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Current “state of the art” on dendritic cell-based cancer vaccines in melanoma

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Abstract (200 words max.):

Purpose of review: Dendritic cells (DCs) are the gatekeepers of our immune system and indispensable in the anti-tumor immune response. In recent years, their classification has been revised considerably using single-cell sequencing approaches. In this review, we focus on their unique role in cancer and how specific DC subsets can be manipulated to induce an effective and durable anti-tumor response.

Recent findings:

Historically, due to the ease of their isolation in sufficient cell numbers from peripheral blood, the utility of monocyte-derived DCs as therapeutic cancer vaccines was explored in the clinic. However, it became clear that naturally circulating myeloid DCs (myDC), exerting their physiological role, are a functionally more powerful cellular source of antigen presenting cells. With the advent of immunomagnetic bead technology to isolate naturally circulating DC subsets, the therapeutic value of these myDC subsets is currently being explored. Since DCs are also needed in the tumor microenvironment in order to "relicense" the activity of anti-tumor T cells, also intratumoral administration routes for DC vaccines are explored. In addition, to circumvent the use of expensive cellular vaccines, approaches to attract DCs to the tumor microenvironment are considered of interest in order to repair a defective cancer-immunity cycle.

Summary: In recent years, the type of DCs used for vaccination and their administration route evolved considerably. Intratumoral vaccination strategies require combination with additional stimuli to ensure proper functioning of DCs in the tumor microenvironment. Moreover, intratumoral administration limits the applicability to patients with accessible lesions.

Keywords

dendritic cell vaccine; natural circulating dendritic cells; intratumoral; melanoma; clinical trials

Text of review

Introduction

Although the treatment of advanced melanoma has been revolutionized through the introduction of immune checkpoint inhibitors (ICI) targeting programmed death ligand 1 (PD-1; e.g., pembrolizumab

and nivolumab) and CTLA-4 (ipilimumab) by significantly increasing survival, benefit resulting from this treatment remains absent in a plethora of patients.(1-11) For these patients the need for effective cancer treatments remains unmet.

An effective immune response against cancer cells requires a series of events that must occur in a certain order and re-occur iteratively, a process referred to as the cancer-immunity cycle.(12) Herein, dendritic cells (DCs) play a central role with their gatekeeper function, as they sense, capture, and process antigens released from tumors (e.g., cancer-testis antigens, differentiation antigens, neoantigens). DCs present these tumor-associated antigens (TAA) via major histocompatibility complex (MHC) I or II molecules to naïve T cells and prime and activate these in the presence of pro-inflammatory cytokines (e.g., type 1 interferons (IFN), interleukin-12 (IL-12), tumor-necrosis factor-α) and damage-associated molecular patterns. Activated TAA-specific effector T cells are able to migrate and infiltrate into the tumor microenvironment (TME), recognizing the TAA presented in a MHC at the cell surface of cancer cells leading to targeted cancer cell killing.(12)

A possible explanation for failure of ICI may be the absence of DCs in the TME, and mechanisms that lead to the exclusion of DCs from the tumor have been recently identified. Among these, the activation of the oncogenic WNT/β-catenin pathway has been identified as an important driver for the exclusion of Batf3-expressing myeloid DCs (myDCs), also termed conventional DCs (cDCs), from the TME as a result of a downregulated production of chemokines that are necessary to attract these myDCs from the blood into the TME.(13-15) *Spranger et al* have shown that the migration of effector T cells depends on the presence of $CD103^+$ myDCs that produce the chemokine CXCL10. Alterations in the CXCR3-CXCL9/10 chemokine axis were seen in β-catenin-expressing tumors leading to defective migration of effector T cells. Additionally, the absence of myDCs at the invasive margin and within metastases has been correlated with defective activation of cytotoxic T lymphocytes (CTL), thereby allowing metastases to escape the antitumor immune response.(16) Additionally, an analysis of data from *The Cancer Genome Atlas* (TCGA) demonstrated that naturally circulating DCs (nDCs), especially myDCs, but not monocyte-derived DCs (moDCs), seem to be associated with improved survival in various

