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Recent Advances and Future Perspective of DC-Based Therapy in NSCLC

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Current treatment for patients with non-small-cell lung cancer (NSCLC) is suboptimal since therapy is only effective in a minority of patients and does not always induce a long-lasting response. This highlights the importance of exploring new treatment options. The clinical success of immunotherapy relies on the ability of the immune system to mount an adequate anti-tumor response. The activation of cytotoxic T cells, the effector immune cells responsible for tumor cell killing, is of paramount importance for the immunotherapy success. These cytotoxic T cells are primarily instructed by dendritic cells (DCs). DCs are the most potent antigen-presenting cells (APCs) and are capable of orchestrating a strong anti-cancer immune response. DC function is often suppressed in NSCLC. Therefore, resurrection of DC function is an interesting approach to enhance anti-cancer immune response. Recent data from DC-based treatment studies has given rise to the impression that DC-based treatment cannot induce clinical benefit in NSCLC by itself. However, these are all early-phase studies that were mainly designed to study safety and were not powered to study clinical benefit. The fact that these studies do show that DC-based therapies were well-tolerated and could induce the desired immune responses, indicates that DC-based therapy is still a promising option. Especially combination with other treatment modalities might enhance immunological response and clinical outcome. In this review, we will identify the possibilities from current DC-based treatment trials that could open up new venues to improve future treatment.

Keywords: dendritic cells, lung cancer, immunotherapy, immunology and lung cancer, non-small cell lung cancer

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INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide (1). This type of cancer is a heterogeneous disease (2). Based on histology, lung cancer is divided into small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC is the most prevalent form, accounting for about 80-85% of the lung cancer cases (3). The five-year overall survival rate (OS) for NSCLC is around 20% in the western world, highlighting the importance to explore the current and future therapeutic approaches in this field (4, 5).

Although surgery remains the cornerstone of therapy for early-stage NSCLC, a wide range of therapeutic options for adjuvant treatment or treatment of advanced stage disease have been introduced over the last decade. Targeted therapy and immunotherapy are examples of these novel therapies (6, 7). Targeted therapy targets specific alterations in NSCLC cells that stimulate tumor growth, for example mutations in the epidermal growth factor receptor (EGFR). Many other specific targets in NSCLC have been identified over time. Targeted therapy often leads to prolonged survival and greatly enhanced quality of life in this subgroup of patients (8, 9). Most patients with NSCLC lack actionable therapy targets. Therefore, immunotherapy, with or without chemotherapy, is the first-line treatment for the majority of NSCLC patients with advanced stage disease. Although targeted therapy and immunotherapy greatly improved clinical outcome in NSCLC, not all patients respond (10). Moreover, the patients who do respond eventually develop therapy resistance. Therefore, a high clinical need for new systemic treatment modalities remains. During this review we will shine our light on the rather unexposed field of dendritic cell (DC)-based therapies, to explore whether these could be a valuable treatment option for NSCLC.

IMMUNOTHERAPY IN NSCLC

The role of the immune system in prevention of cancer development and progression has been widely recognized. Immunotherapy exploits this role by stimulating the patient's immune system to eliminate the tumor. Different immunotherapeutic strategies are being used or currently studied for their use in cancer. These can be largely subdivided into cancer vaccines, cellular therapies, immune stimulatory agonists and immune checkpoint inhibitors (ICIs). NSCLC is a promising potential target for immunotherapeutic approaches due to its high tumor mutational burden, which enhances immunogenicity of the tumor (11). ICIs are the only currently approved immunotherapy option for NSCLC. The most frequently used ICIs are directed against programmed-death receptor 1 (PD-1), expressed on immune effector cells such as T cells and natural killer (NK) cells, or its ligand programmed-death ligand 1 (PD-L1), which is expressed on antigen-presenting cells (APCs) and tumor cells. Receptor binding of PD-1 can lead to inhibition of effector cell function and survival, while it induces T regulatory cells (Tregs) (12–14). Immune cells in tumors frequently demonstrate a non-functional or 'exhausted' phenotype which hampers an anti-cancer immune response. ICIs aim to revert this immunosuppressive phenotype, thereby inducing an efficient anti-cancer immune response (15).

