Chapter

Dendritic Cells and Their Roles in Anti-Tumour Immunity

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Abstract

Dendritic cells are rare cells found in blood and throughout all organs of the body as resident or migrating cell populations. Dendritic cells sense danger signals of pathogens and host cell stress through pattern receptors expressed on the cell surface and within organelles of the cell. Ligation of these receptors leads to activation and production of many different chemokines, cytokines and interferons. Key to the function of dendritic cells is their potent capacity to present antigen and activate naïve T cells. These qualities, potent antigen presentation and cytokine production together allow the dendritic cells to be at the forefront of danger responses, linking innate and adaptive immunity. Research over the last 20 years has clarified a role of dendritic cells in anti-tumour responses, and their location within the tumour environment is clear, with both deleterious and beneficial correlations, depending on the subset and tumour type. Harnessing the qualities of dendritic cells to increase anti-tumour immunity is the ultimate goal, although this will require extensive knowledge of different dendritic cell subsets and their regulation through immune checkpoints.

Keywords: dendritic cells, pattern recognition receptors, immune checkpoints, tumour vaccines, plasmacytoid dendritic cell, conventional dendritic cell

1. Introduction to dendritic cells

Dendritic cells (DCs) are professional antigen presenting cells (APCs), the only cells capable of specifically activating naïve T cells and are key orchestrators of an immune response. They are a rare, heterogeneous population of haematopoietic cells that are equipped to capture, process and present antigen (Ag) to the adaptive immune system.

In a non-inflamed or steady state setting, DCs constantly sample the local environment for Ags and have the potential to induce peripheral tolerance via T cell anergy or deletion [1]. DCs recognise danger via pattern recognition receptors (PRR) on their cell surface, the cytoplasm and within cellular organelles [2]. Ligation of PRRs by pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs), activates DC and licences DC to upregulate co-stimulatory marker expression such as CD86 and CD80 on their cell surface and initiate immunogenic T cell priming.

DCs situated in non-lymphoid tissues, also known as migratory DCs, constantly migrate to draining lymph nodes (LNs), maturing during this process, to present Ag to naïve T cells. Resident DCs in lymphoid organs are immature and maintain tolerance during steady state, but can stimulate naïve T cells when activated *in situ*. The DC maturation process not only involves morphological changes into their characteristic stellate shape with dendritic cytoplasmic processes and increased expression of MHC and co-stimulatory markers, but their Ag acquisition and sampling capabilities are initially upregulated and then rapidly shut down while MHCII expression on the cell's surface is increased due to the simultaneous up- and down-regulation of MHCII synthesis and turnover events respectively [3]. This allows mature DCs to present a snapshot of the Ag profile in its local environment prior to migration and/or activation. Furthermore, activated DCs produce a combination of cytokines that modulate an immune response that is specific to the initial danger signals.

In humans, the majority of DC characterisation studies are of DCs isolated from the blood due to the rarity of the cell type and limited access to human tissue samples, although more investigations on non-lymphoid DCs in the skin, lung and liver have recently emerged [4–7]. DCs in the blood comprise ~1% of total peripheral blood mononuclear cells (PBMCs) and are traditionally identified by the high expression of MHCII (HLA-DR) and the lack of lineage markers CD3, CD14, CD15, CD19, CD20 and CD56, although the latter marker has recently been shown to be expressed on gut and other non-lymphoid DCs [6].

Human blood DCs can be divided into conventional DCs (cDCs) and plasmacytoid DCs (pDCs), which are HLA-DR^{hi}CD11c^{+}123^{-} and HLA-DR^{hi}CD11c^{-}123^{+} respectively. Human blood cDCs are further categorised into cDC1 and cDC2 subsets. Additionally, there are monocyte-derived DCs that originate separately from cDCs and pDC precursors. The recent use of whole population and single cell sequencing techniques has been instrumental in elucidating transcription factors and surface markers that are unique to each DC subset, which has helped identify

	DC subsets		
	cDC1	**cDC2**	**pDC**
Surface phenotype	CD11c^{+}HLA-DR^{+} CD123^{-}CLEC9A^{+} XCR1^{+}Necl2^{+} CD141^{+} CD11b^{-}CD172α^{-}	CD11c^{+}HLA-DR^{+}CD123^{-} CD1c^{+}CD11b^{+}CD172α^{+} CLEC10A^{+} with further subdivision based on CD5^{hi}CD32B^{+}CD163^{-}CD36^{-} or CD5^{lo}CD32B^{-}CD163^{+}CD36^{+}	CD11c^{-}HLA-DR^{+} CD123^{+}CD303^{+} CD304^{+}CD45RA^{+} CD2$^{+/-}$
Transcription factors	*BATF3*, *IRF8*	*IRF4*, *IRF8*	*TCF4*, *SPIB*, *ZEB2*, *IRF4*, *IRF8*, *IRF7*
PRR expression	TLR3, 8	TLR2, cytosolic RNA sensors (RIG-I, MDA-5), STING	TLR7, 9, STING
Ag presentation	Cross-presentation of cellular Ag	Cross-presentation of soluble Ag	CD4^{+} and CD8^{+} T cell priming*
Roles in immunity	Potent producer of Type III IFN (after TLR3 stimulation), CTL priming, Th1 response	Th1, Th17 response	Potent producers of Type I and III IFN and mediating antiviral immunity

**Previous Ag presentation abilities by pDCs are now suggested to be contributed by contaminating AXL^{+}Siglec6^{+} (AS) DCs.*

Table 1.
Key features of human DC subsets.

relationships between DC subsets across species and tissues as well as corroborate DC functional analyses [6–9], summarised in **Table 1**.

2. Conventional dendritic cells 1 (cDC1)

cDC1s constitute ~0.03% of PBMCs and are found in the blood, tonsil, spleen and non-lymphoid tissues such as the skin. They were classically defined by the high expression of CD141 (blood DC antigen 3 (BDCA3) or thrombomodulin) [10]. However, CD141 is not a completely specific marker for cDC1 as it is also expressed on endothelial cells, monocytes and other DC subsets [8]. Using phenotypic, transcriptional and functional assays, these $CD141^+$ DCs have been further characterised as $CD11c^+HLA\text{-}DR^+CD11b^-CD172a^-$ $CLEC9a^+XCR1^+Necl2^+$ cells that lack monocytic markers CD14 and CD16 [4, 11] identifying them as human cDC1 [12–16].