cancers.(17-19) Of interest, the presence of myDCs also correlated more strongly with T-cell infiltration into tumors as compared to neoantigen load in 266 melanomas from TCGA.(20) It has been described that infiltration of type 1 cDCs (cDC1) in human tumors has shown an association with better responsiveness to anti-PD-1 checkpoint blockade.(21) More recently, it has been described that successful anti-PD-1 therapy requires crosstalk between T cells and DCs. Effective treatment with anti-PD-1 checkpoint inhibitors requires DCs to produce IL-12 for which IFN-γ secretion from CTL is required.(22) Animal models indicate that myDCs are essential for priming anti-tumor T-cell responses, with cDC1 (Batf3-dependent $CD103^+/CD141^+$ DCs) mediating $CD8^+$ CTL and conventional type 2 DCs (cDC2) $CD4^+$ T-cell responses.(23) Preclinical experiments in mice have shown that $CD4^+$ T-cell responses are dependent of the transcription factor IRF4 in cDC2s and that exogenous IL-10 and IL-33 can recover the ability of IRF4-deficient cDC2s to promote $CD4^+$ T cells.(24, 25) Altogether, these observations show that DCs are crucial players in the TME to mount an effective anti-tumor immune response. The ability of DCs to mount this adaptive immunity against cancer cells can be harnessed for cancer therapy. Various strategies are currently under evaluation to exploit DCs for therapeutic reasons, all having different costs, applicability, and feasibility. Some of these are the use of free adjuvants or DC-activation factors (e.g., synthetic CpG oligonucleotides), DC-mobilizing and -expanding agents (e.g., FMS-like tyrosine kinase 3 ligand (Flt3L)), the use of viral vectors or vaccines that express TAAs and activating factors for DCs, or the adoptive transfer of DCs.(26) This review focuses on the evolution of DC-based vaccines and strategies to increase their presence in the TME for the treatment of melanoma.

Historical perspective of DC vaccines

Conventional therapeutic DC-applications have relied on the “therapeutic vaccination” paradigm where DCs were injected intradermally, subcutaneously, intravenously, or intranodally. In this approach, the isolation of autologous DCs or *in vitro* generation of DCs is followed by *ex vivo* manipulation including maturation, amplification, and antigen loading before reinfusion to patients.

Most frequently moDCs have been used for this approach. However, only modest response rates were achieved despite continued optimization of various vaccination parameters such as the choice of antigens as well as the maturation protocol. The generation of moDCs requires several days of *ex vivo* culture which increases the cost of such a cellular *advanced therapy medicinal product* (ATMP).

Interestingly, instead of using tumor antigens, one recent first-in-human, randomized phase II study investigated a monocyte-derived type-1-polarized DC vaccine targeting selected tumor blood vessel antigens in combination with the tyrosine kinase inhibitor dasatinib in ICI-refractory advanced melanoma patients.(27) Immunologic responses such as increased immune cell infiltration and/or objective clinical responses have been observed in 46% of evaluable patients. Another approach is the combination of DC vaccination with adoptive tumor-infiltrating T-cell (TIL) transfer. Complete and durable clinical responses have been observed in ICI-resistant, metastasized melanoma patients treated with adoptive TIL combined with autologous tumor lysate-loaded DC vaccination.(28) Also, a randomized phase II trial investigated lymphodepletion plus adoptive cell transfer of TILs, with or without DCs pulsed with MART-1 was performed. However, there was no significant difference between both treatment arms.(29)

Primary, naturally circulating dendritic cells: more potent vaccines?

Bone marrow emergent "natural DCs" are present in the peripheral blood and have recently been classified according to their surface markers and function using functional, transcriptomic and proteomic analyses.(30-34) This classification has been elucidated using sophisticated techniques amongst others single-cell RNA-sequencing of human blood DCs, monocytes, and progenitors.(30) Naturally circulating DCs reflect a heterogenous group of cells both functionally and morphologically, but also concerning their location. In humans, the two major subsets are myDCs and plasmacytoid dendritic cells (pDCs), each with a distinct phenotype and function during an immune response.(35) As myDCs and pDCs both express different pattern recognition receptors, they respond to different stimuli and have different patterns of migration.(36) It has been shown that myDCs and pDCs may act

synergistically in a bidirectional way.(37, 38) Extended preclinical research indicates that myDCs are essential in “re-licensing” antitumor T lymphocytes to eradicate tumor cells within the TME.(17, 19) It has been shown that myDCs play an essential role in the initiation of antigen-specific antitumoral immunity.(13, 17)