Recently, anti-cytotoxic T-lymphocyte associated protein 4 (CTLA-4), which is another ICI, has been registered to be used in combination with a PD-1 inhibitor in the United States and is registered for the combination with a PD-1 inhibitor and chemotherapy in Europe (16, 17). CTLA-4 is expressed on T cells after activation. It is also constitutively expressed on Tregs. CTLA-4 binds CD80 and CD86 on APCs. Receptor binding transmits an inhibitory signal to the T cell. Furthermore, binding of CTLA-4 to CD80 and CD86 blocks their binding to T cell receptors thereby hampering T cell activation (18).

Nowadays, it is known that the tumor and its surrounding microenvironment (TME) can modulate anti-tumor immune responses. Recent data suggest that the low response rate to ICIs could be partly explained by the lack of immune cells in the TME or other regulatory factors that prevent an anti-tumor immune response (19). Therefore, other forms of immunotherapy to enhance the anti-tumor immune response are currently being studied, such as DC-based therapy.

DC-BASED THERAPY

DC-based therapy depends on the fundamental link that DCs form between tumor antigen recognition and an anti-tumor immune response. More specifically, DCs are highly specialized APCs that show the highest antigen-presenting potential when inducing naïve T cell activation (20). In tissue, DCs are constantly scanning their surroundings. Upon antigen encounter in the presence of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMP), DCs get activated, undergo maturation and secrete large amounts of pro-inflammatory cytokines to shape the local inflammatory environment (6, 21, 22). After maturation, DCs migrate to the lymph node where they activate T cells to induce an immune response directed against their presented antigen. In absence of PAMP or DAMP signals during antigen encounter, DCs remain immature, migrate to the lymph nodes and induce antigen-specific tolerance in T cells. DC-based therapies showed promising results in several malignancies such as melanoma, prostate cancer, and glioma (23–25). In NSCLC only early-phase clinical trials have been performed, which show disappointing clinical results but were not powered to evaluate clinical effect. In this review, we will analyse these studies and discuss different possibilities to optimize DC-based therapy in order to improve therapeutic effects.

DC VACCINATION MONOTHERAPY FOR NSCLC

The DC-based therapy in NSCLC consists of the vaccination of patients with DCs. In all studies investigating DC vaccination in NSCLC patients, monocytes derived from autologous peripheral blood mononuclear cells (PBMCs) were differentiated to moDCs *ex vivo* (**Table 1**) (26–36). These DCs were then primed with a combination of several synthetic peptides of commonly expressed tumor antigens in NSCLC or autologous tumor lysate. In the majority of studies, primed DCs were administered *via* multiple subcutaneous injections. *In vivo*, these DCs are supposed to activate cytotoxic T cells that will induce a tumor-directed immune reaction.

All studies showed DC vaccination to be safe. In addition, many studies examined vaccine-specific immunological responses by determining *ex vivo* T cell responses directed

TABLE 1 | Characteristics of studies in which DC vaccination monotherapy was performed in NSCLC.