The dependence of $CD141^+$ DCs on Flt3 ligand (FL), an important DC developmental factor, has been demonstrated *in vitro* and *in vivo* [11, 17–19] and transcription factor *BATF3* is required *in vitro* but not *in vivo* [15]. Another cDC1-defining transcription factor, *IRF8*, is also highly expressed in human cDC1, although patients harbouring mutations in *IRF8* did not exhibit cDC1 deficiencies, suggesting the involvement of other transcription factors as well [6, 20]. Furthermore, genome wide expression profiling and microarray analyses have revealed transcriptional profile clustering between $CD141^+$ DCs in blood and non-lymphoid tissues, as well as between human blood $CD141^+$ DCs and murine $CD8a^+$ and migratory $CD103^+$ DCs [4, 21], firmly establishing $CD141^+$ cDC as cDC1.

PRRs expressed by human cDC1s are predominantly Toll-like receptor (TLR) 3, located in endosomes and which recognises double-stranded RNA and TLR8, also located in endosomes and which recognises bacterial ssRNA and mammalian mitochondrial RNA [10, 22]. In response to TLR3 signals [23] and also HCV *in vivo* [23, 24], the cDC1 produce large amounts of type III interferon (IFN), also known as IFN-lambda (λ).

The cDC1s are superior to other DC subsets in their ability to present ex*ogenous* Ag on MHCI, a process known as cross-presentation [2] and the activation of cytotoxic $CD8^+$ T cells, crucial for anti-tumour responses. In particular, they have a specialised ability to cross-present Ags from dead or necrotic cells to $CD8^+$ T cells, enhanced by Clec9a on cDC1 binding to actin filaments exposed on dead and dying cells [25]. The cDC1 are superior at inducing Th1 differentiation of CD4 helper T cells [11, 16].

3. Conventional dendritic cells 2 (cDC2)

Human cDC2, traditionally known as $CD1c^+$ or $BDCA1^+$ DCs, constitute ~1% of PBMCs and can be identified by the expression of CD11c, CD11b, CD13, CD33, CD172a, HLA-DR and CD45RO [2, 10, 26]. The phenotypic similarities between these DCs and moDCs, as well as the expression of CD1c on B cells and other DC subsets, have made the precise segregation of this subset quite difficult. Although previous studies have used CD64 to exclude monocytes from bonafide $CD1c^+$ DCs in the blood, cDCs express low levels of this marker and cannot be definitively used to separate the cell populations [6, 7]. More recently, the use of single cell RNA sequencing techniques has identified additional surface phenotypic markers, such as *CLEC10A*, *FCGR2B*, *FCER1A*, to distinguish human cDC2 subsets [7, 8]. In particular, *CLEC10A* protein has been proposed as the cDC1 *CLEC9A*-equivalent marker for cDC2s in different species and tissues. However, different

isoforms of Clec10A have been found in mice and should be carefully considered when using it across species [27]. Heterogeneity within the human cDC2 subset has been identified using CD5 or CD32B versus CD163 and CD36. The $CD5^{lo}$ or $CD163^{+}CD36^{+}$ 'cDC2' are transcriptionally more related to monocytes than the other cDC2 subset ($CD5^{hi}$ or $CD32B^{+}$) [8, 28]. Like cDC1, $CD1c^{+}$ cDC2s require FL, but also rely on transcription factors *IRF4* and *IRF8*, for development [20, 29].

The cDC2 DCs highly express TLR2 and also express a range of cytosolic viral RNA sensors such as RIG-I [30, 31]. Different proposed cDC2 subsets also seem to have different PRR expression patterns. For example, $CD5^{hi}$ cDC2 express high levels of TLR7 and 8 compared to $CD5^{lo}$ cDC2 and $CD32B^{+}$ cDC2 express higher levels of *TMEM173* (also known as STING) in comparison to $CD163^{+}$ $CD36^{+}$ cDC2 subset [8, 28].

Activated cDC2s can drive Th17 immune response and can also produce high levels of IL-12p70, potentially inducing Th1 differentiation [2, 29]. However, current data suggests Th17 versus Th1 driven responses may be independently driven by $CD5^{+}$ versus $CD5^{lo}$ cDC2 subsets, respectively [8, 28].

Human cDC2s are able to cross-present *soluble* Ag to naïve and memory $CD8^{+}$ T cells at comparable levels with cDC1s [32–35]. However, the mechanism of cross-presentation differs between both subsets [35] and cDC2 do not possess the potent ability to cross-present Ags from dead cells. Human cDC2 are also potent stimulators of $CD4^{+}$ T cells [8, 10, 16].

4. Plasmacytoid dendritic cells (pDC)

The pDCs constitute ~0.01–0.04% of PBMCs and commonly reside in secondary lymphoid organs localising in the follicular cortex, T cell nodules and around high endothelial venules [36, 37]. As their name suggests, pDCs are similar in morphology to that of plasma cells. Under light microscopy, pDCs are observed to be spherical in shape with a rounded nucleus, often predominant endoplasmic reticulum and present as clusters in T-cell rich regions of lymphoid tissue [36–38].

The pDCs, originally identified as 'natural interferon producing cells' (NIPC), are renowned for their ability to drive immense type I and type III IFN production via TLRs 7 and 9 [39–41]. This IFN production is essential to combat viral infection but pDC-derived IFN is also thought to contribute to disease in autoimmune diseases including systemic lupus erythematosus [42]. They are also thought to play a role in Th2 induction and asthma progression in humans [42]. Conversely, pDC have also been shown to play a major role in tolerance *in vivo*, through their production of IDO and TGF-beta [42].

pDCs are recognised as being $CD11c^{-/lo}CD45RA^{+}CD123^{+}CD303^{+}CD304^{+}$HLA-$DR^{+}$ and can express CD56 (reviewed in [2]). pDCs may also be identified by their transcription factors including; TCF4 (also known as E2-2), SPIB, ZEB2, IRF8, IRF7 and IRF4 [43–45]. Haploinsufficiency in the *TCF4* gene results in Pitts-Hopkins syndrome, which characteristically generates defective pDCs, illustrating a dependence of this factor for normal human pDC development [46].

The pDCs can be divided into 2 subsets based on CD2 expression [47]. Recent single cell transcriptomic profiling of blood DCs from healthy donors has revealed that $CD2^{+}$ 'pDC' also express AXL and SIGLEC6 (known as AS DCs). These AS DCs can stimulate $CD4^{+}$ and $CD8^{+}$ allogeneic T cell proliferation whereas the segregation of pDCs away from contaminating AS DCs demonstrated potent IFN-α production after TLR9 stimulation and a lack of T cell priming attributes [8]. Whether AS DCs and pDC are 2 distinct cell types or differentiation stages of one another is yet to be defined.