For several reasons, harvesting pDCs and/or myDCs for the use as vaccines has gained interest over the past years since their clinical grade isolation by immunomagnetic bead cell sorting has become feasible. Until now, nDCs have been investigated in a small number of early phase clinical trials. The trials have used either “myDC only” vaccines, “pDC only” vaccines or combined “myDC/pDC” vaccines. In addition, the route of administration differed between trials as did the number of DCs administered. The only trial so far that investigated exclusively pDCs in a phase I clinical trial (NCT01690377) was performed in patients with stage IV or unresectable stage III melanoma. After isolation, pDCs were stimulated with the prophylactic Frühsommer-Meningoenzephalitis vaccine and loaded with gp100 and tyrosinase and then administered intranodally three times bi-weekly, followed by two maintenance administrations every 6 months. $CD4^+$ and $CD8^+$ T-cell responses directed against gp100 and tyrosinase have been observed, indicating immunogenicity of the vaccine. This vaccine was safe without any high-grade adverse events.(39)

Plasmacytoid DCs were also investigated in a combined vaccine with myDCs. A phase I/II trial (NCT02574377) in patients with stage III melanoma who underwent a complete lymph node dissection compared intranodal administration of a pDC-vaccine versus myDC-vaccine versus a combined pDC/myDC-vaccine. The activation of both myDCs and pDCs was accomplished using protamine/mRNA and DCs were loaded with gp100, tyrosinase, NY-ESO-1, MAGE-C2, and MAGE-A3. Antigen-specific $CD8^+$ T cells were detected in skin test-derived T cells and in peripheral blood in 80% and 55% of the patients, respectively. In 64% of the patients, functional IFN-γ-producing T cells were found in the skin test. Only low-grade adverse events were observed.(40)

Additionally, a randomized, double-blind, placebo-controlled phase III trial in patients with stage IIIB/IIIC melanoma was set up to evaluate intranodal administration of nDCs loaded with synthetic

peptides as an adjuvant treatment (NCT02993315). However, accrual was stopped prematurely after inclusion of 151 patient due to the availability of adjuvant treatment with anti-PD-1 antibodies in the Netherlands since November 2018. Adjuvant nDC-vaccination in stage IIIB and IIIC melanoma patients showed no benefit over placebo in terms of 2-year recurrence-free survival.(41)

Also, a subcutaneously administered pDC cell line-based vaccine has been investigated in melanoma patients in a first-in-human trial, showing a significant increase in the frequency of circulating anti-tumor specific T lymphocytes and stable disease as best clinical response. (42)

Intratumoral administration to increase DC numbers in the TME

The presence of DCs in the TME is of utmost importance for effective induction of anti-tumor immunity. Thus, intratumoral administration of nDCs could hypothetically turn a "cold", non-inflamed tumor into an inflamed TME if the necessary stimuli are present. Intratumoral treatments such as oncolytic viruses (e.g., talimogene laherparepvec (T-VEC)(Imlygic®, Amgen) or synthetic Toll-like receptor agonists (e.g., tilsotolimod)) have gained interest. DCs can either be administered intratumorally as autologous, non-substantially manipulated CD1c (BDCA-1)$^+$ and CD141 (BDCA-3)$^+$ myDCs (non-ATMP) or autologous manipulated DCs that are loaded *ex vivo* with TAA and/or activated by TLR agonists before injection (ATMP).

Early phase clinical trials have been conducted or are currently ongoing that investigate an intratumoral DC vaccination in combination with other immunotherapeutic agents, including oncolytic viruses, ICI, or prophylactic vaccines.

As a first-in-human study, a phase I basket trial (NCT03707808) for patients with solid tumors that mainly included patients with advanced melanoma investigated intratumoral injection of autologous, unmanipulated CD1c (BDCA-1)$^+$ myDCs in combination with intratumoral injection of ipilimumab and the anti-PD-L1 antibody avelumab plus intravenous administration of nivolumab. In this trial that included nine heavily pretreated patients with advanced, ICI-refractory solid tumors, clinical responses have been observed in injected and non-injected lesions. An increase in tumor-infiltrating CD8$^+$ T

lymphocytes as well as an upregulation of PD-L1 on on-treatment biopsies of a patient who achieved a durable partial response were observed.(43)

In another phase I clinical trial (NCT03747744), intratumoral administration of autologous, unmanipulated CD1c (BDCA-1)$^+$ myDCs or the combination with CD141 (BDCA-3)$^+$ myDCs in combination with the oncolytic virus T-VEC was investigated.(44, 45) In total, 13 patients were included and underwent study treatment; clinical responses were observed in injected as well as non-injected lesions, however, not always translating into an overall response. Two patients with ICI-refractory melanoma have developed durable complete responses persisting more than two years after treatment. Interestingly, on-treatment biopsies revealed a strong infiltration by inflammatory cells in regressing lesions of these two patients. The treatment was also tolerated well with mainly low-grade adverse events and especially local reactions at the injection-site.(44, 45)

Currently, intratumoral injection of CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ myDCs combined with an AS01$_B$ adjuvant, which is a component of a prophylactic shingles vaccine and has preclinically shown DC-maturating properties, as an alternative stimulus for T-VEC is under evaluation in a phase I clinical trial (NCT03707808). The intratumoral administration of AS01$_B$ and CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ myDCs is combined with intratumoral injection of ipilimumab and intravenous administration of nivolumab. First safety and antitumoral results are pending.

A randomized phase II clinical trial is currently investigating stereotactic body radiation therapy and systemic pembrolizumab with or without intratumoral avelumab and ipilimumab plus CD1c (BDCA-1)$^+$/CD141 (BDCA-3)$^+$ myDCs in patients with solid tumors, including advanced melanoma patients who present with oligometastatic progression after PD-1-based therapy (NCT04571632). This trial for the first time also allows the injection of visceral lesions making such an intratumoral approach potential for more patients.(46)

Finally, intratumoral injection of an autologous moDC vaccine is currently being investigated in a phase I study, wherein safety and efficacy of moDCs pulsed with tumor lysate (PV-001-DV) combined with systemic infusion of PV-001-DCs in advanced melanoma patients is investigated (NCT03803397).

Novel strategies to turn tumors into *in situ* vaccines

Besides DC vaccines, also strategies to attract DCs into the TME are under investigation. Radiotherapy has been shown to prime tumor-specific $CD8^+$ T cells, which is dependent on infiltration of cDC1 in the tumor by radiotherapy-induced type I IFNs. *Wennerberg et al* described that CD73 is increased upon radiotherapy and that CD73 blockade with radiotherapy restored radiotherapy-induced cDC1 infiltration in settings where induction of type I IFNs was suboptimal.(47)

Recently, *Bergamaschi et al* proposed a model wherein heterodimeric IL-15 promotes intratumoral CTL and cDC1 accumulation. In response to heterodimeric IL-15 treatment, activated $CD8^+$ T cells and NK cells released IFN-γ and XCL-1. Increased levels of XCL-1 induced recruitment of cDC1 in tumors, which in turn induced secretion of the chemokines CXCL9 and CXCL10 that attract $CD8^+$ T cells and NK cells into the tumor.(48) In advanced tumors, this effector response is largely suppressed but radiation can be used to jumpstart the cancer-immunity cycle by inducing cancer cell-intrinsic type I IFN release to attract cDC1 which in turn produce CXCL9 and CXCL10. IL-15 is expressed by cDC1 and thus fosters the crosstalk between $CD8^+$ T cells, NK cells, and cDC1 to reprogram the tumor immune contexture.(49)

Another cytokine that is capable of increasing the number of intratumoral DCs is Flt3L. Flt3L drives expansion of various bone marrow progenitor populations, however, in mature cells Flt3 expression is only observed in pDCs and myDCs. Flt3L administration expands both DC-subsets and pre-DCs in the blood. However, Flt3L does not affect the maturation state of the DCs.(16, 50) This could be overcome by co-injection of a TLR agonist, which is explored by *Hammerich et al* who combine local administration of Flt3L, radiation therapy and the TLR3 ligand poly-ICLC and showed increased $CD8^+$ T-cell responses in patients suffering from indolent non-Hodgkin's lymphoma.(51)

Conclusions and perspectives

In recent years, nDCs have come to the forefront of cancer vaccines instead of moDCs, especially because of their more mature functional properties. Furthermore, intratumoral administration of nDCs

in combination with TME-priming agents seems to be a feasible and safe alternative for classical DC vaccines and should be investigated more thoroughly (Figure 1). Intratumoral therapies are also mainly feasible for patients with accessible tumor lesions such as cutaneous, lymph node or other soft tissue metastases, since injection of visceral lesions requires experienced radiologists and computed tomography-guidance and the risk for iatrogenic complications remains higher. Strategies using intratumoral injection of non-substantially manipulated nDCs or *in situ* "vaccines" make it possible that all private tumor antigens of an individual patient can be processed by the DCs present in the TME, which stands in contrast to DC vaccines that have been loaded with specific tumor antigens. A possible disadvantage of injecting or attracting DCs in the tumor could be inhibition of DC-activation/function in the TME, thereby skewing them towards a protumoral phenotype. This can potentially be circumvented by rational combination with DC-activating agents.