Ref.	Subject number	Clinical stage	Antigen source	DC maturation status	Type and regimen of DC administration	Most important results after vaccination
Ueda et al., Int. J. Oncol. (26)	N = 3	III and IV[a]	CEA peptide	Immature	5 biweekly i.d. and s.c. vaccinations	• 2 out of 3 patients showed a DTH response.
Hirschowitz et al., J. Clin. Oncol. (27)	N = 16	I, II and III	Irradicated tumor lysate or lysate of a NSCLC cell line	Mature	2 i.d. vaccinations	• 6 out of 16 patients showed tumor-specific IFN-γ T cell responses. • No correlation between immunological response and OS or DFS was determined.
Chang et al., Cancer (28)	N = 6	III and IV	Tumor lysate	Not fully mature[b]	4 weekly vaccinations followed by 2 biweekly boost vaccinations in the inguinal lymph nodes	• 2 out of 6 patients demonstrated increased tumor-specific IFN-γ T cell responses. • These 2 patients demonstrated stable disease.
Hirschowitz et al., Lung cancer (29)	N = 14	I, II and III	Irradicated tumor lysate or lysate of a NSCLC cell line	Immature	2 i.d. vaccinations	• 10 out of 14 patients showed tumor-specific IFN-γ T cell responses. • No correlation between immunological response and OS or DFS was demonstrated.
Um et al., Lung cancer (30)	N = 9	IIIB and IV[a]	Tumor lysate	Mature	3 i.d. DC vaccinations at 2 weeks interval	• 5 out of 9 patients showed increased tumor-specific IFN-γ T cell responses. • All patients demonstrated disease progression.
Perroud et al., J. Exp. Clin. Cancer Res. (31)	N = 5	III and IV	Peptides of WT-1, MAGE-1, and Her-2/neu	Unknown[c]	2 biweekly s.c. and i.v. vaccinations	• *Ex vivo*, T cell responses directed towards the DC vaccine were increased. • 2 out of 5 patients showed an unexpectedly long OS.
Engell-Noerregaard et al., World J. Of Vaccine (32)	N = 22	III and IV	Lysate of a melanoma cell line expressing among others MAGE-A/B	Mature	A weekly s.c. vaccination for 5 weeks, followed by a booster vaccination after 6 weeks, with s.c. IL-2, COX-2 inhibitors, and TLR7 agonist[d]	• Ex vivo, vaccination-specific IFN-γ T cell responses were mostly observed in patients showing stable disease. • Some patients showed unexpected long survival.
Takahashi et al., Eur. J. Cancer (33)	N = 47	II, III and IV[e]	Tumor lysate or multiple peptides of WT-1, MUC1, and CEA	Immature	≥ 1 biweekly s.c. vaccination[f]	• Patients who received WT-1 vaccine showed increased OS.
Takahashi et al., Cancer Immunol. Immunother. (34)	The above study group was extended to N = 240	II, III and IV[b]	WT-1 and/or MUC-1 peptide	Immature	≥ 5 biweekly s.c. vaccinations[f]	• Having a DTH response was correlated with increased survival. • No difference in OS between patients vaccinated with WT-1 DCs and patients vaccinated with other DC vaccines was determined.
Ge et al., BMC Cancer (35)	N = 15	I, II and IIIA	Survivin and MUC-1 peptides[g]	Partly mature[h]	3 weekly i.v. vaccinations	• Circulating Tregs were significantly decreased 2 weeks after vaccination. • Improved quality of live was reported.
Li et al., Oncol. Lett. (36)	N = 16	I, II and III	MAGE-A3 and Survivin peptides	Mature	16 rounds of two monthly i.d. vaccinations	• All patients showed a DTH response. • In 15 out of 16 patients, tumor-specific IFN-γ T cell responses were increased.

Studies are displayed in order of publication (old to new). [a]*Patients without response to first-line treatment or who declined first-line treatment were included,* [b]*DCs were HLA-DR*$^{+}$*CD86*$^{+}$*CD40*$^{+}$*CD80*^{low}*CD83*$^{-}$*CCR7*$^{-}$*,* [c]*No established maturation method was used and no data that showed the maturation status of the DCs was available,* [d]*When patients showed no disease progression after vaccination, 1 boost vaccination per 4 weeks was administered,* [e]*Patients who had inoperable tumors or relapsed quick after surgery,* [f]*When patients showed no disease progression, vaccination was repeated.* [g]*DCs were also incubated with inhibitors of suppressor of cytokine signalling 1 (SOCS1),* [h]*DCs were HLA-DR*$^{+}$*CD80*$^{+}$*CD83*$^{+}$*CD86*$^{+}$*CD40*$^{+}$*CD14*$^{-}$*CCR7*$^{-}$*; MUC-1, Mucin-1; CEA, carcinoembryonic antigen; i.d., intradermal; s.c., subcutaneous; DTH, delayed-type hypersensitivity; WT-1, Wilms' tumor protein 1; MAGE-1, melanoma-associated antigen 1; her-2/neu, human epidermal growth receptor 2; i.v., intravenous; IFN, interferon; Tregs, T regulatory cells; IL-2, interleukin 2; COX-2, cyclo-oxygenase 2; TLR-7, Toll-like receptor 7; DFS, disease-free survival.*