A rare and highly aggressive acute leukaemia known as Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) involves the malignancy of pDC precursors [48], driven, at least in part by the juxtaposition of the pDC-specific RUNX2 enhancer and the MYC promotor due to the chromosomal translocation (6;8) (p21;q24) [49]. The BPDCN can be reliably identified by immunohistochemical staining with TCF4 and CD123 antibodies [50]. BPDCNs most commonly present as skin lesions and may be accompanied by swelling of other organs such as the lymph nodes, bone marrow or spleen. Standard chemotherapy treatments for myeloid neoplasms often result in poor prognosis [51] although a toxin-conjugated anti-CD123 drug, tagraxofusp-erzs, has recently been approved as the first FDA-approved BPDCN-specific treatment [52].

5. Monocyte derived DCs

Monocyte derived DC (moDC) refers to DCs induced from monocytes with GM-CSF *in vitro.* These tissue culture systems originated in the early 1990s based on work showing varying combination of cytokines with GM-CSF could induce the acquisition of antigen presentation capacity in stem cells and $CD34^+$ blood precursors [53–56], and this was optimised with the addition of IL-4 [57]. These systems have been an immensely popular tool for more than two decades for *in vitro* research pertaining to conventional DC biology and immunological function. They have been particularly useful in human research due to the difficulties in obtaining large numbers of *ex vivo* primary human DC for research. However, the feasibility of these models has recently been questioned, detailed analyses of GM-CSF induced DC cultures reveal a heterogeneous population of macrophages and conventional DCs, with the $MHCII^{hi}$ cells the most DC-like [58–61].

It still remains unclear whether the moDC actually represent an *in viv*o equivalent cell subset. They potentially represent an *in vitro* equivalent of an inflammatory monocyte known as TNF/iNOS producing DCs (TipDCs), based on their surface phenotype [62], cytokine profile and a shared precursor [62]. Importantly, high intra-tumoral expression of CD40L, TNF-α and iNOS, key phenotypes of TipDCs, were strongly correlated with substantially higher long term disease free survival rates over 10 years in patients with colorectal cancer [63]. Therefore, moDCs may represent a useful and relevant *in vitro* model of inflammatory DCs.

5.1 MoDC and cancer vaccines

While the *ex vivo* induced moDC do not recapitulate *bona fide* DC subsets, the ease of isolation and culture has made the moDC a popular vaccine candidate in human clinical trials since the late 1990s. However, results from clinical trials using moDC in cancer immunotherapies for various cancer types have been modest at best [64, 65]. In a more recent phase II trial of patients with surgically resectable liver metastatic colon adenocarcinoma, vaccination of patients with autologous tumour lysate pulsed moDC conferred interim protection, demonstrating a 3-fold increase in the median disease free survival compared to the control arm of the study [66]. The continued refinement of moDC preparations and the choice of antigens, may see future improvements of DC cancer vaccines.

The ability to present Ag and activate the adaptive immune response makes DCs an attractive target to re-invigorate anti-cancer immunity. There are different types of DC vaccines, with the most common type involving the *ex vivo* maturation

of autologous DCs. In this method, DCs are isolated from patient peripheral blood mononuclear cells (PBMCs) obtained via leukapheresis, incubated with maturation stimuli and tumour Ags, and vaccinated back into the patient. Because this method requires a large number of DCs, and naturally circulating blood DCs are rare, the majority of clinical trials have previously used moDCs for this type of DC vaccine and have been extensively characterised [67, 68].

Thus far, a wide variety of moDC vaccine strategies have been trialled [68]. moDCs have been differentiated and matured using monocyte conditioned medium with various supplements of cytokines (TNF-α, GM-CSF, IL-4, IFN-α), TLR agonists (LPS) and other factors such as prostaglandin E2 [67–69]. There is also variety in the type of Ags loaded into DCs such as peptides from tumour-associated Ags (TAA), TAA-encoding mRNA and whole tumour lysates [67]. More recently, the electroporation of synthetic mRNA encoding DC-maturation factors such as CD40 ligand, constitutively active TLR4 and CD70 together with fusion proteins DC-LAMP and melanoma-associated Ags into autologous moDCs (TriMixDC-MEL) have proven safe and immunogenic in phase 1 clinical trials in metastatic melanoma [70]. However, the variation in the aforementioned vaccine factors as well as the route of DC administration (intranodal, i.v.) and lack of standardised method of moDC generation has shown variable efficacies of moDC vaccines in clinical outcomes.

6. DC vaccines

More recent clinical trials using naturally circulating blood DCs have turned to CliniMACS system by Miltenyi to isolate different DC subsets from patients (**Figure 1**). Two completed Phase I clinical trials have used $CD1c^+$ DCs (cDC2) loaded with TAA peptides in hormone refractory metastatic prostate cancer and metastatic melanoma and observed good safety and immunogenicity [71, 72]. Another completed Phase I trial using pDCs showed the induction of tumour-Ag specific CTL response as well as an IFN signature [33]. On-going clinical trials, as summarised by Bol et al., are not only isolating single DC subsets for vaccination, but are also trying combination vaccines comprised of cDC2 and pDC subsets and using dual-activating maturation agonists such as single stranded RNA that stimulates TLR8 on cDC2 and TLR7 on pDCs (NCT-02993315, NCT-02574377, NCT-02692976) [67]. However, there are still many challenges in using naturally circulating blood DCs in tumour vaccinations. The methodology for isolation of sufficient $CD141^+$ cDC1 DCs, which comprise only 0.03% PBMCs, is still lacking and will be important to harness due to their superior ability to cross-present dead and necrotic Ag. Furthermore, although improved over the years, the duration of DCs spent *ex vivo* can drastically affect DC viability and functionality and the personalised nature of these vaccines can limit the quantity of patient access to these treatments.

