologous tumor cell-pulsed DC vaccine administration [65]. Three of the 12 patients who completed the treatment schedule demonstrated durable complete responses (average duration 35 months), three had partial responses and the remaining six had progressive disease. In addition, clinical effects have been observed after tumor cell lysate-pulsed DC vaccination of renal cell carcinoma patients [81]. Among 27 evaluated patients, two showed a complete response (CR), one a partial response (PR), and seven with stable disease were reported [81]. Clinical trials using DC-based immunotherapy for prostate cancer have shown encouraging results (extensively reviewed by Thomas-Kaskel *et al.* [78]). Approximately 500 prostate cancer patients have been treated with tumor antigen-loaded DCs and immune responses have been reported in two-thirds of these patients. Clinical responses were observed in almost half of the treated patients [78].

Idiotypic-based vaccines

Inducing tumor-specific immunity by immunizing patients with tumor cells or their antigenic components has been successfully implemented against hematological malignancies. A given patient's B-cell malignancy is usually derived from a single expanded B-cell clone that expresses an immunoglobulin (Ig) with a unique idiotype (Id; variable regions of Ig). Therefore, Id can be regarded as a tumor-associated antigen and a potential target in clinical vaccination approaches [82]. The use of idiotypic vaccines has been hampered by the fact that autologous Id is patient-specific and, thus, the vaccine must be individually prepared for each patient [82]. Custom-made monoclonal antibodies against tumor Id partially solved this problem and have led to some long-lasting complete tumor regressions in clinic [83]. However, some patients were refractory to this type of therapy owing to the outgrowth of a mutated tumor Id variant that had lost the epitope recognized by the monoclonal antibody [84]. In the meantime, vaccines were made from the tumor Id that were intended to induce long-lasting, polyclonal as well as mixed – humoral (antibody-based) and cellular (T-cell-mediated) – immune responses against the tumor [85]. The tumor-specific Id from B-cell lymphomas was successfully 'rescued' from the tumor cells by fusing them to a myeloma cell line. These hybrid cells secrete the tumor-derived Igs, which are then purified and prepared into a vaccine [85]. However, since the native Id is a self-protein, induction of anti-Id immune responses remain difficult. Therefore, numerous studies have been conducted combining this approach with DC vaccination to promote the uptake and presentation of the antigen to the immune system in a recognizable form. Timmerman *et al.* evaluated Id-pulsed DCs in 35 patients with B-cell lymphoma. Among ten initial patients with measurable lymphoma, eight mounted T-cell proliferative anti-Id responses, and four had clinical responses, two demonstrated CRs, one PR was observed and one molecular response was reported [86]. Subsequently, 25 additional patients were vaccinated after first chemo therapy, and 15 of 23 who completed the vaccination schedule mounted T-cell or humoral anti-Id responses.

Unlike lymphomas, Id produced by myeloma cells is mostly secreted into the serum and the malignant plasma cells express low surface Id protein as a potential target for idiotype vaccines. However, it has been reported that multiple myeloma patients can mount T-cell responses to tumor Id when vaccinated with DCs pulsed with Id-protein (three out of 17 CR; two out of 17 PR) [82].

Chaperone-based vaccines

The demonstration that tumor cell lysate or necrotic tumor cell immunogenicity is associated with members of the chaperone protein family (heat shock proteins [HSP]) has led to the development of tumor-derived HSP-based vaccines [87]. The specific immunogenic properties

of chaperone proteins stem from their function as carriers of a wide repertoire of tumor-derived antigenic peptides [87,88]. They also constitute natural adjuvants *per se* capable of activating DCs [89]. Work by our group [90] and others [91-95] has demonstrated that individual purified chaperone proteins – HSP70, HSP90, GRP94/gp96 and calreticulin – are each capable of generating immune responses against their tumors of origin. The immune response induced by these vaccines is specific of the tumor from which the chaperones were purified since vaccines prepared from one tumor type are not capable of protecting against tumors of another origin [75]. The immunological identity of the HSP-based vaccines appeared to rely on the peptide-binding capacity of at least some of the chaperones as well as the speculated ‘peptide antigen fingerprint’, which presumably differed from tumor to tumor. HSP70 and GRP94/gp96 are currently used in clinical trials [96-98].