towards the vaccine or by performing a delayed-type hypersensitivity (DTH) test. The principle of a positive DTH test is that if T cells are activated by DC vaccination, this DC vaccine will be recognized upon injection in the skin. This will cause a local immune reaction resulting in erythema. Most studies initially confirmed expression of their used tumor antigen in the tumor or used autologous tumor lysate for DC priming. Interestingly, vaccine-specific immunological responses

were demonstrated in most studies after vaccination. However, this induced tumor-specific immune response was almost never linked to a radiological response or improved survival.

DC VACCINATION COMBINATION THERAPIES FOR NSCLC

Investigators have also been focusing on the effect of combining DC vaccination with other therapies. For instance, chemotherapy and radiotherapy are hypothesised to enhance anti-tumor immunity and could therefore synergize with immunotherapy. A well-described effect of chemotherapy and radiotherapy is immunogenic cell death of cancer cells, exposing high levels of tumor antigen and DAMP molecules to immune cells in the TME (37–39). The superior immune-activating ability that chemotherapy and radiotherapy induce in cancer cells is highlighted in DC vaccination studies of cancer mouse models exploiting this strategy. In these studies, DC vaccination of DCs loaded with radiation-treated or chemotherapy-treated cancer cells, resulted in reduced tumor volume compared to mice vaccinated with DCs loaded with untreated tumor cells (40–43). Moreover, chemotherapy and radiotherapy were reported to stimulate human leukocyte antigen I (HLA-I) expression of the tumor, making tumor cells more sensitive to cytotoxic killing by $CD8^+$ T cells (38). It is important to define the optimal dose of chemotherapy or radiotherapy for combination treatment with immunotherapy, as high doses of chemo- and radiotherapy can induce cell death of immune cells as well (37). The synergistic effect of chemotherapy to DC vaccination was recently validated in a human melanoma study (44). For NSCLC, this synergistic effect of both chemotherapy and radiotherapy with DC vaccination was confirmed in mouse models (45–47). In human NSCLC, only one study examined the combination of chemotherapy with DC monotherapy, but many studies investigated the combined effect of chemo- and/or radiotherapy, DC vaccination and cytokine-induced killer cells (CIK) (**Table 2**) (48, 50–55). CIK cells consist of a heterogeneous group of T cells, NK cells, and NKT cells. CIK cells are derived from autologous PBMCs, activated and expanded *ex vivo* under influence of anti-CD3 and cytokines, such as interferon γ (IFN-γ) and interleukin 2 (IL-2) (56). CIK therapy was shown to be safe and had a response rate of 39% in various tumors. Moreover, CIK treatment was associated with increased survival (57). Co-culture of CIK cells and DCs enhanced cytolytic function of CIK cells and increased both IL-12 secretion by DCs and levels of immunostimulatory receptors on DCs as well as CIK cells (58).

Importantly, all studies showed that combination therapy was safe and well-tolerated. From all studies that combined DC-based therapy with radio- and/or chemotherapy in NSCLC, four out of seven demonstrated improved OS in the combination therapy group compared to radiotherapy or chemotherapy alone. Two studies of combined DC-CIK therapy that showed no differences in OS between groups, did show improved disease-free survival (DFS) in the combination therapy group. Unfortunately, the study investigating the effect of chemotherapy and DC vaccination alone included no control group to compare treatment efficiency or clinical outcome.