Apart from the *ex vivo* maturation of autologous DCs, another strategy of DC vaccines has been receptor targeting (**Figure 1**). This involves the administration of a monoclonal Ab (mAb) specific for endocytic receptors on various DC subsets to deliver tumour Ags to DCs directly *in vivo* [73]. Tumour Ags are conjugated to these DC-targeting mAb either chemically, through genetic fusion, or attachment to nanoparticles and liposomes [74]. Importantly, the administration of adjuvant, such as TLR3 agonist poly I:C, in conjunction with Ag delivery, is necessary to induce immune priming instead of tolerance, as shown in mice [75–77]. Moreover, the targeting of cross-presenting DC subsets has been particularly attractive, due to their ability to activate CTLs. DEC-205, a C-type lectin that is highly expressed on cDC1 can cross-present Ag when targeted and induce tumour Ag NY-ESO-1-specific

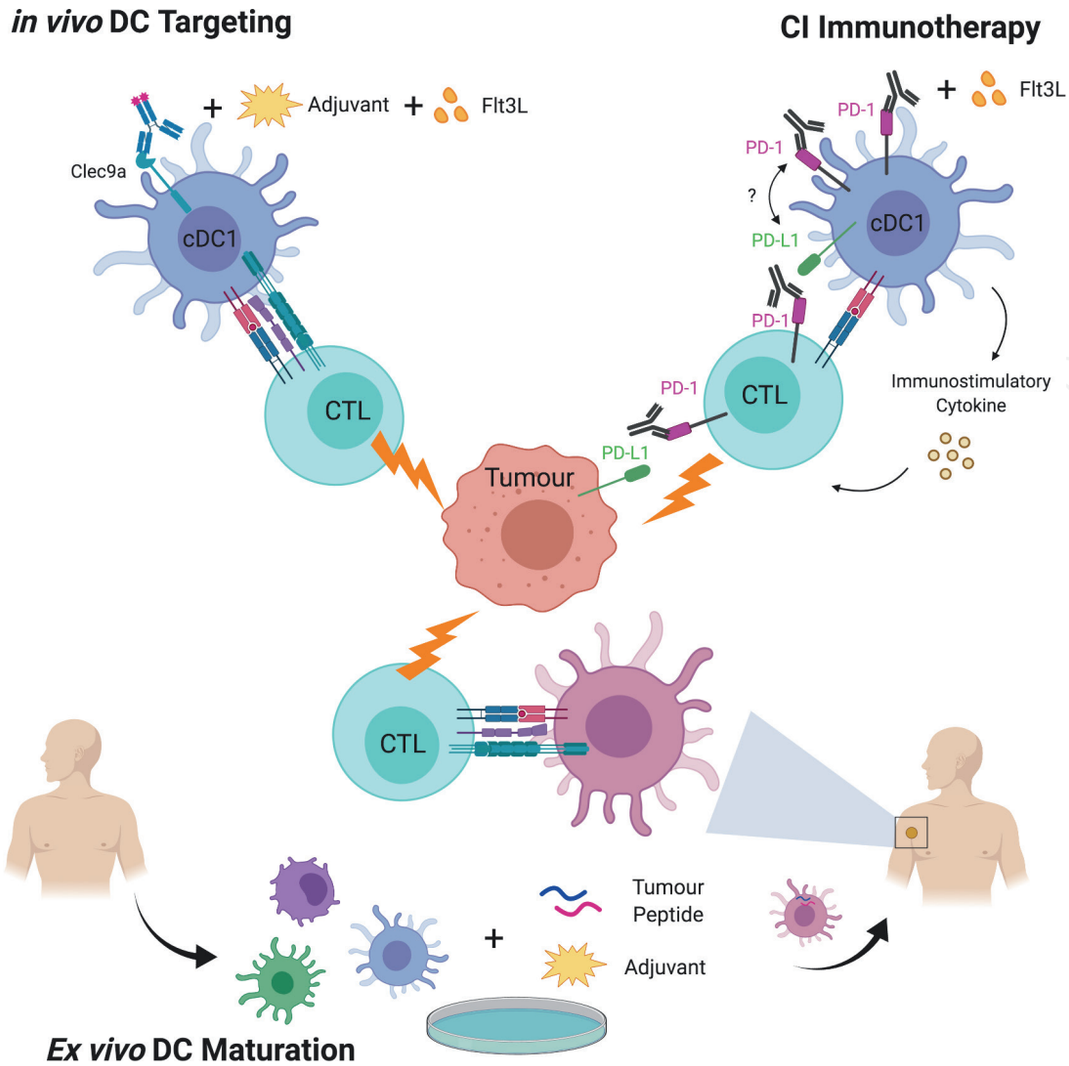

Figure 1.
Overview of potential roles of DC in cancer therapies. To improve current cancer treatments and the activation of tumour-specific CTL, DC may be directly targeted in vivo *(Section 6) or may themselves be the targets of checkpoint immunotherapies (Section 8).* Ex vivo *manipulation of DC (Section 6) may also be beneficial in some cancer patients.* In vivo *targeting strategies may also be combined with Flt3-L treatment to enhance DC numbers, and adjuvants targeting specific PRR to ensure the DC subset of interest are activated. Created with Biorender.com.*

cellular and humoral responses in patients with solid cancers [78, 79]. However, DEC-205 is also expressed on many other cell-types including $CD1c^+$ DCs, pDCs and monocytes which can affect targeting specificities and efficiencies [79–81]. In contrast, another C-type lectin, Clec9a (also known as DNGR-1), is specifically expressed on cDC1 and strategies targeting this molecule have demonstrated highly immunogenic responses without adjuvant in non-human primates, and also superior Ag-specific cross-presentation when targeted *in vitro* and *in vivo* [79, 81, 82]. Based on these pre-clinical studies, the progression of vaccines targeting Clec9a into clinical trials is much anticipated.

7. DC in the tumour microenvironment

The tumour microenvironment (TME) is a complex niche of tumour cells, stromal cells and tumour infiltrating myeloid and lymphoid immune cells. The dynamic nature of this niche varies with different types and stages of cancer, as well as between patients themselves. It has been established that the infiltration of $CD8^+$ cytotoxic T cells have been associated with better treatment outcomes with

checkpoint blockade therapies in a number of cancer types including metastatic melanoma [83]. However, the phenotype and role of tumour-infiltrating DCs (TIDCs) are less clear, possibly due to the lack of consistent markers probing DCs within the TME and the lack of distinctions between monocyte and putative DC subsets [84].

Using immunohistochemistry staining, many studies have previously used CD1a and S100 proteins to identify TIDCs. The higher density of these cells within tumours correlated with better clinical outcomes in melanoma and head and neck cancers [84, 85]. However, discrepancies in this correlation were reported in colon, breast, gastric, nasopharyngeal, lung and ovarian cancers [84, 86–88]. One major factor that could explain these reported discrepancies is the markers used to identify DCs. CD1a and S100 are expressed at different levels on Langerhans cells (LCs), interdigitating DCs and moDCs, but not on cDCs or pDCs and the expression of these markers on epithelial-tropic DCs such as LCs could account for the strong correlations observed in only the epithelial cancers [84]. Furthermore, DC activation markers CD83 and DC-LAMP were used to identify mature DCs, though CD83 is not expressed in all DC subsets [7, 84, 89]. In breast adenocarcinoma patients, immature DCs were found to localise within the tumour whereas CD83/DC-LAMP^{+} mature DCs localised in the peri-tumour edges [90]. Some studies have reported significant correlations between the intratumoral infiltration of mature DCs with better clinical outcomes. For example, a recent report showed that the recruitment of DC-LAMP^{hi} cells into the tumour stroma exhibited strong correlations with significantly higher overall and relapse-free survival in high-grade serous ovarian carcinoma [91]. However, this correlation has also been inconsistent in a number of different cancers [85, 90, 92–94].