Within this immunological arena, our laboratory had developed a novel anticancer vaccine named chaperone-rich, cell lysate (CRCL). CRCL is generated by a free-solution isoelectric-focusing technique (FS-IEF) using tumor lysates that results in an enrichment for chaperone proteins rather than a purification of them [88,99-106]. CRCL contains HSP90 and HSP70 family members, the endoplasmic reticulum chaperones GRP94/gp96 and calreticulin. In addition, HSP40, HSP60, GRP75 and GRP78 are also contained in the CRCL vaccine preparation. CRCL preserves its antigenic components, while excluding some presumed immunosuppressive factors present in unfractionated lysates [107]. CRCL combines the relative simplicity of lysate preparations, along with a high yield and an extensive antigen repertoire of chaperone proteins. CRCL vaccines have a more pronounced immunologic effect per unit of protein than any of the individual chaperone proteins used as a vaccine alone [108,109]. FS-IEF is a relatively simple, rapid and efficient procedure, allowing one to obtain 1–2 mg of CRCL vaccine per gram of tumor tissue, which is 10–25-times as much vaccinating material from the same quantity of tumor as with the conventionally purified HSP (i.e., HSP70 and GRP94/gp96) currently used in clinical trials [96,110,111]. This makes the FS-IEF method of multiple chaperone complex enrichment desirable from a clinical standpoint in terms of high yield from a potentially limited tumor source, and with a rapid turn-around time from tumor harvest to treatment of the patient. CRCL presents tumor antigens to DCs and, by virtue of its adjuvant effects, triggers DC activation (expression of CD40, CD80/86 and CD70, all of which are costimulatory molecules that are fundamental for T-cell activation) [25,109,112,113,114]. We have recently demonstrated that the molecular signaling events associated with CRCL-mediated activation of DCs involve the induction of the MAP kinase pathway, evidenced by increased phosphorylation of ERK1/2 and p38, the activation of the transcription factor NF- κ B and the phosphorylation of STAT1, STAT5 and AKT [114]. Interestingly, further supporting the use of CRCL as a potent anti-tumor vaccine, we have demonstrated that DCs loaded with tumor-derived CRCL resist regulatory T-cell [115] and TGF- β -mediated suppression [114]. CRCL has been shown to be effective against a variety of cancers in mice, including 12B1 (a murine BCR-ABL⁺ leukemia), A20 lymphoma (a murine B-cell lymphoma), B16 (a murine melanoma), and TUBO (a murine breast cancer) [108,116,117]. Furthermore, human ovarian cancer-derived CRCL has demonstrated superior activating effects on DCs compared with tumor lysate, and DCs pulsed with human ovarian cancer-derived CRCL were capable of generating tumor-specific cytotoxic T lymphocytes [113]. Thus, the enhanced immunogenicity arising from CRCL-pulsed DCs as a vaccine indicates that CRCL may represent an antigen source of choice for DC-based personalized anticancer immunotherapies [75].