In addition to chemotherapy and radiotherapy, one study investigated the combination of DC-CIK and the EGFR tyrosine kinase inhibitor erlotinib in patients with advanced stage NSCLC (**Table 2**) (49). This study demonstrated increased progression-free survival (PFS) in the combination therapy group, while OS did not differ between the groups. This synergistic effect is particularly interesting considering that EGFR-mutated NSCLC is insensitive to anti-PD-1/anti-PD-L1 therapy (15, 59, 60). Erlotinib is normally not combined with other systemic treatment, because it shows no benefit in survival to monotherapy, while toxicity potentially increases (61, 62).

Whereas the current DC-based monotherapy studies could not show clinical benefit in NSCLC patients, combinations with chemotherapy, radiotherapy, and targeted therapy showed to improve clinical outcome. However, in almost all combination studies CIK cells were administered simultaneously with the DC vaccine. Hence, whether the observed clinical advantage of combination therapy over the standard therapy is an effect of the DC vaccine, the CIK cells, or the combination of both cannot be discerned from those studies.

A combination of therapies that was not studied in NSCLC before is DC-based therapy and other immunotherapy, such as ICIs. Several studies have pointed out that when there is no anti-tumor immune response, 'releasing the brakes' by checkpoint inhibition will not lead to improved clinical results. Hence, a combination with an immune strategy that actively induces an anti-tumor immune response might improve therapy response rate (63). Vice versa, therapies that actively stimulate the immune response, often result in increased expression of immune checkpoint molecules and might therefore also benefit from a combination treatment with ICIs. In addition, in single-cell RNA sequencing data from NSCLC tissue a mature DC subset with high expression of regulatory molecules, such as PD-L1, was identified which could be targeted by anti-PD-L1 therapy (64).

The potential synergistic effect of ICIs and DC vaccination is currently examined in advanced stage melanoma patients. Accordingly, two studies showed that a combination strategy of anti-CTLA-4 and DC vaccination resulted in an improved clinical response compared to similar cohorts that received anti-CTLA-4 treatment alone, without causing additional toxicity (65–68). In addition, ICI therapy was shown to be effective in advanced stage melanoma patients with recurrent disease after adjuvant DC vaccination (69). Until date, no results are available of studies that examine whether the synergistic effect of combined ICI and DC-based therapy also applies for NSCLC.

FROM PERIPHERAL IMMUNE ACTIVATION TOWARDS A LOCAL RESPONSE

An important question is whether the current administration route for DC vaccination in NSCLC can induce a tumor-specific

TABLE 2 | Characteristics of studies in which DC vaccination combination therapy was performed in NSCLC.