More recently, with the establishment of The Cancer Genome Atlas (TCGA) program, scientists are able to compare DC-specific signatures with a publicly available molecular and clinical database of a vast array of cancers. In melanoma and breast cancer patients, DC-specific genes such as *BATF3*, *IRF8, CLEC9A* and *FLT3* were associated with higher CTL scores and better overall survival [95–97]. They also exhibited positive correlations with chemokines *CXCL9*, *10* and *11* and chemokine receptor *CCR7* expression [95, 96]. Furthermore, Broz et al. [98] observed strong associations between cDC1-derived genes within the tumour and better overall survival in breast cancer, head-neck squamous cell carcinoma and lung adenocarcinoma. This corroborates mouse tumour models showing that migratory cDC1 subsets are required for cross-presenting tumour Ag in tumour-draining lymph nodes and priming of cytotoxic CD8^{+} T cells [97, 99].

Whilst the recent data above points towards a benefit of the infiltration of conventional DC into tumour sites, the correlation between tumour infiltrating pDCs and poor survival prognosis is clear. This has been described in breast, head and neck, ovarian and lung cancers [100–103] where it is thought that pDC-induced tolerance and impaired IFN-α production contributes to a suppressive, non-immunogenic TME. Indeed mouse studies point to a role of TGF-β in the tumour environment in preventing an activatory phenotype of pDC and favouring a tolerising, IDO producing phenotype [104].

Further factors within the TME that have been illustrated to correlate with DC infiltration or function include for example, vascular endothelial growth factor (VEGF), a tumour angiogenic factor, inversely correlated with DC density and overall survival in gastric adenocarcinoma tissues [87, 105]. High serum VEGF levels were also associated with low blood cDC1 and cDC2 numbers in colorectal and non-small cell lung cancers and treatment of VEGF decoy

receptor, VEGF-Trap, increased the proportion of mature DCs, but not overall numbers or DC priming function in various solid cancer patients [106–108]. Direct evidence of VEGF-induced DC inhibition was also reported in DCs differentiated from $CD34^+$ precursors and moDCs [105, 106, 109]. Other cytokines such as IL-6, IL-10 and TGFβ have also demonstrated DC-inhibitory effects in the TME [104, 110–114].

In metastatic melanoma patients, higher active β-catenin signalling within the tumour was associated with low cDC1 signatures and T cell signatures [115]. Furthermore, the expression of fatty acid synthase was inversely correlated with $CD11c^+$ DC signatures in ovarian, prostate and bladder cancers [116].

8. DC and immune checkpoint inhibitors

Chemotherapy and radiotherapy have remained the core pillars of cancer treatments. However, the combination of these traditional therapies with immunotherapies targeting immune checkpoint receptors has greatly enhanced patient clinical outcomes, especially in patients with immunogenic cancers, summarised in **Table 2**.

Immune checkpoints consist of a family of co-stimulatory and co-inhibitory receptors expressed by T cells that modulate their immune responses. Signalling from these receptors depends on their interaction with specific ligands present at the surface of various immune and non-immune cells. These regulatory pathways are a major cause of immune suppression during cancer due the high levels of co-inhibitory ligands being expressed in the tumour microenvironment, resulting in T cell immunosuppression. Monoclonal antibodies (mAb) blocking programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4), two co-inhibitory immune checkpoint receptors have become routine treatment against many malignancies and more therapeutic molecules against members of the immune checkpoint family are being trialled. Here we review the role of DC in the response to immune checkpoint therapies.

8.1 DC and PD-1

PD-1 is expressed by activated T cells and interacts with two ligands, PD-L1 (B7-H1/CD274) and PD-L2 (B7-DC/CD273). PD-1 engagement results in down-regulation of T cell proliferation and function [117]. This inhibitory pathway is harnessed by tumour cells to escape attack by T cells through expression of PD-L1 on their cell surface. Anti-PD-1/PD-L1 therapies have shown considerable effects on patients with high PD-L1-expressing tumours, boosting the effector functions of tumour-associated $CD8^+$ T cells inducing tumour regression. To date, two anti-PD-1 mAb (Pembrolizumab, Nivolumab) and three anti-PD-L1 mAb (Atezolizumab, Durvalumab, Avelumab) have been approved for the treatment of cancers including advanced melanoma, non-small-cell lung cancer, head and neck squamous cell carcinoma, Hodgkin lymphoma and renal carcinoma [118].

The ligands for PD-1 are abundant on DC. PD-L1 expression is on pDC and cDC subsets and upregulated in response to inflammatory stimuli and following exposure to platinum-based chemotherapy drugs [84, 119]. Furthermore, PD-L1 is also highly expressed on DC that infiltrate tumours as exemplified by the high PD-L1 expression measured on both pDC and multiple myeloma cells isolated from the bone-marrow of multiple myeloma patients [120]. PD-L2 is detectable at low

Checkpoint inhibitor (CI)	CI cell expression	Ligand	Ligand cell expression	Anti-CI mAb clinical name	Clinical outcome
PD-1	T, B, NK cells, DC	PD-L1/2	PD-L1: DC, monocytes, Treg, cells, tumour; PD-L2: Activated cDC, moDCs	Pembrolizumab, Nivolumab	Approved for metastatic melanoma, renal cell carcinoma, squamous-cell carcinoma of head and neck, Hodgkin's lymphoma, metastatic colorectal, non-small cell lung, Merkel cell and ovarian cancers Improved clinical outcomes in combination with peptide/vector vaccines for advanced solid cancers, metastatic melanoma and HPV-16-related cancers
CTLA4	T cells, activated moDCs	CD80/86 (B7.1/2)	APC	Ipilimumab, Tremelimumab	Approved for metastatic melanoma, renal cell carcinoma and colorectal cancer treatments Mixed results in combination with peptide and moDC vaccines
TIM-3	T, B cells, cDC, myeloid cells	Galectin-9, CEACAM-1, HMGB1, phosphatidylserine	Tumour	— (pre-clinical)	—
LAG-3	Activated T, NK cells, pDCs	MHCII	APC	LAG-3Ig fusion protein	Elevated clinical activity Phase I/II trial in combination with paclitaxel for metastatic breast carcinoma
ICOS	Treg cells, activated T cells	ICOS-L	APC (especially activated pDCs)	MEDI-570	Phase I Trial for T cell lymphoma (National Cancer Institute Clinical Trial NCT02520791)

Table 2.
List of checkpoint inhibitors, their ligands, cell expression and clinical associations.

levels on cDC only after activation and is highly expressed by moDC [121]. Whether PD-L2 is also expressed by DC in different TMEs and the effect of anti-PD-L2 therapies is yet to be defined.

cDC1 play a critical role in the efficacy of anti-PD-1/PD-L1 mAb therapies. Single cell mass spectrometry analyses of PBMC from patients with advanced melanoma, before and after anti-PD-1 therapy revealed that CD141 and CD11c, both expressed by cDC1 are strong predictive biomarkers of clinical response to anti-PD-1 treatments [122]. This is consistent with several mouse studies reporting that cDC1-deficient mice do not respond to immune checkpoint blockade using anti-PD-L1 or a combination of anti-PD-1 anti-CTLA4 mAb [123, 124]. Furthermore, mice that possess cDC1 defective in antigen cross-presentation fail to establish CTL responses and do not respond to anti-PD-1 blockade [125].

The success of anti-PD-1 therapy also depends on a cross-talk between cDC1 and T cells in the TME. In mouse models anti-PD-1 treatment induces IL-12 production by tumour-infiltrating cDC1 [124, 126] which amplifies T cell effector functions. In melanoma patients, the clinical electroporation of an IL-12 plasmid in the tumour lesions enhances the CTL gene signature, thus validating the role of this cytokine in supporting CTL responses [126], **Figure 1**.

In addition to its ligands, expression of the PD-1 receptor on DC has been reported during cancer. In hepatocellular carcinoma patients, detectable levels of PD-1 were reported on peripheral blood cDC1, cDC2 and pDC whereas PD-1 was only present on cDC1 in healthy donors. This was confirmed with microscopy analyses of cancerous liver tissues showing co-expression of PD-1 and the DC marker CD11c [127]. In line with this data, co-expression of PD-1 and PD-L1 was detected on $CD11c^+$ DC isolated from the tumours of non-small cell lung cancer patients [128]. However, in this case, PD-1 was absent from DC isolated from the PBMC of either cancer patients or healthy donors, suggesting that PD-1 is upregulated locally on DC in response to the immunosuppressive tumour environment [128].

Mouse studies support an inhibitory role of PD-1 on DC [127]. This finding however contrasts with a recent study revealing that PD-1 can establish *cis*-interactions with both PD-L1 and PD-L2 at the cell membrane. PD-L1/PD-1 *cis*-interaction disrupts PD-L1 binding to PD-1 on T cells, thus resulting in increased T cell activities. However, whether this mechanism exists in DC in the setting of cancer remains unknown [128]. Similarly, several reports have shown that PD-L1 can interact in *cis* with the immune checkpoint ligand CD80/B7.1 [129–131] and this occurs on several types of APC, including cDC1 and cDC2 [131]. The PD-L1/CD80 *cis*-interaction limits the binding of PD-L1 to PD-1 on T cells and ultimately promotes T cell immune responses [131]. Altogether, these data show that, while *trans*-interactions between PD-L1 and PD-1 at the interface of DC and T cells promote T cell immune suppression, *cis*-interactions between PD-L1 and other molecules on DC show opposite effects and could potentially promote cancer immunity.

Combining anti-PD-1/PD-L1 therapy with DC-based vaccines, or vaccines that target DC *in situ*, or include a DC growth factor, is a logical strategy to increase responses to checkpoint blockade in cancer patients. Several studies in mice have reported that such combination leads to higher protection by boosting the antigen-specific T cell immune response induced by different type of vaccines [18, 123, 132–134]. Several vaccines containing peptides or viral vectors, in combination with anti-PD-1 mAb Pembolizumab or Nivolumab, have shown encouraging results in early clinical trial with patients with advanced solid cancers, melanoma and Human Papillomavirus 16-Related Cancer [135–138].

8.2 DC and CTLA4

The co-inhibitory immune checkpoint CTLA4 (CD152) is constitutively expressed by regulatory T cells (Treg) and by effector T cells upon activation. CTLA4 is highly homologous to the co-stimulatory receptor CD28 and binds the same ligands CD80 and CD86 (B7.2), however with a much higher affinity. As such, CTLA4 outcompetes CD28 for ligand binding and reduces CD28-mediated co-stimulation of T cell functions. CTLA4 blockade promotes anti-tumour immunity by increasing the activation of effector T cells and by depleting Treg in the TME. The CTLA4 blocking mAb Ipilimumab and Tremelimumab have been approved for the treatment of metastatic melanoma, renal cell carcinoma and colorectal cancer [118].

CTLA4 on T cells directly alters DC functions by removing the CTLA4 ligands (CD80/86) from their cell surfaces. When human moDC are co-cultured with $CTLA4^+$ T cells, CD80/86 levels on DC decrease rapidly in a CTLA4-dependent manner. This mechanism, named trans-endocytosis, involves the physical capture of CTLA4 ligands by the receptor and their degradation. This process is upregulated by TCR engagement [139, 140]. Mouse *in vivo* studies show that trans-endocytosis is primarily carried out by regulatory T cells and impacts the migratory cDC1 and cDC2 [141]. In addition, CTLA4 interaction with CD80/CD86 on DC induces immunosuppression through reverse signalling. MoDC stimulated with soluble CTLA4 or agonistic anti-CD80/86 Ab produced indoleamine 2,3-dioxygenase (IDO), which is able to inhibit allogenic T cell activation [142]. IDO is expressed by human pDC [143], hence similar immunosuppressive pathways are likely to be induced downstream of CD80/86 in this subset, as reported in mouse pDC [144].

Besides their regulation through CTLA4-CD80/86 interaction, moDC also express the CTLA4 molecule upon activation by TLR stimuli. Treatment of these cells with an agonistic anti-CTLA4 Ab induced increased production of IL-10, reduced expression of IL-8 and IL-12 and decreased T cell stimulation capacity [145]. MoDC are also able to secrete CTLA4 in extracellular microvesicles. Microvesicular CTLA4 has been shown to downregulate CD80 and CD86 on moDC [146].

Combinatorial approaches of anti-CTLA4 mAb with cancer vaccines have been tested in clinics and have yielded mixed results. In melanoma patients, peptide vaccines, in combination with anti-CTLA4 Ipilimumab did not show better clinical outcomes compared to Ipilimumab alone [127, 147, 148]. However, other strategies using DC vaccines have provided promising results. For instance, the co-administration to melanoma patients of autologous moDC that have been pulsed with tumour peptide, together with Tremelimumab, resulted in objective and durable tumour responses [149]. Furthermore, a phase II study using Ipilimumab and moDC electroporated with synthetic mRNA (TriMixDC-MEL) has been tested in advanced melanoma patients and has shown an encouraging rate of highly durable tumour response [150].

8.3 DC and TIM-3

T cell immunoglobulin mucin-3 (TIM-3) is a co-inhibitory immune checkpoint receptor expressed by all T cell populations as well as B cells and a large variety of myeloid cells. Four TIM-3 ligands have been identified, including Galectin-9, CEACAM-1, HMGB1 and phosphatidylserine. Engagement of TIM-3 on

tumour-infiltrating T cells induces exhaustion and suppresses tumour immunity. Preclinical studies have reported high therapeutic activities of blocking anti-TIM-3 antibodies against various types of malignancies and clinical trials with TIM-3 inhibitors are currently underway [128].

High TIM-3 expression has been reported on cDC1 and cDC2 from peripheral blood [151–153] and on tumour-associated cDC1 and cDC2 from mammary tumour biopsies [152]. Mouse models indicated that blocking TIM-3 on cDC1 leads to an increase in the T cell chemoattractant CXCL9. Moreover, cDC1 expressing TIM-3 correlated with CXCL9 expression in human breast cancer biopsies and was positively associated with $CD8^+$ T cell infiltration. These data suggest that TIM-3 blocking in these cancers could potentially enhance $CD8^+$ T cell recruitment to the TME [152].

8.4 DC and LAG-3

Lymphocyte activation gene-3 (LAG-3) is a co-inhibitory immune checkpoint receptor expressed on activated T cells and NK cells that recognise MHCII molecules on APCs as a ligand. LAG3 negatively regulates T cell activation and is frequently co-expressed with PD-1 on exhausted T cells in the TME. Several LAG-3-targeting cancer immunotherapies are currently in different phases of clinical development [154].

The interaction between MHCII and LAG-3 not only has effects in T cells, but also induces reverse signalling in DCs that is stimulatory. This was shown using the soluble LAG-3-Ig fusion protein that activates moDC, as indicated by the upregulation of co-stimulatory molecules, the production of several pro-inflammatory cytokines and chemokines and increased allogenic T cell activation. However, Ab-mediated MHCII ligation does not activate moDC, thus showing that the MHCII: LAG-3 interaction is required in this process [155–157]. Soluble LAG-3-Ig fusion protein in combination with the chemotherapy drug Paclitaxel has demonstrated elevated clinical activity in metastatic breast carcinoma during a phase I/II trial. This treatment also strongly stimulated the patients' APC, as evidenced by the increase in the number and activation of monocytes, pDC and cDCs [158].

Notably, LAG-3 itself has been found expressed by DC, specifically by a subpopulation of circulating pDC in healthy donors. $LAG\text{-}3^+$ pDC are also found in the tumour lesions and in the tumour-draining lymph nodes of melanoma patients and are thought to contribute to the immunosuppressive environment. Engagement of LAG-3 on pDC provides an activating signal, independent of TLR signalling, inducing low IFN-α and high IL-6 expression [159]. Hence, LAG-3-specific mAb in cancer immunotherapies may enhance the anti-tumour immune response by inhibiting LAG-3 signalling in both T cells and DC.

8.5 DC and ICOS

Inducible T cell costimulatory (ICOS) belongs to the co-stimulatory immune checkpoint receptor family and similarly to CD28, enhances the proliferation and effector functions of T cells. ICOS is expressed on activated T cells and constitutively on a subpopulation of Treg [160] while ICOS-L is present at the surface of APC. High ICOS expression on T cells has been particularly observed during anti-CTLA4 therapies and the co-administration of agonistic ICOS-specific mAb further improves the efficacy to CTLA4 blockade [161].

pDC are able to induce immunosuppression though ICOS stimulation. ICOS-L is strongly upregulated by human blood pDC, but not $CD11c^+$ cDC, in response to TLR stimuli or IL-3 [162]. Co-cultures of pDC with allogenic T cells induced IL-10 expression through a mechanism mediated by ICOS-L-ICOS interaction [162] and similar observations were reported with pDC isolated from ovarian carcinoma [163]. Furthermore, pDC are able to induce Treg proliferation though ICOS stimulation [160] and this mechanism likely explains the dramatic accumulation of $ICOS^+$ Treg in ovarian, breast, liver and gastric tumour tissues, in close proximity with $ICOS\text{-}L^+$ pDC [101, 164–166].

9. Summary

DCs are rare, heterogeneous cells with clear roles in anti-tumour immunity. As summarised in **Figure 1**, understanding how best to activate DC to gain optimal anti-tumour adaptive immune responses will likely involve careful optimisation of adjuvants, checkpoint immunotherapies and DC targeting strategies. Emerging studies will likely examine checkpoint receptors and their ligands on DC, lymphocytes and other cells in tumour environments, in order to design targeted therapies for optimal antigen presentation, DC activation and anti-tumour response.

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References

[1] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;**392**:245-252. DOI: 10.1038/32588

[2] Macri C, Pang ES, Patton T, O'Keeffe M. Dendritic cell subsets. Seminars in Cell & Developmental Biology. 2018;**84**:11-21. DOI: 10.1016/j.semcdb.2017.12.009

[3] Villadangos JA, Schnorrer P, Wilson NS. Control of MHC class II antigen presentation in dendritic cells: A balance between creative and destructive forces. Immunological Reviews. 2005;**207**:191-205. DOI: 10.1111/j.0105-2896.2005.00317.x

[4] Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human tissues contain CD141hi cross-presenting dendritic cells with functional homology to mouse CD103+ nonlymphoid dendritic cells. Immunity. 2012;**37**:60-73. DOI: 10.1016/j.immuni.2012.04.012

[5] Baharom F, Thomas S, Rankin G, Lepzien R, Pourazar J, Behndig AF, et al. Dendritic cells and monocytes with distinct inflammatory responses reside in lung mucosa of healthy humans. The Journal of Immunology. 2016;**196**:4498. DOI: 10.4049/jimmunol.1600071

[6] Guilliams M, Dutertre C-A, Scott CL, McGovern N, Sichien D, Chakarov S, et al. Unsupervised high-dimensional analysis aligns dendritic cells across tissues and species. Immunity. 2016;**45**:669-684. DOI: 10.1016/j.immuni.2016.08.015

[7] Heidkamp GF, Sander J, Lehmann CHK, Heger L, Eissing N, Baranska A, et al. Human lymphoid organ dendritic cell identity is predominantly dictated by ontogeny, not tissue microenvironment. Science Immunology. 2016;**1**:eaai7677. DOI: 10.1126/sciimmunol.aai7677

[8] Villani A-C, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science. 2017;**356**:eaah4573. DOI: 10.1126/science.aah4573

[9] See P, Dutertre C-A, Chen J, Günther P, McGovern N, Irac SE, et al. Mapping the human DC lineage through the integration of high-dimensional techniques. Science. 2017;**356**:eaag3009. DOI: 10.1126/science.aag3009

[10] O'Keeffe M, Mok WH, Radford KJ. Human dendritic cell subsets and function in health and disease. Cellular and Molecular Life Sciences. 2015;**72**:4309-4325. DOI: 10.1007/s00018-015-2005-0

[11] Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen J-L, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8α+ dendritic cells. The Journal of Experimental Medicine. 2010;**207**:1261. DOI: 10.1084/jem.20092618

[12] Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JCY, et al. The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. Blood. 2008;**112**:3264. DOI: 10.1182/blood-2008-05-155176

[13] Huysamen C, Willment JA, Dennehy KM, Brown GD. CLEC9A is a novel activation C-type Lectin-like receptor expressed on BDCA3+ dendritic cells and a subset of monocytes. Journal of Biological Chemistry. 2008;**283**:16693-16701. DOI: 10.1074/jbc.M709923200

[14] Sancho D, Mourão-Sá D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, et al. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. The Journal of Clinical Investigation. 2008;**118**:2098-2110. DOI: 10.1172/JCI34584

[15] Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. Blood. 2012;**119**:6052. DOI: 10.1182/blood-2012-01-406967

[16] Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. The Journal of Experimental Medicine. 2010;**207**:1247. DOI: 10.1084/jem.20092140

[17] Proietto AI, Mittag D, Roberts AW, Sprigg N, Wu L. The equivalents of human blood and spleen dendritic cell subtypes can be generated in vitro from human CD34+ stem cells in the presence of fms-like tyrosine kinase 3 ligand and thrombopoietin. Cellular and molecular immunology. 2012;**9**:446. DOI: 10.1038/cmi.2012.48

[18] Ding Y, Wilkinson A, Idris A, Fancke B, O'Keeffe M, Khalil D, et al. FLT3-ligand treatment of humanized mice results in the generation of large numbers of CD141+ and CD1c+ dendritic cells In vivo. The Journal of Immunology. 2014;**192**:1982. DOI: 10.4049/jimmunol.1302391

[19] Galibert L, Diemer GS, Liu Z, Johnson RS, Smith JL, Walzer T, et al. Nectin-like protein 2 defines a subset of T-cell zone dendritic cells and is a ligand for class-I-restricted T-cell-associated molecule. Journal of Biological Chemistry. 2005;**280**:21955-21964. DOI: 10.1074/jbc.M502095200

[20] Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. IRF8 mutations and human dendritic-cell immunodeficiency. New England Journal of Medicine. 2011;**365**:127-138. DOI: 10.1056/NEJMoa1100066

[21] Robbins SH, Walzer T, Dembélé D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome Biology. 2008;**9**:R17. DOI: 10.1186/gb-2008-9-1-r17

[22] Krüger A, Oldenburg M, Chebrolu C, Beisser D, Kolter J, Sigmund AM, et al. Human TLR8 senses UR/URR motifs in bacterial and mitochondrial RNA. EMBO Reports. 2015;**16**:1656-1663. DOI: 10.15252/embr.201540861

[23] Lauterbach H, Bathke B, Gilles S, Traidl-Hoffmann C, Luber CA, Fejer G, et al. Mouse CD8α+ DCs and human BDCA3+ DCs are major producers of IFN-λ in response to poly IC. The Journal of Experimental Medicine. 2010;**207**:2703. DOI: 10.1084/jem.20092720

[24] Yoshio S, Kanto T, Kuroda S, Matsubara T, Higashitani K, Kakita N, et al. Human blood dendritic cell antigen 3 (BDCA3)+ dendritic cells are a potent producer of interferon-λ in response to hepatitis C virus. Hepatology. 2013;**57**:1705-1715. DOI: 10.1002/hep.26182

[25] Zhang JG, Czabotar PE, Policheni AN, et al. The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. Immunity. 2012;**36**(4):646-657. DOI: 10.1016/j.immuni.2012.03.009

[26] Collin M, Ginhoux F. Human dendritic cells. Seminars in Cell & Developmental Biology. 2019;**86**:1-2. DOI: 10.1016/j.semcdb.2018.04.015

[27] Heger L, Balk S, Lühr JJ, et al. CLEC10A is a specific marker for human CD1c+ dendritic cells and enhances their toll-like receptor 7/8-induced cytokine secretion. Frontiers in Immunology. 2018;**9**:744. DOI: 10.3389/fimmu.2018.00744 [Published: 27 April 2018]

[28] Yin X, Yu H, Jin X, Li J, Guo H, Shi Q, et al. Human blood CD1c+ dendritic cells encompass CD5high and CD5low subsets that differ significantly in phenotype, gene expression, and functions. The Journal of Immunology. 2017;**198**:1553. DOI: 10.4049/jimmunol.1600193

[29] Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity. 2013;**38**:970-983. DOI: 10.1016/j.immuni.2013.04.011

[30] Luber CA, Cox J, Lauterbach H, Fancke B, Selbach M, Tschopp J, et al. Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. Immunity. 2010;**32**:279-289. DOI: 10.1016/j.immuni.2010.01.013

[31] Worah K, Mathan TSM, Vu Manh TP, Keerthikumar S, Schreibelt G, Tel J, et al. Proteomics of human dendritic cell subsets reveals subset-specific surface markers and differential Inflammasome function. Cell Reports. 2016;**16**:2953-2966. DOI: 10.1016/j.celrep.2016.08.023

[32] Mittag D, Proietto AI, Loudovaris T, Mannering SI, Vremec D, Shortman K, et al. Human dendritic cell subsets from spleen and blood are similar in phenotype and function but modified by donor health status. The Journal of Immunology. 2011;**186**:6207. DOI: 10.4049/jimmunol.1002632

[33] Tel J, Aarntzen EHJG, Baba T, Schreibelt G, Schulte BM, Benitez-Ribas D, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. Cancer Research. 2013;**73**:1063. DOI: 10.1158/0008-5472.CAN-12-2583

[34] