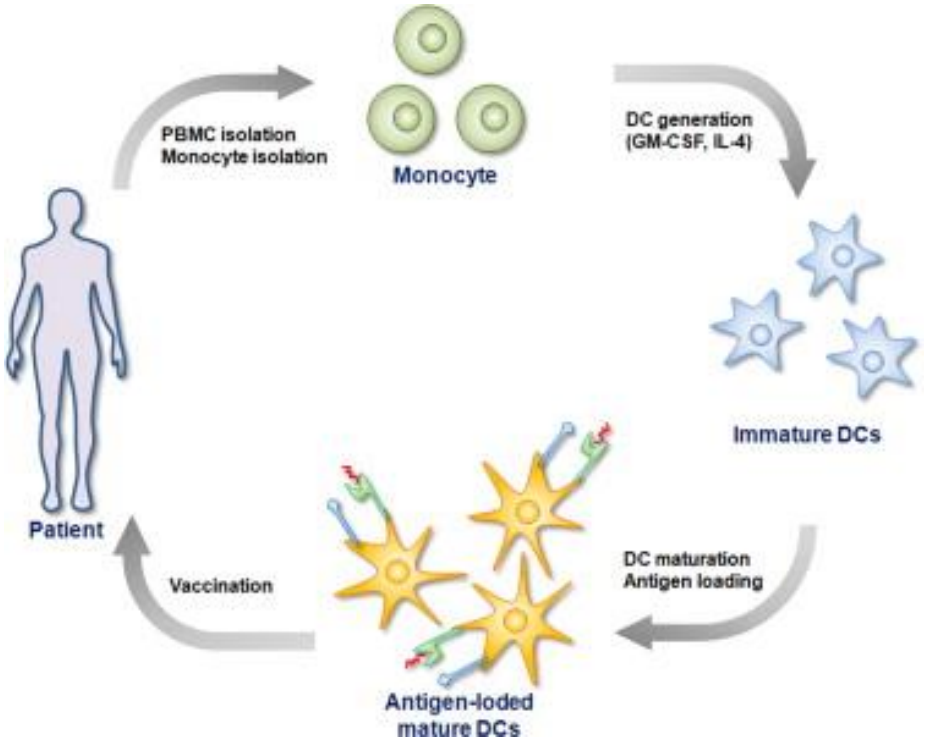
Dendritic cell vaccinations and optimal antigen presentation



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Outline

Summary

Dendritic cells (DC) form the bridge between non-specific innate and antigen-specific adaptive immunity. As antigen presenting cells, the main role of these cells is the uptake of antigens, both foreign and self, and the presentation of these antigens to T cells. DCs can be found in the blood, in the skin but also in interstitial tissues of many organs where they continuously probe their environment for antigens. In case of an infection, for example by bacteria or viruses, DCs take up molecules from the microbes and undergo maturation before migrating to lymph nodes and presenting the antigens to naïve T cells. . The antigens-specific T cells, both $CD4^+$ T_{Helper} and $CD8^+$ cytotoxic T cells, become activated and mediate clearance of the infection. Additionally, DCs help maintaining immunologic tolerance to host tissues by presenting self-antigens to suppressor T_{Reg} cells. DCs are also important in tumour immunology in both beneficial and detrimental manners. By presenting tumour-associated antigens (TAA) tumour-specific T cells can be induced which can kill tumour cells. However, malignant cells can evolve mechanisms to drive DCs into mediating tolerance to TAAs, thereby suppressing immune responses to the tumour. Injecting patients with DCs loaded with TAAs is currently under investigating for their potential as an anti-cancer vaccine aimed to boost the patient's anti-tumour immunity. Malignant melanoma and multiple myeloma are some of the cancers in which the application of DC vaccination is studied in clinical trials. Despite the fact that DC vaccination still needs optimization, in some patients tumour-specific immune responses where observed and these patients generally showed a better survival, implying that vaccination with DCs could improve the health status of patients with cancer. DC vaccination might also be harnessed in patients receiving allogeneic haematopoietic stem cell transplantation (allo-HSCT). Allo-HSCT is currently the only curative therapy against some haematological cancers. The success of the therapy depends on the induction of graft-versus-leukaemia (GVL) responses by donor lymphocytes attacking remaining malignant haematopoietic cells. However, the application of allo-HSCT is complicated by the occurrence of graft-versus-host (GVH) responses mediated by donor lymphocytes attacking host tissues. These responses can result in a severe and life-threatening condition called graft-versus-host disease (GVHD). Due to the immunomodulatory role of DCs, vaccines with these cells might be used to both enhance the GVL effect and reduce GVH responses. As a possible strategy, DCs presenting both TAAs can be used to activate T cells before donor lymphocyte infusion (DLI). DLI is a procedure in which donor lymphocytes are injected into the patient and is normally used to boost anti-leukaemia responses in relapsing disease after allo-SCT. This strategy might stimulate GVL responses whereas GVH reactions are dampened.

Introduction

The immune system comprises two systems: the innate immune system and antigen-specific adaptive immune system. The innate immune system responds quickly after invasion by pathogenic organisms, but is nonspecific and builds no memory against re-encounters with the same pathogen [1]. The adaptive immunity takes longer to respond, but is antigen-specific and memory is developed to allow quicker responses to recurring invasion by an antigen encountered earlier. Dendritic cells (DC) are antigen-presenting cells (APC) that form the bridge between the innate and adaptive immunity. DCs are generated from hematopoietic stem cells in the bone marrow differentiating into myeloid progenitors. Four major categories of DCs are known: Langerhans DCs in epidermal layers of the skin, interstitial DCs in most organs, monocyte-derived DCs (moDC) and plasmacytoid-derived DCs (pDC) in blood and peripheral lymphoid organs [1]. The first three are all myeloid DCs (mDC) while the latter is of lymphocyte origin.

Dendritic cells and immunity

The main function of DCs is the capture and presentation of processed antigens on both class I and II MHC molecules. DCs are continuously monitoring the extracellular environment for foreign antigens [2–5]. DCs express different types of pattern recognizing receptors (PRRs), such as Toll-like receptors TLR, C-type lectins (CLR) and intracellular helicases like retinoic acid-inducible gene I (RIGI). These receptors detect different types of microbial conserved components distinct from the host, also called pathogen-associated molecular pattern (PAMP), such as cell-wall components, like lipopolysaccharides (LPS), lipopeptides, or nucleic acids like viral or bacterial RNA and DNA. Triggering of such receptors results in the uptake of a foreign particle. Upon capturing and processing foreign antigens, DCs mature and undergo a change that makes them lose their antigen-uptake capacity but gain functions of APCs, such as enhanced class I and II MHC expression. Protein antigens are processed into smaller peptides which can be loaded on class I and II MHC molecules while lipid antigens are loaded on CD1d family molecules, a non-classical set of MHC molecules. DCs in the lymph node capture and process antigens from the lymph and present them to naïve $CD4^+$ T cells to prime them and inducing secretion of IL-2, proliferation and clonal expansion of the T cells. DCs from other tissues migrate to lymph nodes after antigen-processing and maturation to present the antigens to the primed T cells. Naïve $CD4^+$ T cells can differentiate into antigen-specific helper T cell (T_H cells) of different types upon interaction with a DC, such as T_H1, T_H2, T_H17 and follicular T_{FH} cells. The type of T_H cell depends on the co-stimulatory molecules and interleukins expressed by the DCs, which in turn is dependent on the kind of antigen the DC has captured (bacterial, viral etc.). T_H cells regulate other immune cells. Naïve $CD8^+$ T cells can become antigen specific cytotoxic T lymphocytes (CTL) specialised in killing malignant or virally infected cells.