Ref.	Subject number	Clinical stage	Antigen source	DC maturation status	Type and regimen of DC administration	Most important results after vaccination
Zhong et al., Cancer Immunol. Immunother. (48**)**	N = 28 (DC-CIK + chemotherapy = 14; chemotherapy = 14)	III and IV	CEA peptide	Immature	• All patients received 4 cycles of vinorelbine with cisplatin chemotherapy. • The DC-CIK + chemotherapy group in addition received 4 monthly cycles of i.v. DC-CIK vaccinations.	• Patients in the DC-CIK + chemotherapy group demonstrated significantly increased PFS compared to the chemotherapy only group. • There was no difference between 1-, 2-, and 5-year OS between the different groups.
Shi et al., J. Immunother. (49**)**	N = 54 (erlotinib + DC-CIK = 27, erlotinib = 27)	III and IV	Tumor lysate	Immature	• All patients received erlotinib. • The DC-CIK + erlotinib group in addition received 4 s.c. DC vaccinations and 5 i.v. CIK vaccinations within the erlotinib treatment. • Patients received treatment cycles until disease progression or withdrawal from the study (≥ 2 cycles).	• Circulating CD4 T cells, CD8 T cells and the CD4/CD8 ratio were significantly increased after erlotinib + DC-CIK treatment, while there were no differences in these parameters in the erlotinib only group.. • PFS was significantly increased in the DC-CIK + erlotinib group compared to the erlotinib only group. • There was no difference in OS between both treatment groups.
Hu et al., Med. Oncol. (50**)**	N = 27[a]	III and IV	Tumor lysate	Immature	• Patients received pemetrexed chemotherapy followed by i.d. DC vaccination at day 12. • Patients received multiple rounds of DC vaccination until disease progression (≥ 2 cycles) or up to a maximum of 6 rounds.	• Primary endpoint was safety and combination therapy was shown safe. • No clinical nor immunological effect could be determined, since no control group was available.
Zhao et al., Exp. Ther. Med. (51**)**	N = 157 (DC-CIK + chemotherapy = 79; chemotherapy = 78)	IIIA	–	Immature	• All patients received surgery. • Chemotherapy consisted of four cycles of gemcitabine and cisplatin. • 2 i.v. DC-CIK vaccinations were administered after the second cycle and after the fourth cycle of chemotherapy in the DC-CIK + chemotherapy group.	• The 3-year cumulative recurrence rate was significantly reduced in the DC-CIK + chemotherapy group. • The 3-year cumulative survival was significantly increased in the DC-CIK + chemotherapy group.
Zhu et al., Genet. Mol. Res. (52**)**	N = 65 (DC-CIK + radio-/ chemotherapy = 30; radio-/ chemotherapy = 35)	IIIB	-	Unknown[b]	• All patients received 4 cycles of docetaxel and cisplatin chemotherapy combined with a total dose of 60-70 Gy radiotherapy. • The DC-CIK + radio/chemotherapy group received 4 rounds of 2 or 3 i.v. DC-CIK vaccinations in between the chemo- and radiotherapy cycles.	• Patients in the DC-CIK + radio/ chemotherapy group demonstrated significantly increased CD3 and CD4 T cells 4 weeks after treatment. This difference was not observed in the radio/ chemotherapy group only. • Patients in the DC-CIK + radio/ chemotherapy group demonstrated significantly increased 6-months and 12-months OS compared to the radio-/ chemotherapy alone group.
Zhang et al., Oncol. Lett. (53**)**	N = 507 (DC-CIK + standard therapy = 99; standard therapy = 408)	III and IV	NSCLC cell line lysate	Unknown[b]	• Standard therapy consisted of surgery, chemotherapy, and radiotherapy. • DCs were administered i.v. once a week for 3 weeks. • In the first week of treatment, patients received i.v. CIK vaccinations once a day for 4 days. After 3 weeks, patients received i.d. DC vaccinations once a week for 3 weeks.	• 59 out of 97 patients from the combination therapy group demonstrated a DTH response (the control group was not tested). • Patients who received DC-CIK showed significantly improved survival compared to patients who received standard therapy.
Zhang et al., Radiot. Oncol. (54**)**	N = 82 (DC-CIK + radiotherapy = 21; radiotherapy = 61)	III and IV	MUC-1 peptide	Unknown[b]	• All patients received a total dose of 60-66 Gy radiotherapy. • The DC-CIK + radiotherapy group received 4 s.c. DC vaccinations and 4 i.v. CIK vaccinations between radiotherapy fractions.	• Peripheral blood of before and after treatment was available for 20 patients. No differences in circulating CD8 T cells, CD4 T cells and NK cells were observed between before and after treatment in both treatment groups.

(Continued)

TABLE 2 | Continued

Ref.	Subject number	Clinical stage	Antigen source	DC maturation status	Type and regimen of DC administration	Most important results after vaccination
						• Patients in the DC-CIK + radiotherapy group showed a significantly increased PFS compared to the radiotherapy only group. • No difference in OS between both treatment groups was observed.
Zhao et al., Clin. Transl. Oncol. (55)	N = 135 (DC-CIK = 45; chemotherapy = 40; DC-CIK + chemotherapy = 50	III and IV	–	Partly mature[c]	• Chemotherapy consisted of pemetrexed or docetaxel. • DC-CIK was administered i.v. daily for 3 days. • Patients of all groups received ≥ 2 rounds of treatment.	• In multivariate analysis, combination therapy of DC-CIK and chemotherapy was an independent prognostic factor for increased 1-year PFS and OS. • There was no difference in 1-year OS between the DC-CIK only and the chemotherapy only group.