Limitations of current DC-based anticancer vaccines & combination strategies

Despite encouraging results, numerous clinical studies have demonstrated the limitations of DC therapy in generating effective and durable clinical anti-tumor responses in patients with

advanced-stage diseases [11,12,21,56,118]. One explanation for these disappointing clinical results may partly stem from the end-stage nature of the cancer patients included in these DC vaccination protocols. Indeed, the immune system of these patients is considerably altered by the immunosuppressive environment established during cancer progression. Tumors are capable of inducing immune tolerance by exploiting multiple immune regulatory mechanisms that, in turn, may impair DC function. Consequently, even if appropriately loaded with tumor antigens and activated *in vitro*, the efficiency of DC-based vaccines may be significantly impaired *in vivo* by the tumor environment. A number of biologically active agents (e.g., TGF- β , IL-10, IL-13, VEGF, indoleamine 2,3 dioxygenase and PGE₂) synthesized by tumor or stromal cells may exert suppressive effects on the immune system [24,119-125]. Tumor-derived factors alter DC differentiation and promote accumulation of immature DCs, plasmacytoid DCs, immunosuppressive regulatory DCs as well as myeloid-derived suppressor cells (MDSCs) [22,24,126-130]. Accumulation of several populations of regulatory DCs in the spleen and the lymph nodes of tumor-bearing mice inhibits CTL responses but induces the development of immuno suppressive CD4⁺CD25⁺FoxP3⁺ Tregs, which may also hinder the efficiency of DC-based vaccines (FIGURE 2) [131-136].

Therefore, combining DC-based therapy with approaches aimed at overcoming tumor-induced tolerance (inhibition of immunosuppressive molecules or cells such as TGF- β , Tregs, myeloid-derived suppressor cells, etc.) may further enhance the clinical efficiency of DC-based anticancer vaccines. For instance, several TGF- β antagonists (neutralizing monoclonal antibodies, fusion proteins, antisense TGF- β oligonucleotides) and TGF- β receptor kinase inhibitors have been shown to promote the anti-tumoral immune responses induced by antigen-pulsed DCs or adoptive T-cell transfer [137-139]. In line with this concept, immunotoxins such as the recombinant IL-2 diphtheria toxin conjugate (ONTAK) and LMB-2 have been shown to enhance the immunostimulatory effect of tumor-antigen pulsed DC while selectively depleting Tregs, leading to the stimulation of helper and cytotoxic T-cell responses [140, 141]. Treg depletion upon treatment with immunotoxins has also been observed in patients with metastatic renal carcinoma and melanoma [140,141]. Cyclophosphamide facilitates adoptive immunotherapy of established tumors through the elimination/inactivation of suppressor T cells [115,142,143]. We have recently reported, in an established lymphoma model, that the efficiency of DCs pulsed with total tumor cell lysates is significantly enhanced by imatinib mesylate, a chemotherapeutic drug used to treat BCR-ABL⁺ murine myelogenous leukemia [136]. This synergistic effect involves the inhibition of Tregs by imatinib, allowing the stimulation of effector CD4⁺ and CD8⁺ T cells by the tumor-lysate pulsed DC vaccine. We have also demonstrated that CRCL-loaded DCs can be effectively combined with imatinib mesylate to treat established leukemia [144]. Thus, combination therapies may be of interest to enhance the efficacy of personalized DC-based vaccination.

Conclusion & future perspective

Dendritic cell-based vaccines for cancer immunotherapy have been studied and tested for more than a decade. Significant progresses have been made in their design, optimization and translation. Interestingly, in most pre-clinical and clinical trials, the side effects of DC-based therapies were minimal or absent. Specific immune responses raised by tumor-antigen-pulsed DCs were detected in a large number of patients with various types of cancer. However, the absence of objective tumor regression in most vaccinated patients has dampened the initial enthusiasm for DC-based immunotherapy. The reason of this relative lack of clinical response primarily stems from the induction of immune tolerance by progressive tumors, especially in patients with terminal-stage disease. Personalized DC-based cancer vaccines may overcome some of these immunosuppressive phenomena as they contain virtually all the antigens harbored by a given tumor and are, therefore, endowed with the capability to trigger the activation of a wider repertoire of tumor-specific T cells. Such polyclonal immune responses

may prevent the outgrowth of tumor escape variants. However, a main limitation of these personalized DC based vaccines stems from the small size of many tumor specimens obtained by biopsy, which makes it difficult to obtain enough material for vaccine preparation, especially when multiple immunizations are required. In some instances, it is possible to propagate tumor cells *in vitro* to increase the number of cells available, but prolonged cell culture may significantly alter the characteristics of the tumor cells.

A future avenue for cancer immunotherapy consists of overcoming the phenomena of tumor-induced immunosuppression, which may unleash the full therapeutic efficiency of DC-based vaccines. This goal may be achieved by using combination strategies, associating personalized DC vaccination with chemotherapeutic drugs that may not only directly target cancer cells but that may also eliminate or impair the function of immunosuppressive cells or inhibit tumor-induced suppressive factors. Further evaluation and standardization of such approaches are still needed but may have the potential to significantly improve the outcome of cancer immunotherapy.

Executive summary

Dendritic cells-based vaccines efficiently induce anti-tumor immune responses

- The choice of the nature of tumor antigens impacts the specificity and efficiency of the immune response raised by the vaccine.
- Peptides derived from defined tumor antigens induce a restricted repertoire of T-cell clones, which may foster the emergence of tumor escape variants.
- Personalized tumor vaccines induce polyclonal immune responses against a wide repertoire of tumor antigens, which may avoid the outgrowth of tumor-escape variants.

Personalized DC-based anticancer vaccines

- Whole tumor cells, tumor cell lysates and apoptotic tumor cells.
- Idiotypic-based vaccines.
- Individual chaperone proteins (e.g., heat shock protein).
- Multichaperone lysates (chaperone-rich cell lysate).

Limitations & combination therapy

- There is only a limited amount of vaccine material available from individual patients; and limited access to tumor-derived material.
- Mechanisms of tumor-induced tolerance that may impair the function and efficiency of dendritic cells (DCs) administered to patients with advanced diseases.
- Combination chemoimmunotherapeutic strategies to overcome tumor-induced immunosuppression may foster the efficacy of DC-based immunotherapy.

Conclusion

- Personalized tumor immunotherapy has an advantage over peptide-based vaccines as it may avoid the occurrence of tumor immune-escape variants.
- Personalized DC-based vaccines are safe and capable of inducing polyclonal immune responses targeting multiple tumor-specific antigens.
- DC-based combination chemoimmunotherapies may have the potential to significantly improve the treatment of cancer patients.

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Figure 1. *Ex vivo* preparation of dendritic cell-based anticancer vaccines

Vaccine generation includes: choosing the source of DC, their *ex vivo* expansion, loading with tumor antigen, maturation/activation and *in vivo* delivery. The primary source of DC currently used in clinical trials and in most animal studies consists of myeloid DCs derived from the blood (for clinical trials) or bone marrow precursors (for most animal studies). Further activation with cytokines/chemokines, TLR-ligands or growth factors leads to maturation of the differentiated DCs. Different approaches have been evaluated to load DCs with tumor-specific antigens that include tumor-derived peptide/protein, RNA/DNA, total lysates, apoptotic or necrotic tumor cells, chaperone proteins, exosomes or tumor cell–DC fusion. DCs may be administered intravenously, intradermally, subcutaneously or by intranodal or intratumoral injection.

DC: Dendritic cell; TLR: Toll-like receptor.

Figure 2. Tumor-induced immunosuppression affects DC-based vaccine efficiency

The tumor microenvironment (including immunosuppressive factors produced by tumor cells such as TGF- β , IL-10, VEGF and IDO) may promote the generation and expansion of immunosuppressive cells (e.g., Tregs, iDC, pDC and MDSCs). These immunoregulatory cells or these immunosuppressive factors impair the therapeutic efficiency of DC-based vaccines and foster tumor progression.

DC: Dendritic cell; iDC: Immature DC; IDO: Indoleamine 2,3-dioxygenase; mDC: Mature DC; MDSC: Myeloid-derived suppressor cell; pDC: Plasmacytoid DC; Treg: Regulatory T cell.