DCs are also reported to be important in B cell development [6]. Mouse DCs were able to present antigens to B cells and induce class-switch independent of T_H cells, while the latter was still required for antibody production. It was also showed that DCs stimulate proliferation and survival of the B cells. Whether human DCs have a similar role in B cell development is unknown.

Dendritic cells and tolerance

Immature DCs are not inactive. They sample the environment and scavenge cellular debris and dying cells, thereby capturing and processing self-antigens and harmless foreign antigens [2, 7, 8]. Together with IL-10 stimulation and activation of the WNT-β-catenin pathway, the DC develops a tolerogenic phenotype. Like their pro-inflammatory counterparts, the tolerogenic DCs migrate into the lymphatic system to present them to naïve $CD4^+$ T cells. Under these circumstances, T cells specific for self-antigen are deleted or induced to differentiate into regulatory T (T_{Reg}) cells. T_{Reg} cells are important in the prevention of auto-immune responses, the attack of own tissues by the immune system, by secreting IL-10 and TGFβ which inhibits T cell function and proliferation. Ligation of PD1 (Programmed cell Death Protein 1) on $CD8^+$ T cells with PD-L1 (Programmed cell Death Ligand 1) expressed by tolerogenic DCs also directly inhibits function of $CD8^+$ T cells [9]. Therefore, DCs are essential in mediating tolerance to own tissues as well.

Tumour immunity and dendritic cells

Dendritic cells are also important in tumour immunity. DCs take tumour-associated antigens (TAA) from captured apoptotic tumour cells and living cells and process them for antigen presentation. By presenting the TAA to naïve T cells, the generation of tumour antigen-specific $CD8^+$ CTLs is induced. This way, DCs are able to generate anti-tumour immune responses [2]. It is reported that dendritic cells are even required for the induction of anti-tumour immunity *in vivo*[10, 11].

However, in many cancers tumour cells are able to evade immunity [2]. By secretion of IL-6 and macrophage colony-stimulating-factor (M-CSF), monocytes are skewed to differentiation into macrophages instead of monocyte-derived DCs. Thymic stromal lymphopoetin (TSLP) expressed by tumour cells interferes with DC maturation by inducing the expression of OX40L in DCs, which results in a switch of $CD4^+$ T cells to pro-tumorigenic T_H2 cells. T_H2 cells can accelerate tumour growth by secreting IL-4 and IL-13, thereby inhibiting apoptosis and inducing macrophages to secrete epidermal growth factor (EGF), which can stimulate breast cancer cell proliferation. Ligation of ILT7 on pDCs with bone marrow stromal antigen 2 (BST2) results in DCs inducing T cells to produce IL-10, thus suppressing anti-tumour responses. Additionally, DCs can directly promote survival and angiogenesis in certain types of tumours [2].

Although DCs can have pro-tumour effects and are often misused by tumour cells, several studies are on-going on harnessing these antigen-presenting cells to mediate anti-tumour CTL responses.

Advantages of this novel kind of strategy are that immune response against primary and metastatic tumours are elicited and that long-lasting memory is induced, thereby possibly developing immunity against relapsing malignancies [2, 12–14]. In this thesis, clinical trials of DC vaccination and methods to optimise antigen presentation in melanoma and multiple myeloma (MM) are discussed. We aim to find which strategy is most suitable for use in combination with allogeneic hematopoietic stem cell transplantation (allo-HSCT) as a therapy for leukaemia and other haematological malignancies.

Dendritic cell vaccination against malignant melanoma

Several clinical trials on DC vaccination against solid tumours are performed. In this thesis, we discuss several trials in melanoma patients. Melanoma is a type of cancer derived from melanocytes, the cells responsible for pigment production. In early stages, it is well treatable by surgical removal of the tumour, but the emergence of metastases means a poor prognosis. We chose for this type of cancer because many DC vaccination trials with different strategies are performed with melanoma patients. We discuss the type of DCs, maturation status, antigen loading of DCs, route of administration and the clinical responses to the vaccinations if available.

Type and maturation of DCs in vaccines

As mentioned previously, there are different types of DCs. The main types are monocyte-derived DCs (MoDC) and plasmacytoid DC (pDC). In many clinical trials of DC vaccination in melanoma patients, MoDCs are generated ex vivo by stimulating peripheral blood mononucleated cells (PBMC) with granulocyte-monocyte colony-stimulating factor (GM-CSF) and IL-4[15–21]. In the cooperative studies of Banchereau *et al*. 2001, Palucka *et al*. 2003 and Paczesny *et al*. 2004, $CD34^+$ progenitor-derived DCs were used [12–14]. Recombinant GM-CSF was administered to patients to enrich $CD34^+$ hematopoietic progenitor cells (HPC). Leukapheresis was performed to collect $CD34^+$ HPCs and the cells were enriched. $CD34^+$ DCs were generated by incubating HPCs with autologous serum, β-mercaptoethanol and L-glutamine and subsequent stimulation with GM-CSF and tumour-necrosis factor (TNF). In most studies the DCs were stimulated to maturation after the loading with TAAs. In all studies the generated DCs were capable of inducing immune responses to the vaccinated TAAs.

In one study it was demonstrated that DC subsets varies in their capacity of cross-presentation and the presentation of exogenous soluble antigens on MHC class I and II [22]. $CD1c^+$ DCs and MoDCs were both able to cross-present full-length tumour antigen NY-ESO-1 to $CD8^+$ T cells. Cross-presentation was not observed in pDCs, since these cells were only able to present MHC class I epitopes after infection with life virus and could not present antigens when they were pulsed with inactivated whole virus[22]. PDCs were also found to be less efficient in $CD4^+$ T cell activation. When DCs generated from $CD34^+$ stem cells where compared with MoDCs, it was observed that MoDCs could take up both soluble NY-ESO-1 antigen and antigen-antibody immune complexes and present them on MHC class I, while $CD34^+$ derived DCs could not [23]. However, similar as in the cooperative studies by Banchereau, Palucka and Paczesny, $CD34^+$ DCs were able to present pre-processed peptides [12–14, 23]. Apparently, the DC subpopulations chosen for vaccination is dependent on the kind of antigens, but also on the method of antigen loading, as both determine the proteolytic pathway the antigens will follow before being presented on MHC molecules [22].

Besides type of DCs, the maturation state is also crucial for induction of effective anti-tumour immunity. This was demonstrated in a trial by De Vries *et al*., 2003 [17]. In a clinical trial the patients were divided into two groups. One group received vaccination with mature DCs, while immature DCs were administered to the second group. DCs were loaded with several gp100- and tyrosinase peptides and KLH. A strong proliferative response against KLH was observed in patients receiving mature DCs after the first vaccination, which was not enhanced by subsequent vaccinations, suggesting that one vaccination was sufficient. No clear responses against KLH were observed in patients receiving immature DC vaccination. Cytokines IFN-γ, TNF-α and IL-2 were produced by PBMCs from patients vaccinated with mature DCs upon KLH challenge, in contrast with immature DC-vaccinated patients. The cytokine profile also indicates that the type of immune response induced by DC vaccination is T_{h1}, as no IL-4 production was produced. In 9 out of 10 patients receiving mature DC vaccination, antibodies against KLH are detected and the antibody titres increased after multiple vaccinations. The antibodies detected were mainly of the IgG2 isotype, another indication of T_{h1} responses. In not any of the patients vaccinated with immature DCs were antibodies against KLH detected after three vaccinations. Delayed-type hypersensitivity (DTH), an indicator of immune responses induced by DC vaccination was observed in all patients receiving vaccination with mature DCs. In contrast, DTH was not observed in any of the immature DC vaccinated patients.

In conclusion, both the subset and the maturation state of DCs are important in DC vaccination. The subsets differ in their capacity to process and cross-present different kinds of antigens, implying that the choice of DC subset depends on the nature of the antigen. Vaccination with mature DCs showed better capacity to elicit anti-tumour responses than immature DCs.

Targeting melanoma tumour antigens

To induce anti-tumour immune responses, DCs needs to be loaded with TAAs.

In many studies on melanoma DC vaccines, short peptide antigens derived from TAAs were used [12–17, 21]. In all studies, DCs loaded with short peptides showed capacity to induce peptide-specific $CD8^+$ T cells which could kill both T2 cells transfected with TAAs and melanoma cell lines expressing the TAA. However, these premade peptides are HLA-restricted, which explains why in most studies only HLA $A*0201^+$ patients were included [19]. Additionally, while DCs are capable of taking up exogenous peptides and present them on MHC class I, the presentation has a short duration due to recycling of MHC molecules [19]. On the other hand, whole protein-based vaccines show reduced DC capacity to prime $CD8^+$ T cells [19]. Loading with peptides from a single TAA can also result in immune escape by tumour cells, which was observed in the study by Thurner *et al*. 1998 [16]. In this trial, patients were vaccinated with DCs loaded with melanoma-associated antigen 3 (MAGE-3). In patients with partial responses the remaining lesions consisted completely of MAGE-3-negative tumour cells with no infiltration of peptide-specific $CD8^+$ T cells. Possibly because of this, DCs in other studies are loaded with peptides from multiple melanoma-TAAs, such as melanoma antigen recognized by autologous T cells (MART-1/MelanA), gp100, tyrosinase and MAGE-1 and MAGE-3.

Banchereau, Palucka and Paczesny *et al*. performed several clinical studies in a group of 18 patients vaccinated with peptide loaded $CD34^+$ DCs[12–14]. These DCs were loaded with short peptides derived from MelanA/MART1, MAGE-3, tyrosinase and gp100 and control antigen keyhole limpet hemocyanin (KLH). It was observed that T cell responses against the peptides was induced by the vaccine, as proliferation and IFN-γ production was observed upon stimulation with peptides [12]. Additionally, it was observed that TAA peptide-specific $CD8^+$ T cells were induced, although there was variation among the patients in the number of vaccines required to induce such responses [13]. Importantly, in some patients a peak of T cell responses was followed by a decline, possibly due to migration to tumour sites or cell death due to restimulation during activation [13]. The $CD8^+$ T cells induced by the vaccine with peptide-loaded $CD34^+$ were capable of lysing peptide-pulsed T2 cells, melanoma cell lines and were also capable of binding to MHC-peptide tetramers, indicating recognition of both peptide-MHC complexes and tumour cells [14]. However, in a few patients the vaccine failed to induce $CD8^+$ T cell responses. Further research is warranted to determine whether this is vaccine-related or has something to do with the health status of the patients, but all of these patients without responses showed progressive disease at the end of the study [14].

Other strategies to load DCs are multiepitope polypeptides (MEP), long peptides or electroporating DCs with TAA mRNA [15, 19, 20, 24]. A multiepitope polypeptide for melanoma (MEP-mel) is generated containing four antigenic peptides: two from gp100, one from MART-1 and one of

tyrosinase [19]. Protein cleavage and antigen presentation is enhanced by linking the peptides to each other with proteasome recognize sequences. DCs either transfected with MEP-mel or loaded with peptides were capable of inducing IFN-γ production. IFN-γ production by T cells lasted longer when stimulated with DCs loaded with MEP-mel than peptide-loaded DCs. Additionally, inhibition of the proteasome by lactacystin reduced antigen presentation in DCs loaded with MEP-mel, as observed by decreased T cell activation, indicating that MEP-mel requires proteasomal cleavage to be presented on MHC class I molecules [19].

DCs loaded with a long peptide derived from melanoma TAA MELOE-1 containing multiple $CD4^+$ and $CD8^+$ T cell epitopes were *in vitro* capable of inducing both MELOE-1-specific $CD4^+$ T cells and $CD8^+$ CTLs [20]. Clinical studies with DCs loaded with long peptides in melanoma patients are to be conducted yet.

Both Nestle *et al.* (1998) and Palucka *et al*. (2006) investigated vaccination with DCs loaded with tumour lysates or whole dead tumour cells [15, 18]. As previously mentioned, exogenously peptide-loaded DC vaccines are HLA restricted; while DCs loaded with tumour lysates do not have this drawback. By taking up tumour lysates, multiple antigens are processed and presented by the DCs, possibly allowing a broader repertoire of melanoma-specific T cells. It was observed that immunity against melanoma TAAs could be induced by DCs loaded with tumour lysates. An association between occurrence of DTH reactivity to TAA peptides and clinical responses upon vaccination was observed. Large infiltration of $CD8^+$ T cells were seen in DTH sites analysed by immunochemistry and *in vitro* tests showed specific cytotoxicity for MART-1 and gp100 but not tyrosinase. In the 2006 study by Palucka *et al*. autologous MoDCs were loaded with killed cells from the melanoma cell line Colo829 [18]. In a few (3 out of 13) tested patients $CD8^+$ T cell responses were observed against peptides derived from MART-1 (melanoma antigen recognized by T cells), a melanoma TAA, and 2 of them showed IFN-γ production *in vitro* upon stimulation with MART1 peptides. In one of them even $CD8^+$ T cell responses against a novel MART-1 peptide was observed, suggesting possible cross-presentation of TAA induced by the DC vaccine. While these studies showed that DCs loaded with killed tumour cells instead of peptide-antigens can induce anti-tumour responses, the clinical responses were poor. An important remark on this study is that only stage IV melanoma patients were included, while in other studies it was demonstrated that stage III patients had both stronger immune responses and better clinical responses to DC vaccination. Nevertheless, possible cross-presentation by DCs loaded with killed tumour cells might be an advantage over HLA-restricted peptide-loaded DC vaccines. Although no severe signs of auto-immunity was seen in patients vaccinated with tumour cell-loaded DCs, question remains whether immune responses against non-tumour-specific antigens are also induced in a significant level, as tumour cells are derived from host cells and contains many self-antigens. In an early animal study by Ludewig *et al*., 1999, severe auto-

immunity was observed in mice vaccinated with DCs with tumour-antigens shared with normal host cells [25]. This might indicate that careful selection of TAAs is necessary to prevent auto-immunity while maintaining anti-tumour capacity.