Studies are displayed in order of publication (old to new). DC-CIK therapy or DCs for DC vaccination were derived from autologous PBMCs. [a]All patients failed gefitinib or erlotinib maintenance therapy. [b]No established maturation method was used and no data that showed the maturation status of the DCs was available, [c]showed a $CD80^+CD86^+$ population > 80% in their vaccine; SCC, squamous cell carcinoma; TNF-α, tumor necrosis factor α; CEA, carcinoembryonic antigen; i.v., intravenous; s.c., subcutaneous; MUC-1, Mucin-1; PFS, progression-free survival.

immune response in the lungs. The most promising results from human DC-based therapies are achieved in melanoma. In these trials, DCs were injected in the skin and migrated to cutaneous lymph nodes in which maturated DCs can initiate an anti-tumor immune response. For melanoma, this is often in close proximity to the tumor. For NSCLC this same route of administration is chosen, although the tumor is located at a large distance from the cutaneous lymph nodes. This difference in environment of T cell activation might lead to a decreased amount of T cells that reach the tumor. This is illustrated in a pancreatic cancer mouse model in which intraperitoneal administration of a DC vaccine suppressed tumor growth and inhibited tumor progression to a larger extent compared to subcutaneous injections of the same DC vaccine (70). It might therefore be interesting to study the effect of DC-based therapy that is administered into the local lymph nodes. Although this is more invasive, it might induce a more locally effective anti-tumor immune response.

The local environment during T cell activation might not be the only element causing the suggested suboptimal lung T cell infiltration. In an inflammation mouse model, it was shown, that after local immunization with the immunogenic ovalbumin protein (OVA), DCs isolated from lung-draining mediastinal lymph nodes induced increased lung homing of CD4 T cells compared to DCs isolated from muscle-draining inguinal lymph nodes (71). The authors linked this increased ability to induce lung-homing of CD4 T cells to a $CD24^+$ DC subset that is highly expressed in the mediastinal lymph node compared to the inguinal lymph node. To induce a local immune reaction in the lung, it therefore seems pivotal to specifically target this DC subset. Interestingly, in another inflammation mouse model it was demonstrated that this specific DC subset is probably not induced in the lung-draining lymph node itself, but rather in the lungs before lymph node migration (72). This is illustrated by an experiment in which DCs isolated from lung tissue, lung-draining lymph nodes, and other lymph nodes received antigen and were co-cultured with T cells *ex vivo*. DCs isolated from lung were superior at inducing lung-homing T cells, while there was no difference between DCs originating from lung-draining lymph nodes and other lymph nodes. This finding that lung-derived DCs induced superior homing of T cells to the lung was also confirmed in another mouse model of viral infection. In this model, mice were intranasally challenged with viral particles after which DCs were isolated from both lung and lung-draining lymph nodes at multiple time points after infection. Their results demonstrate that at 30 minutes after infection lung DCs were superior at inducing T cell homing compared to DCs originating from lung-draining lymph nodes, while at 24 hours after infection this difference was abolished. Since transport of soluble antigen from the lung towards the local lymph node already occurs within a few minutes after infection, this experiment validates that DCs that migrated from the lung are the main stimulants for lung-homing T cells. Although these are all pre-clinical data, a lung-derived DC subset paramount for optimal local tumor-specific T cell immune response might also be present in humans. DCs used for DC vaccination in NSCLC are not lung-derived. Therefore, means to equip DCs with an optimal capacity to induce lung-homing T cells should be developed.

IN VIVO TARGETING OF DCS

The clinical trials using DC-based therapy in NSCLC were performed with DCs that were earlier isolated from the patient's peripheral blood, after which the DC vaccine was finalized in the lab. This procedure to construct DC vaccines has disadvantages: it is demanding for patients, laborious and expensive. For that reason, targeting DCs *in vivo* is a promising approach. Moreover, specific DC subsets in the tumor could be directly targeted when a monoclonal antibody directed against specific endocytic DC receptors would be used as guide for antigen delivery. For example, C-type lectin domain containing 9A (CLEC9A) on conventional type 1 DCs (cDC1s). Since CLEC9A is involved in cross-presentation, this specific

targeting could also skew the antigen-processing towards this direction (73). This strategy of specific DC-targeting is not yet performed in humans, but some mouse studies show promising results.

In melanoma mouse models, receptors targeting among others, CLEC9A, CD11c, and DEC-205 (receptor on circulating DCs of mice and human, involved in cross-presentation) were bound to OVA (74–77). With concurrent activation, such as Polyinosinic:polycytidylic acid (poly I:C) and anti-CD40, the administered tumor antigen-receptor complex was shown to elicit effective immune responses and inhibit tumor growth. Hence, in the study using DEC-205 it was even demonstrated that *in vivo* vaccination showed larger inhibition of tumor growth compared to vaccination with *ex vivo* spleen-derived DCs which were also primed with OVA and maturated using anti-CD40 (74).

In the previous examples, antigens were chemically conjugated to a monoclonal antibody, or the DNA sequence of the antigen was genetically fused to the monoclonal antibody, while DC activation signals were injected separately. Currently, however, many studies focus on more complex manners of antigen delivery *in vivo* that could improve antigen uptake and DC activation efficiency. Examples are lipid vesicles or nanoparticles with surface-bound DC targeting receptors, and containing tumor antigens and DC activation signals (78). An important advantage of this technique is that the maturation signals are selectively delivered to the DCs. This is important because maturation molecules have been shown to have a tumor-supporting function when binding to other cells in the TME (79–81).

There are also studies that only focus on *in vivo* DC activation, without loading the DC with antigen. In a lymphoma mouse model, it is shown that intratumoral injection of TriMix mRNA, encoding costimulatory molecules CD70, CD40 ligand, and constitutively active toll-like receptor 4 (TLR4), induces systemic tumor-specific T cell responses independent of the co-delivery of tumor antigen (82). Moreover, in animal cancer models they show increased survival after injection of TriMix mRNA. The uptake of Trimix mRNA relies on the ability of DCs to rapidly and selectively internalize free RNA and avoids off-target effects. These results suggest that tumor-infiltrating DCs may have acquired antigen, and that the problem in their malfunctioning phenotype is rather the lack of sufficient activation signals in their surroundings.

CONCLUSION

Nowadays, DC-based therapy in NSCLC is still at a developmental stage. The DC vaccination studies performed are all early-phase studies that demonstrated low toxicity of the treatment, but were underpowered to show clinical benefit. However, most of these clinical trials showed that DC vaccination can induce the desired immune response. The latter highlights the potential of DC-based therapy in NSCLC and encourages further research that could advance the peripheral immunological effect to a radiological response or improved survival. In particular, studies that examine whether the anti-tumor immune response in peripheral blood or skin could also be induced in the tumor environment could provide more insight. The immune-activating ability of DC vaccination and the low toxicity of treatment make this therapy an excellent candidate for combination with other anti-cancer treatment. Clinical success of combination therapies is illustrated by the results of combination studies of chemotherapy and/or radiotherapy and even targeted therapy with DC-CIK vaccination in NSCLC. Likewise, studies in melanoma demonstrated the synergizing effect of DC-based therapy and ICIs. Especially this latter combination with ICIs, which inhibit the immunosuppressive TME, could allow the optimal immune-activating potential of DC based therapy to be revealed in NSCLC.

AUTHOR CONTRIBUTIONS

IAEvdH wrote the manuscript. GFG and BP wrote and reviewed the manuscript. IJMdV and MMvdH reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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