Finally, strategies that attract DCs into the TME are explored to circumvent the expensive cell isolation and/or *ex vivo* culture procedures for DC-vaccines.

Key Points

(3-5 key points/sentences that summarize your article)

- In the recent years, naturally circulating DCs such as myeloid and plasmacytoid dendritic cells are earning more interest to be used as cancer vaccine instead of monocyte-derived DCs, mainly for their more mature functional properties.
- Intratumoral administration of naturally circulating DCs in combination with tumor microenvironment-priming agents seems to be a feasible and safe alternative for classical DC vaccines.
- To obviate the need for expensive cellular vaccines, approaches to attract DCs into the tumor microenvironment are being developed.

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Conflicts of interest

JKS reports non-financial support from MSD and Amgen; personal fees from Novartis. Bart Neyns reports personal financial compensation from Roche, Bristol-Myers Squibb, Merck Sharp & Dohme, Novartis, AstraZeneca for public speaking, consultancy, and participation in advisory board meeting. The institution (UZ Brussel) received research funding related to research projects conducted by Bart Neyns from Pfizer, Novartis, Roche, Merck-Serono. The remaining authors have no conflicts of interest.

Figure legends:

(Attach figures and tables separately)

Fig 1.

Heading: **Evolution of DC-based vaccines for treatment of melanoma patients**.

Legend: **Evolution of DC-based vaccines for treatment of melanoma patients**. Naturally circulating DCs and monocytes are isolated from blood through leukapheresis and immunomagnetic cell sorting, and in case of moDCs differentiated *in vitro* from monocytes. Over the past years, the type of DCs and administration route for DC vaccination evolved considerably. More recently focus shifted from traditional and intratumoral vaccines to *in situ* vaccines. Figure created in *BioRender*.

Flt3L: FMS-like tyrosine kinase 3; ICI: immune checkpoint inhibitors; IL-15: interleukin 15; moDC: monocyte-derived dendritic cell; myDC: myeloid dendritic cell; OV: oncolytic virus; pDC: plasmacytoid dendritic cell; TAA: tumor-associated antigens; TLR: toll-like receptor

References

1. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23.

2. McDermott D, Haanen J, Chen TT, Lorigan P, O'Day S, investigators MDX. Efficacy and safety of ipilimumab in metastatic melanoma patients surviving more than 2 years following treatment in a phase III trial (MDX010-20). Ann Oncol. 2013;24(10):2694-8.

3. Robert C, Thomas L, Bondarenko I, , et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517-26.

4. Maio M, Grob JJ, Aamdal S, et al. Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. J Clin Oncol. 2015;33(10):1191-6.

5. Ascierto PA, Del Vecchio M, Robert C, et al. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. Lancet Oncol. 2017;18(5):611-22.

6. Ascierto PA, Del Vecchio M, Mackiewicz A, et al. Overall survival at 5 years of follow-up in a phase III trial comparing ipilimumab 10 mg/kg with 3 mg/kg in patients with advanced melanoma. J Immunother Cancer. 2020;8(1).

7. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908-18.

8. Hamid O, Puzanov I, Dummer R, et al. Final analysis of a randomised trial comparing pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory advanced melanoma. Eur J Cancer. 2017;86:37-45.

9. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med. 2015;372(26):2521-32.

10. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320-30.

11. Robert C, Long GV, Brady B, et al. Five-Year Outcomes With Nivolumab in Patients With Wild-Type BRAF Advanced Melanoma. J Clin Oncol. 2020;38(33):3937-46.

12. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39(1):1-10. ** This seminal paper describes the cancer-immunity cycle which is now recognized as the fundament for induction of anti-tumor responses.

13. Spranger S, Dai D, Horton B, Gajewski TF. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. Cancer Cell. 2017;31(5):711-23 e4. ** This paper highlights that the presence of DC in the tumor microenvironment is crucial for the induction of effective anti-tumor immune responses. In addition, the authors show that the beta-catenin signaling pathway is responsible for exclusion of DCs from the tumor microenvironment.

14. Spranger S, Gajewski TF. A new paradigm for tumor immune escape: beta-catenin-driven immune exclusion. J Immunother Cancer. 2015;3:43.

15. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature. 2015;523(7559):231-5.

16. Salmon H, Idoyaga J, Rahman A, et al. Expansion and Activation of CD103(+) Dendritic Cell Progenitors at the Tumor Site Enhances Tumor Responses to Therapeutic PD-L1 and BRAF Inhibition. Immunity. 2016;44(4):924-38.

17. Broz ML, Binnewies M, Boldajipour B, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell. 2014;26(5):638-52.

18. Bottcher JP, Bonavita E, Chakravarty P, et al. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. Cell. 2018;172(5):1022-37 e14.

19. Roberts EW, Broz ML, Binnewies M, et al. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. Cancer Cell. 2016;30(2):324-36.

20. Spranger S, Luke JJ, Bao R, et al. Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. Proc Natl Acad Sci U S A. 2016;113(48):E7759-E68.

21. Barry KC, Hsu J, Broz ML, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nat Med. 2018;24(8):1178-91.

22. Garris CS, Arlauckas SP, Kohler RH, et al. Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-gamma and IL-12. Immunity. 2018;49(6):1148-61 e7.

23. Binnewies M, Mujal AM, Pollack JL, et al. Unleashing Type-2 Dendritic Cells to Drive Protective Antitumor CD4(+) T Cell Immunity. Cell. 2019;177(3):556-71 e16.

24. Schlitzer A, McGovern N, Teo P, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity. 2013;38(5):970-83.

25. Williams JW, Tjota MY, Clay BS, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. Nat Commun. 2013;4:2990.

26. Wculek SK, Cueto FJ, Mujal AM, et al. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020;20(1):7-24.

27. Storkus WJ, Maurer D, Lin Y, et al. Dendritic cell vaccines targeting tumor blood vessel antigens in combination with dasatinib induce therapeutic immune responses in patients with checkpoint-refractory advanced melanoma. J Immunother Cancer. 2021;9(11).

28. Lovgren T, Wolodarski M, Wickstrom S, et al. Complete and long-lasting clinical responses in immune checkpoint inhibitor-resistant, metastasized melanoma treated with adoptive T cell transfer combined with DC vaccination. Oncoimmunology. 2020;9(1):1792058.

29. Saberian C, Amaria RN, Najjar AM, et al. Randomized phase II trial of lymphodepletion plus adoptive cell transfer of tumor-infiltrating lymphocytes, with or without dendritic cell vaccination, in patients with metastatic melanoma. J Immunother Cancer. 2021;9(5).

30. Villani AC, Satija R, Reynolds G, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science. 2017;356(6335). **In this paper, single-cell RNAseq was used to map the different subtypes of DCs in human blood. The data have led to the identification of novel DC subtypes of which the function still needs to be explored.

31. Laoui D, Keirsse J, Morias Y, et al. The tumour microenvironment harbours ontogenically distinct dendritic cell populations with opposing effects on tumour immunity. Nat Commun. 2016;7:13720.

32. Crozat K, Guiton R, Guilliams M, et al. Comparative genomics as a tool to reveal functional equivalences between human and mouse dendritic cell subsets. Immunol Rev. 2010;234(1):177-98.

33. Worah K, Mathan TSM, Vu Manh TP, et al. Proteomics of Human Dendritic Cell Subsets Reveals Subset-Specific Surface Markers and Differential Inflammasome Function. Cell Rep. 2016;16(11):2953-66.

34. Haniffa M, Shin A, Bigley V, et al. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. Immunity. 2012;37(1):60-73.

35. Wimmers F, Schreibelt G, Skold AE, et al. Paradigm Shift in Dendritic Cell-Based Immunotherapy: From in vitro Generated Monocyte-Derived DCs to Naturally Circulating DC Subsets. Front Immunol. 2014;5:165. * This excellent review provides an overview of the development of dendritic cell immunotherapy.

36. Bol KF, Schreibelt G, Rabold K, et al. The clinical application of cancer immunotherapy based on naturally circulating dendritic cells. J Immunother Cancer. 2019;7(1):109.

37. Bakdash G, Schreurs I, Schreibelt G, Tel J. Crosstalk between dendritic cell subsets and implications for dendritic cell-based anticancer immunotherapy. Expert Rev Clin Immunol. 2014;10(7):915-26.

38. van Beek JJ, Gorris MA, Skold AE, et al. Human blood myeloid and plasmacytoid dendritic cells cross activate each other and synergize in inducing NK cell cytotoxicity. Oncoimmunology. 2016;5(10):e1227902.

39. Tel J, Aarntzen EH, Baba T, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. Cancer Res. 2013;73(3):1063-75.

40. Bloemendal M BK, Boudewijns S, Gorris MAJ, et al. Immunological responses to adjuvant vaccination
with combined CD1c+ myeloid and plasmacytoid dendritic cells in stage III melanoma patients. ONCOIMMUNOLOGY.2022, VOL. 11, NO. 1, e2015113 (12 pages).

41. Bol K BM, van Willigen W, Schreibelt G, et al. 1078MO - MIND-DC: A randomized phase III trial to assess the efficacy of adjuvant dendritic cell vaccination in comparison to placebo in stage IIIB and IIIC melanoma patients. ESMO Virtual Congress 2020: Annals of Oncology (2020) 31 (suppl_4): S672-S710. 10.1016/annonc/annonc280; 2020.

42. Charles J, Chaperot L, Hannani D, et al. An innovative plasmacytoid dendritic cell line-based cancer vaccine primes and expands antitumor T-cells in melanoma patients in a first-in-human trial. Oncoimmunology. 2020;9(1):1738812.

43. Schwarze JK, Awada G, Cras L, et al. Intratumoral Combinatorial Administration of CD1c (BDCA-1)(+) Myeloid Dendritic Cells Plus Ipilimumab and Avelumab in Combination with Intravenous Low-Dose Nivolumab in Patients with Advanced Solid Tumors: A Phase IB Clinical Trial. Vaccines (Basel). 2020;8(4).

44. Schwarze JK, Tijtgat J, Awada G, et al. 962MO - A phase I clinical trial on intratumoral injection of autologous CD1c (BDCA-1)+/CD141 (BDCA-3)+ myeloid dendritic cells (myDC) in

combination with talimogene laherparepvec (T-VEC) in patients with advanced pretreated melanoma. ESMO Congress 2021.

45. Schwarze JK, Tijtgat J, Awada G, et al. Intratumoral administration of CD1c (BDCA-1)(+) and CD141 (BDCA-3)(+) myeloid dendritic cells in combination with talimogene laherparepvec in immune checkpoint blockade refractory advanced melanoma patients: a phase I clinical trial. J Immunother Cancer. 2022;10(9).

46. Tijtgat J, Schwarze JK, Vandenbroucke F, et al. Early interil results from a randomized phase II clinical trial of stereotactic body radiation therapy (SBRT) and systemic pembrolizumab with or without intratumoral avelumab/ipilimumab plus CD1c (BDCA-1)+/ CD141 (BDCA-3)+ myeloid dendritic cells in non-small cell lung cancer (NSCLC). ImmunoRad: The International Conference on Immunotherapy Radiotherapy Combinations; New York2022.

47. Wennerberg E, Spada S, Rudqvist NP, et al. CD73 Blockade Promotes Dendritic Cell Infiltration of Irradiated Tumors and Tumor Rejection. Cancer Immunol Res. 2020;8(4):465-78.

48. Bergamaschi C, Pandit H, Nagy BA, et al. Heterodimeric IL-15 delays tumor growth and promotes intratumoral CTL and dendritic cell accumulation by a cytokine network involving XCL1, IFN-gamma, CXCL9 and CXCL10. J Immunother Cancer. 2020;8(1).

49. Pilones KA, Charpentier M, Garcia-Martinez E, Demaria S. IL15 synergizes with radiotherapy to reprogram the tumor immune contexture through a dendritic cell connection. Oncoimmunology. 2020;9(1):1790716.

50. Cueto FJ, Sancho D. The Flt3L/Flt3 Axis in Dendritic Cell Biology and Cancer Immunotherapy. Cancers (Basel). 2021;13(7).

51. Hammerich L, Marron TU, Upadhyay R, et al. Systemic clinical tumor regressions and potentiation of PD1 blockade with in situ vaccination. Nat Med. 2019;25(5):814-24.