Electroporation of DCs with mRNA of melanoma TAA is another strategy investigated by Aarntzen *et al*., 2012 [24]. MoDCs were electroporated with mRNAs encoding melanoma TAAs tyrosinase and gp100. By this method, the mRNAs are translated into proteins which are then processed to small peptides which can be presented as antigen on MHC molecules, thereby not having the disadvantage of HLA restriction and allowing a larger T cell repertoire. This might also be a strategy to circumvent the selection of TAA negative tumour cells as seen in the study by Thurner *et al*, 1999, where DCs were loaded with only one TAA peptide [16]. TAA-specific $CD8^+$ T cells were detected in the blood of some patients, which were not present before vaccination in three patients, indicating that TAA mRNA-electroporated DCs can newly induce or enhance $CD8^+$ T cell immunity. TAA-specific $CD4^+$ T cells were observed in PBMC cultures of some patients, indicating that electroporated DCs can also induce antigen-specific $CD4^+$ T cell responses. None of the samples tested FOXP3 positive, excluding a suppressor phenotype of the $CD4^+$ T cells. In most patients $CD8^+$ T cells were detected in SKIL cultures and in some of them these were TAA-specific. Most stage III and some stage IV patients showed IFN-γ production upon stimulating with protein- or peptide-loaded target cells, indicating that TAA-specific T cells capable of recognizing tumour cells were induced by the vaccine. Similar to other studies, it was observed that stage III patients overall showed better immune responses than stage IV patients, an indication that disease progression is important for the efficacy of DC vaccination [12–14, 24].

A vaccine can also be developed using DCs fused with tumour cells. An animal study in mice is performed by Wang *et al*. in 1998 to study the potential of DC-melanoma hybrid cells to induce anti-melanoma immunity [26]. It was observed that mice vaccinated with DC-B16 melanoma hybrid cells showed longer survival and lower tumour incidence upon challenging with B16 melanoma cells. Additionally, T cells isolated from mice immunized with these hybrid cells were able to lower tumour burden in mice with pulmonary metastases upon adoptive transfer. Both observations indicate that DC-tumour fusion cells can induce therapeutic and protective anti-tumour immunity.

Route of administration of DC vaccine

DC vaccines can be administered intradermally (in the dermis of the skin), subcutaneously (underneath the skin), intranodally (directly into a lymph node) and intravenously, possibly influencing efficacy of the vaccine.

Lesterhuis *et al*., 2011, performed a comparative study on intradermal and intranodal administered vaccination. [21]. Additionally, it was evaluated if low-dose IL-2 injections might enhance immune responses to the DC vaccine, as this cytokine stimulates T cell activation and proliferation. A more constant redistribution of DCs after vaccination was seen among patients injected intradermally, while intranodally injected patients show in some patients a higher percentage of redistribution but in seven out of 24 patients no redistribution was seen at al. Incorrect injection might be the cause of this observation. Also, redistribution of DCs in patients receiving an intranodal vaccine might be partially the cause of passive lymph flow instead of active migration. A possible explanation for the differences in redistribution between intradermal and intranodal vaccination might be that in the case of the first only the most viable DCs actively migrate to lymph nodes. No clear differences were observed in the number of targeted lymph nodes between both routes of administration. Levels of KLH-specific T cell proliferation were similar between patients receiving intradermal or intranodal vaccinations with or without concomitant low-dose injection of IL-2. The anti-KLH antibody responses were also similar between the four groups of patients, indicating that KLH-specific immune responses are not influenced by route of administration of the vaccination or concomitant injection of IL-2. In the majority of patients tested, TAA peptide-specific T cells are found in DTH sites. No differences were seen in antigen recognition between intradermally or intranodally vaccinated patients, but TAA-specific $CD8^+$ T cells from intradermally vaccinated patients show higher capability of tumour cell recognition indicated by increased IFN-γ production and cytolytic activity. A higher number of patients receiving intranodal vaccination show tetramer positive and antigen-specific T cells in patients upon IL-2 treatment. Also, the number of tetramer-positive T cells increased but no differences in peptide and tumour cell recognition were observed. In intradermally vaccinated patients IL-2 treatment did not affect the number of tetramer-positive T cells or IFN-γ production upon stimulation with peptides, protein or tumour cells. These observations suggest that IL-2 treatment does not augment the recognition of tumour cells after DC vaccinations and thus has no beneficial effect. Moreover, an increased number of regulatory T cells ($CD4^+$, $FOXP3^+$) is observed after both intradermal and intranodal vaccination with concomitant administration of IL-2, which may even weaken the anti-tumour responses induced by vaccination with DCs. In this study, intravenously and subcutaneously administered vaccinations were not discussed. In the earlier study by Thurner *et al*., the amount of TAA-specific T cells decreased when patients were subsequently receiving intravenous vaccination after being intradermally vaccinated [16]. This might indicate that

intravenous vaccination result in overstimulation and subsequent T cell deletion. In studies by Banchereau *et al*., Palucka *et al*. and Paczesny *et al*., the DC vaccines were administered subcutaneously [12–14]. These trials brought evidence that their vaccine was able to induce TAA peptide-specific $CD8^+$ T cell responses capable of cytolysis of tumour cells. In the study by De Vries *et al*., patients were initially vaccinated both subcutaneously and intravenously. The observation that most DCs remained in the fat tissue upon subcutaneous vaccination was reason to switch to a combination of intradermal and intravenous administration of the DC vaccine. Whether this switch results in improved induction immune responses was unclear, as most patients were already immunized by the subcutaneous vaccine before intradermal vaccination was used.

In conclusion, based on these studies, intradermal vaccination seemed to be more effective in inducing anti-tumour $CD8^+$ T cell responses, as it results in better cytolytic activity when compared with intranodal vaccination [21]. Co-administration of IL-2 does not result in enhanced anti-tumour responses, even though the number of tetramer-positive cells in intranodally vaccinated patients increased. The observation that IL-2 treatment could results in higher amounts of regulatory T cells might even indicate a detrimental effect on the efficacy of DC vaccination. Intravenous vaccination with DCs could result in a decline of TAA-specific $CD8^+$ T cells as observed by Thurner *et al*. when patients were already vaccinated intradermally [16]. Additionally, it was observed in multiple myeloma patients receiving intravenous vaccination that the majority of the DCs accumulated in the liver, spleen and lungs, indicating this route to be less effective in inducing T cells [27]. Subcutaneously administered vaccines might result in the majority of DCs remaining in the fat tissue, but it remains unclear whether this affects efficacy in comparison to intradermal vaccinations[17].

Clinical responses and toxicity

Besides the apparent induction of anti-tumour immunity by DC vaccination by various studies, both *in vivo* and *in vitro*, most relevant is whether it induces clinical responses such as partial or complete regression of tumours, clearance of disease, prolonged disease free or median overall survival. Also, the vaccine requires being safe and tolerated for clinical use.

In not any of the clinical trials with DC vaccination against melanoma were severe signs of toxicity or auto-immunity observed. In some studies, mild effects such as fever were seen [12–18, 21, 24]. In one study it is observed that DC vaccination against melanoma antigen can induce progression of pre-existing vitiligo [12]. The absence of toxicity of DC vaccines indicates this method of immunotherapy is feasible, save and well-tolerated. Delayed-type hypersensitivity (DTH) was observed in most studies at the injection site and this is demonstrated to be a marker for immune reactions to the vaccine, as a higher degree of DTH correlates with better clinical responses. In the various studies on DC vaccines, it was demonstrated that patients who showed immune responses to the vaccine *in vitro* generally showed better clinical responses as well. This observation indicates that DC vaccination can induce anti-melanoma immunity which can result in partial or complete regression of tumour mass, and might even prolong time of relapses due to induction of immunological memory to melanoma TAAs. In some studies it was seen that patients with stage III melanoma showed better responses to the vaccine than stage IV patients, indicating that DC vaccination is more effective when melanoma is less advanced. Possible explanations can be that advanced melanoma has a stronger degree of immune suppression, the patients become tolerant to melanoma antigens or the patient's immune system is weakened by high tumour burden or a combination.

Table 1 - Overview DC vaccination studies in melanoma: Studies on DC vaccination in melanoma patients and/or *in vitro*. Listed are type of DC used, antigen, route of administration (s.c. = subcutaneous, i.d. = intradermal, i.v. = intravenous, i.n. = intranodal), immune responses to TAA (or KLH) and clinical responses (PD = progressive disease, NPD = non-progressive disease, NE = non-evaluable, MxR = mixed response, PR = partial response).

Study	DC	Antigen	Route of administration	Immune responses to TAA	Clinical responses
Nestle *et al*. 1998 [15]	Immature MoDC	Peptides (n=12)	i.n.	9/12	7 PD, 2 PR, 1 CR, 1 MR
		Tumour lysate (n=4		2/4	2 PD, 1 CR, 1 PR
Thurner *et al*. 1999 [16]	Mature MoDC	MAGE-3A1 peptide	3 s.c./i.d. + 2 i.v.	8/11 patients	6/11 patients
Bancherau *et al*. 2001 [12]	Mature $CD34^+$ hemapoetic progenitor cell-derived DC	Peptides from MART-1, gp100, tyrosinase, MAGE3	4 s.c.	16/18 patients	2 patients rapid PD, 6/7 patients PD in 10 weeks, 7/10 patients regression of tumour size
Palucka *et al*. 2003 [13]				5/18 (first vaccination)	7/18 NPD, 3/18 no evidence of disease, 7/18 PD., 1/18 NE.
Paczesny *et al*. 2004 [14]					9/12 NPD, 3/12 PD
De Vries *et al*. 2003 [17]	Immature MoDC	Peptides from gp100, tyrosinase	3 s.c. + i.v. or 3 i.d. + i.v.	4/9 (T cell only, to KLH)	8/9 PD, 1 NE
	Mature MoDC			10/10 T cell to KLH, 8/10 Ab to KLH	3/10 SD, 1/10 PR, 1/10 MxR, 4/10 PD
Palucka *et al*. 2006	Mature MoDC	Tumour lysate	s.c.	3/13 $CD8^+$ T cell response;	1/20 CR, 1/20 PR Median overall survival 22.5 mo.
Levy *et al*. 2008 [19]	Mature MoDC	Multiepitope polypeptide	n/a (*in vitro*)		n/a (*in vitro*)
Lesterhuis *et al*. 2011 [21]	Mature MoDC	Peptides from gp100, tyrosinase	i.d. (11 patients)	91% tetramer-positive T cells,	Median time to recurrence 32 mo.
			i.d. + IL-2 (10 patients)	89% positive T cells	Median time to recurrence 27 mo.
			i.n. (12 patients)	58% tetramer positive T cells, 50% peptide specific, 42% protein recognition, 17% tumour recognition	Median time to recurrence 42 mo
			i.n. + IL-2 (10 patients)	88% tetramer positive T cells, 86% peptide specific, 29% protein recognition, 14% tumour recognition	Median time to recurrence 14 mo.
Rogel *et al*. 2011 [20]	Mature MoDC	Long peptide MELOE-1	n/a (*in vitro*)	7/7 (healthy donors)	n/a (*in vitro*)
Aarntzen *et al*. 2012 [24]	Mature MoDC	Gp100, tyrosinase mRNA	i.n.	17/26 stage III (15/17 $CD8^+$ T cell); 11/19 stage IV (3/11 $CD8^+$ T cell)	Stage III (n=26): 1 PD, 25 NED; stage IV (n=19): 11 PD, 6 SD, 1 MxR, 1 PR

Dendritic cell vaccination against multiple myeloma

The potential of DC vaccination is also tested against multiple myeloma (MM). MM (also known as Kahler's disease) is a cancer originated from plasma cells, the mature B cells responsible for antibody production that normally reside in the bone marrow [28]. The cancer spreads from the bone marrow via the lymphatic system to lymph nodes, spleen and other organs. It is considered to be treatable but incurable. Chemotherapy, steroids, bortezomib (a proteasome inhibitor), thalidomide and autologous stem cell transplantation might result in remission of the disease [28]. The median survival is up to three years and complete remission is reached in 5% of the patients with conventional chemotherapy [29]. In one third of MM patients, a combination of high dose chemotherapy and autologous stem cell transplantation result in long-lasting remission. However, treatment often fails due to relapses which are refractory to chemotherapy. Therefore, alternative therapies are currently under investigation. Even though most MM patients have type I $CD4^+$ T cells and $CD8^+$ T cells specific for MM cells in their blood, these cells failed to control the disease [27]. Advanced MM patients showed more tumour-specific $CD4^+$ T_{H2} cells compared with T_{H1} cells, the former being detrimental as they stimulate growth of tumour cells [27]. DC vaccinations might enhance the anti-myeloma responses and break immunologic tolerance to myeloma-antigens, thereby enabling controlling or eradicating the disease. However, the results from various studies showed that improvement is necessary [29, 30].

Type of DC

There are different protocols to generate DCs *ex vivo*, each possibly resulting in DCs with different properties regarding antigen uptake and presentation [31]. Commonly in most studies, they are derived by stimulating monocytes from patients PBMCs with GM-CSF and IL-4. From PBMCs both $CD34^+$ and $CD34^-$ precursor cells can be induced to differentiate into DCs, depending on culture conditions. An advantage of $CD34^-$ cells is that they can be purified by removing all non-adherent cells, thereby reducing contamination by lymphocytes. Human $CD14^+$ monocytes can also be a source of DCs. Most DC precursors are dependent on GM-CSF for DC induction, but there are other combinations of cytokines which can induce differentiation into DCs without GM-CSF. The source of DCs and the culturing conditions is important to consider when DCs are used for cancer immunotherapy, as different types of DCs varies in their functional capacity[22]. As mentioned previously on DC vaccination against melanoma, some DCs are able to actively process and cross-present exogenously loaded antigens, whereas others can only present exogenous pre-processed peptide antigens or internally generated antigens through transfection or transduction with tumour

DNA or mRNA [22]. Therefore, it can be assumed that the type of DC used in anti-myeloma DC vaccination also depends on both the nature of the antigen and the route of antigen loading, as different subsets of DCs differ in their antigen processing and presentation machinery.

In most early studies on DC vaccination in multiple myeloma patients, for example Titzer *et al*. (2000) and Wen *et al*. (2001) immature DCs derived from patients PBMCs were used [29, 32]. While TAA specific cellular and humoral responses were induced, the clinical results were disappointing. As described previously on DC vaccination against melanoma, maturation of DCs is essential for inducing potent anti-tumour immunity, as mature DCs have a higher capacity to present antigens and prime T cells due to enhanced expression of MHC and co-stimulatory molecules [17]. Immature MoDCs revert back to their monocyte phenotype upon IL-4 and GM-CSF withdrawal [27]. Also, immature DCs can hamper antigen-specific immune responses by inducing IL-10 producing T cells due to the lack of co-stimulatory factors [33]. The importance of DC maturation was also confirmed by Yi *et al*. who performed clinical studies in MM patients [34]. Although the cohort size was small (n=5), in most patients (four) an increase in IFN-γ secretion by PBMCs was detected after vaccination, while no relevant differences were seen in IL-4 production, indicating dominantly T_{H1} type responses to the vaccine. In these patients an increase in the number of Id-specific B cells was detected as well. This further confirms that DC vaccination with Id as target can induce both cellular and humoral immune responses and maturation of DCs improves efficacy of DC vaccines.

In many cancers, including MM, DCs are defective in their capacity to induce anti-tumour responses [35, 36]. As previously mentioned, tumour cells harness multiple mechanisms to evade host immunity, including interfering with DC development. DC functioning of bone marrow-derived DCs (BMDC) impaired by exposure to either myeloma cells or tumour culture conditioning medium (TCCM) could be restored by inhibition of p38 mitogen activated protein kinase (MAPK) [35]. In other studies it was observed that TCCM activates Janus kinase (JNK) and inhibits extracellular regulated kinase (ERK). These observations might imply possible strategies to improve efficacy of DC vaccination by inhibiting p38 MAPK and JNK and activating ERK, since DC functioning is often impaired in patients with cancer, especially when disease is at an advanced state.

Targeting multiple myeloma tumour antigens

Myeloma cells produce a paraprotein consisting of a monoclonal Ig molecule with specific antigen determinant structures called Idiotype (Id). These Id structures could thus be a candidate TAA for use in DC vaccination. In multiple early studies on immunotherapy against MM, it was observed that immunity against Id can be induced in MM patients [29, 32, 34, 37–39]. Results of early DC vaccination were disappointing, possibly due to the use of immature DCs, although it was observed that DCs loaded with both Id peptides or protein could induce both humoral and cellular immune responses capable of killing tumour cells in some patients.

However, Id-specific responses observed in these studies were only seen in less than half of the patients and clinical responses were rarely observed. Upon high tumour burden and high concentrations of Id in the serum of the patients, peripheral tolerance of T cells to Id is established, hampering Id-specific anti-myeloma immunity [30, 39]. An animal study in mice by Hong *et al*., 2008, demonstrated that Id-pulsed DCs can induce both T_{H1} and T_{H2} responses [40]. Both $CD8^+$ T cells and $CD4^+$ T_{H1} cells were able to kill and suppress growth of myeloma cells, while no killing was seen by T_{H2} cells. It was even demonstrated that T_{H2} cells promoted proliferation of myeloma cells. This indicates that DC vaccination in order to be effective should induce $CD8^+$ T cells and T_{H1} cells, but not T_{H2} cells. A similar pro-tumorigenic effect of T_{H2} cells is seen in breast cancer [2]. Due to the weak immunogenicity of Id, other TAAs possibly expressed by myeloma are investigated for their potential to be used in DC vaccines as a target.

Qian *et al*. investigated heat shock protein (Hsp) gp96 as a myeloma antigen [41]. $CD8^+$ cytotoxic T cells generated by stimulation with HLA-A*0201^+ gp96-pulsed DCs were able to lyse both HLA-A*0201^+ myeloma cell lines expressing gp96 and primary myeloma cells isolated from HLA-A*0201^+ patients *in vitro*. Additionally, HLA-A*0201^- myeloma cells and HLA-A*0201^+ normal B cells were not lysed, indicating specificity of the T cells. It was suggested that gp96-peptides restricted to HLA-A*0201 are shared among MM patients with the same HLA allele, thus allowing development of a universal vaccine that can be effective in many patients which are HLA-A*0201^+ [41]. This might be another advantage over Id as a targeted myeloma TAA, were vaccines needs to be prepared for each individual patient. In a later animal study it was observed that pooled gp96 from myeloma cell lines or allogeneic myeloma cells could be as effective as vaccines made from autologous myeloma cells, but single allogeneic gp96 was ineffective [42]. This implies that vaccines developed from pooled gp96 could be used as an effective vaccine and could be a cost- and time-effective alternative to custom-made gp96-derived vaccines for each patient. Furthermore, combinations of pooled gp96 and CpG with anti-IL-10 or anti-PD-1L (B7H1) treatment were effective in mice with large tumour burden. By treating patients with anti-IL-10 or anti-PD-1L, tolerance in the environment of advanced tumour lesions could be abated, increasing the efficacy of anti-tumour immunity.

Dickkopf-1 (DKK1) is another antigen that is commonly expressed on myeloma cells in most patients and rarely expressed in healthy tissue [43, 44]. In mouse studies it was demonstrated that active immunization protects against developing MM and helps eradicating established disease. However, in these studies the animals were vaccinated with peptides only, without DCs. The fact that both $CD4^+$ T cells and $CD8^+$ T cells specific for DKK1 were observed, might indicate that this antigen can be used as a target of DC vaccination as well.

Various other antigens expressed by multiple types of cancer, such as melanoma and testis cancer, might also be expressed on myeloma cells. Examples of these are MAGE-3 and NY-ESO-1 [45]. Other antigens expressed on myeloma cells are mucin 1, sperm protein 17 (Sp17) and HM1.24 [27]. However, further research is necessary to evaluate their applicability and safety to use in DC vaccines, as for example Sp17 might also be expressed on healthy T and B cells [46].

Tumour lysates are also evaluated for their use in loading DCs for vaccination in MM patients. Wen *et al*. and Hong *et al*. both conducted studies on DCs pulsed with myeloma lysates in 2002 and 2012 respectively [30, 47]. In the first study, a clinical trial, lysates from autologous myeloma cells were used to load immature MoDCs. It was observed that DCs pulsed with tumour lysates were able to induce $CD4^+$ and $CD8^+$ T cell responses against various myeloma antigens in a MHC class 1 and 2-dependent manner, including Id. Cytokine profiles indicated that the T cells were mainly of a type-1 phenotype. Cytotoxicity to both tumour lysate-pulsed autologous DCs and autologous primary myeloma cells was seen as well. Hong *et al*. performed an animal study in which the efficacy of vaccination with either Id peptide- or tumour lysate-pulsed DCs was compared in myeloma mouse models [30]. With both vaccines, mice were protected against developing myeloma, regression of existing tumour masses was induced and the surviving mice were immunized against rechallenge. Additionally, strong humoral responses were seen. $CD8^+$ T cells, $CD4^+$ T_{H1} cells and memory T cells were all observed. Although both strategies of loading DCs with melanoma TAAs succeeded in inducing anti-myeloma immunity, DCs loaded with tumour lysates show to be more potent then Id pulsed DCs, as mice vaccinated with these DCs showed a higher survival rate. Possible explanations of these observations are the weak immunogenicity of Id structures, tolerance in Id-specific T cells, and the polyclonal expansion of anti-myeloma T cells specific for multiple different antigens upon vaccination with tumour lysate-pulsed DCs [30].

Fusion of DCs and myeloma cells is also investigated. Rosenblatt *et al*., 2011, performed a study in which patients were immunized with autologous mature MoDCs fused with myeloma cells from the bone marrow [36]. In the majority of the patients, $CD4^+$ and $CD8^+$ T cells were induced and stabilization of disease was demonstrated in most of the patients with advanced MM. This study demonstrates that fusing DCs with tumour cells might be effective in inducing anti-myeloma immunity.

Route of administration of DC vaccines

In many early studies on DC vaccination in MM patients, the vaccines were administered intravenously [32, 38]. However, it was observed that DCs injected intravenously accumulate in lungs, liver and spleen [34]. This possibly renders this route of administration less potent to induce anti-tumour responses, similarly to the observations in melanoma patients. Subcutaneous injected DCs were found to migrate to lymph nodes and induce protective immune responses.

In a clinical trial by Curti *et al.* patients were vaccinated three times subcutaneously and subsequently two times intravenously with DCs pulsed with either whole Id protein or VDJ-derived peptides restricted to MHC class I [48]. It was concluded that subcutaneously administered DCs were more potent in inducing anti-myeloma responses than when DCs are intravenously injected. However, in this study patients receiving vaccines by either one of the routes were not compared to each other. In melanoma patients it was observed that intravenous administration of DCs in patients already immunized through intradermal vaccination decreases TAA-specific T cells, possibly due to over stimulation [16]. It might be possible that the same was happening in the MM study [48]. Intranodal vaccination is also investigated by Yi *et al*. and turned out to be more effective in eliciting Id-specific immune responses then subcutaneously injected DCs [49]. The reason for discrepancies between this observation and the observations in melanoma patients intranodally injected with DCs is unclear. Additionally, there is evidence from mice studies that the route of administration influences the type of immune response [50]. Intradermally injected DCs mainly elicit T_{H1} responses, desired in the setting of anti-tumour immunity, and DCs administered intravenously elicited T_{H2} cells as well, which are detrimental to anti-myeloma therapy [50].

In a study with DC/myeloma fusion based vaccines, it was observed that tumour-specific $CD8^+$ T cells and Langerhans cells were recruited at the site of subcutaneous injection [36]. This possibly implies that fusions of DCs and tumour cells not necessarily need to migrate to draining lymph nodes for the induction of immune responses, but that patients DCs can take up antigens from injected dead DCs and present them to T cells.

In summary, subcutaneous and intradermal administration of DC vaccines appears to be more effective than administering DCs intravenously

Immune competence of MM patients

Most patients included in early clinical trials on DC vaccination against MM either had a high tumour burden or shortly underwent high dose chemotherapy and subsequent autologous stem cell transplantation. In both situations, the patient's immune system could be compromised, possibly hampering the potential of the vaccine to induce anti-myeloma responses. Therefore, the time point of administration of DC vaccines pre- or post-chemotherapy and transplantation could also be important to consider. Possible options are vaccination of patients before high dose chemotherapy and transplantation, followed by isolation of T cells from the blood and injecting the T cells back after the chemotherapy. Additional restimulation with DC vaccines can be done to enhance T cell immunity. Another option is performing the DC vaccination after the stem cells re-establish the bone marrow so the patient's immune system is functional, as performed by Yi *et al*. In this study, DC vaccines were administered at least 4 months after high dose chemotherapy and autologous stem cell transplantation.

In a study by Reichardt *et al*. in 1999 patients were vaccinated with Id-peptide-pulsed MoDCs three to seven months post high dose chemotherapy and autologous peripheral blood stem cell transplantation (PBSCT) it was observed that the majority of patients developed strong responses to control antigen KLH, but only few patients developed responses against Id-peptides [39]. This might imply that the length of the period after chemotherapy and PBSCT is not the main reason for the limited efficacy of DC vaccines against MM, but as previously described the immunogenicity of Id as a TAA. However, in the same study it was seen that the patients who developed a detectable Id-specific immune responses where the patients in complete remission after autologous PBSCT. This implies that DC vaccination can result in therapeutic immunity against MM, but that optimization is still necessary in regard of antigen choice.

Clinical responses and toxicity

DC vaccination against MM is not as much clinically tested as in patients with melanoma. Therefore, limited data of clinical responses is available. Some strategies are only test in animal studies and the efficacy has still to be confirmed in patient studies.

The clinical responses to DC vaccination in various trials in MM patients were poor [29, 32, 34, 37, 38, 51]. In most early studies, this could possibly be accounted to the use of immature DCs, choice of intravenous administration and the weakly immunogenic myeloma antigen Id [27]. In some studies the patients who developed immunity against Id also show better clinical responses. In more recent studies with a focus on the optimization of DC vaccination against MM, more efficient *in vitro* and *in vivo* induction of anti-myeloma immune responses were observed. However, this still did not directly results in an improved outcome of disease progression. The most promising results came from a study in which patients were vaccinated with DC-myeloma cell fusion products [36]. In the majority of the patients (11 of 16) both immune responses and stabilization of disease was observed. The latter was assessed on serum and urine paraprotein concentration and bone marrow involvement.

No significant toxicity or auto-immunity was observed in patients receiving DC vaccines against MM, proving DC vaccination feasible, safe and well-tolerated by the majority of the patients. However, the safety of GM-CSF administration in patients with a thrombotic history has to be considered, as in one patient an embolus in the lung occurred after vaccination.

The best responses were seen in patients with lower tumour burden or patients in remission after high dose chemotherapy and autologous SCT. This is similar as was seen with melanoma, of which stage IV patients responded worse than stage III patients. As previously described, this can be explained by a higher extent of immune evasion which renders DC vaccination ineffective in controlling or eradicating tumour growth.

Dendritic cell vaccination after allogeneic stem cell transplantation

Myeloablative conditioning therapy followed by allogeneic stem cell transplantation (allo-SCT) is currently the only curative treatment for many hematologic cancers, such as leukaemia but also MM. After allo-SCT, a new immune system of the donor is established in the recipient. The rationale of this therapy is that lymphocytes from the donor stem cells react against residual leukaemia cells, the so called graft-vs.-leukaemia (GVL) effect. However, this effect is related to the dangerous the graft-vs.-host-disease (GVHD), in which donor lymphocytes attacks the tissues of the host. Both reactions are dependent of the degree of discrepancies in major histocompatibility complexes (MHC) and minor histocompatibility antigens (mHAgs) between donor and recipient. MHAgs are polymorphic proteins with variation in amino acid sequences among populations. When a transplant donor and recipient are HLA-matched differences in mHAgs in the host versus graft direction can cause rejection of the graft. Both GHVD and GVL reactions are consequences of mHAg mismatches in the graft versus host direction. The optimal situation would be that GVL reactions are maintained with only limited GVHD. This is possibly because some mHAgs are specifically expressed on haematopoietic cells, whereas others are ubiquitously present. Early attempts with donor lymphocyte infusion (DLI) in mixed and full chimeras are investigated both in clinical trials and animal studies [52]. In mixed chimeras, patients received allogeneic bone marrow transplantation (BMT) following non-myeloablative conditioning therapy, resulting in both donor and recipient hemapoetic systems existing together. In full chimeras, patients undergo allogeneic SCT after myeloablative therapy, thus only a donor immune system will develop. In these studies, it was found that delayed DLI in mixed chimera mice might result in strong GVL while GVHD was inhibited upon the conversion to full-donor chimera [52]. It was suggested that host hematopoietic cells, especially APCs, could play a crucial role in this effect in a MHC class I-dependent manner [52]. Possibly, recipient APCs present antigens of the tumour cells to donor lymphocytes, mainly $CD4^+$ T_H cells and $CD8^+$ T cells, as is observed in patients with chronic myeloid leukaemia (CML) receiving DLI. This is especially the case in HLA-matched allo-SCT, as donor T cells can recognize antigens presented by host APCs on MHC molecules [53]. In a mixed chimera setting, host APCs are still present, which might explain the superior GVL effect observed. Host APCs could also be important in inducing regulatory T cells which might protect against GVHD reactions. In mice it was demonstrated that alloantigen presentation by host APCs to donor T_{Reg} cells is essential for suppression of GHVD responses[53]. Early reconstitution of circulating DCs after allogeneic SCT is found to correlate with improved survival and reduced risk of acute GVHD, demonstrating the importance of DCs[54]. Due to the immunomodulatory properties of DCs, DC vaccination might improve treatment of hemapoetic malignancies by tipping the balance from GHVD towards GVL.

Donor or recipient dendritic cells

When DC vaccination is used in conjunction with allo-SCT and possibly also DLI, either recipient or donor DCs could possibly be used.

Some studies are performed on vaccination with recipient DCs following after partial T cell depleted allo-SCT and DLI [36,37]. In one study by Levenga *et al*. limited toxicity to the vaccination was seen and not any of the patients receiving DCs showed GHVD [55]. However, no significant effect on tumour burden was demonstrated. Although the DCs were only loaded with KLH as a control antigen, molecules such as minor histocompatibility antigens (mHAgs) expressed on the surface of DCs might be shared with host hematopoietic cells and residual malignant cells [57–59]. In a following study by Broen *et al*., 2012, it was found that injection of recipient DCs can induce mHAg-specific T cells capable of lysing primary myeloma cells and a myeloma cell line [56].

Donor derived DC vaccination can also be utilized following allo-SCT. It has been observed that post-transplant patients vaccinated with HLA-matched donor PBMC-derived DCs loaded with irradiated autologous tumour cells develop tumour-specific immune responses [60]. Although tumour burden in responding patients decreased, it remained unclear whether the tumour-specific T cells were *in vivo* induced by the infused DCs cells after injection or were contaminating T cells present in the DC vaccine primed *ex vivo* before vaccination.

In another study, a patient with relapsing acute myeloid leukaemia (AML) after allo-SCT received vaccination with donor DCs loaded with KLH and Wilms' tumour 1 (WT1) antigens [61]. The choice for donor DC was based on the observation that monocytes from the post-transplant patient were defective in their ability to differentiate into DCs. However, the vaccination proved to be ineffective because only responses to KLH and not to WT1 were detected and disease progressed after vaccination. Despite the poor clinical responses, the fact that immune responses against KLH were induced implies that donor DCs are capable of eliciting immune responses in post-transplant patients treated. Possibly loading donor DCs with more immunogenic TAAs might result in better responses.

Targets of DC vaccination in the setting of allogeneic stem cell transplantation

In an ideal situation, the recipient and donor are HLA-matched but differ in the expression of mHAgs in the hematopoietic system. In such setting, possibly the risk of GHVD in peripheral tissues is reduced but GVL is enhanced. Therefore, hematopoietic cell and tumour cell-specific mHAgs might be potential targets for DC vaccinations. However, it is suggested that an effective GVL effect cannot be elicited without some GVHD reactions [62]. Expression of some mHAgs is restricted to the hematopoietic system and malignant cells, while others are generally expressed in host tissues. Therefore, immunotherapy directed against mHAgs after allo-SCT requires careful selection of targets to induce an optimal GVL reaction while GHVD is manageable. A mHAg restricted to the hematopoietic system but also expressed in breast and lung cancer is HA-1.

DCs can be loaded with whole mHAg protein, short peptides or long peptides. When DCs loaded with long peptides of mHAg HA-1 are compared with short HA-1 peptide loaded DCs in their *in vitro* capacity to present antigen and prime T cells, it was found that long peptides were mainly presented by activated DCs and not by other hematopoietic cells, while presentation of short peptides did not show this restriction [63]. No significant differences between long and short peptides were seen in the decay of antigen presentation. The efficacy of DC vaccination with short or long HA-1 peptides after allo-SCT in clinical trials is to be tested yet.

Discussion

In the various studies on DC vaccination against cancer, mature DCs are observed to be more effective in inducing potent anti-tumour responses than immature DCs [17, 27]. This might be accounted to the conversion from optimal antigen uptake to more efficient antigen presentation and enhanced expression of co-stimulatory molecules, which are both necessary for the induction of effective antigen-specific immune responses.

The subset of DCs used for vaccination seem to depend on the nature of the antigens, as some DCs are capable of cross-presentation of exogenous antigens whereas other DCs can only present internal synthesized or pre-processed peptide antigens or mRNAs isolated from tumour cells or by fusion with tumour cells [64]. Short peptide-antigens, while demonstrated to be applicable for DC vaccination, has the disadvantages of HLA-restriction and short half-life time of antigen presentation. Whole proteins, long peptides, mRNAs and lysates or whole dead cells from tumours or DC-tumour cell fusion products have the advantage that both $CD4^+$ T cell and $CD8^+$ T cell epitopes are presented, allowing broader anti-tumour responses.

DC vaccinations administered intradermally seemed to be more effective than intranodally or intravenously injected vaccines [21, 27]. Not only the number of tumour cell-specific T cells was larger, but the $CD4^+$ T cells were mainly of the T_{H1} type as well. Intravenous vaccination could possibly result in T cell anergy or induces more T_{H2} cells, both detrimental to anti-tumour immunity [16, 50]. Whether subcutaneous and intradermal DC vaccinations differ in their potency remains unclear.

DC vaccination against melanoma and multiple myeloma appears to be safe, feasibly and tolerated as no severe signs of toxicity was observed [12–18, 21, 24, 29, 32, 34, 37, 38, 41, 51, 65]. Despite the fact that not all patients responded to DC vaccination, patients in which immune responses were observed *in vitro* or *in vivo* generally showed better clinical responses as well, implying that this immune therapy strategy can be effective in controlling cancer. However, optimization remains necessary, especially in the case of multiple myeloma.

Not much clinical work is published on the application of DC vaccines against haematological malignancies in conjunction with allogeneic HSCT. Nevertheless, due to the immunomodulatory functions of DCs, these cells might be potent in mediating a proper balance between GVL and GVHD. Injecting recipient DCs after allo-HSCT and DLI could induce mHAg-specific T cells capable of lysing myeloma cells *in vitro*, but clinical responses were not observed. The efficacy of vaccines based on donor DCs remains unclear.

Suggestion for further research

A possibly strategy for DC vaccination in patients with leukaemia or MM, is the use of the products of fusion of mature donor DCs with malignant haematopoietic cells from the patients. The patients will be first conditioned with non-myeloablative therapy and allo-HSCT from a HLA matched donor which is different in haematopoietic system-specific mHAgs, thus creating a mixed-chimera situation. The fusion products of DCs and tumour cells are co-incubated with donor lymphocytes to induce tumour-specific T cells. The patients then receive DLI with these activated lymphocytes to induce immune responses against both the remaining recipient haematopoietic cells and malignant cells. The choice for fusion productions of DCs and tumour cells is based on the observation that MM patients showed the best clinical responses upon vaccination with these fusion cells [36]. Additionally, it is not fully clear which patient-specific TAAs are sufficiently immunogenic to induce potent anti-tumour responses and which are specific for haematopoietic cells. By the use of haematopoietic cells as the fusion partner for DC various antigens specific to the haematopoietic system are presented to T cells. The application of DLI in mixed chimera is based on a mouse study in which it was shown that DLI following non-myeloablative conditioning therapy could result in enhanced GVL effects with minimal GVH responses [52]. However, these observations were not confirmed in human trials yet. Careful monitoring of the patients for the occurrence of GHVD, bone marrow aplasia and other signs of toxicity remains necessary. For so far, no severe signs of toxicity and auto-immunity were observed following DC vaccination, but it cannot be excluded that stimulating T cells with DCs fused with malignant tumour cells before DLI might also induce responses against antigens not specific to the haematopoietic system of the recipient.

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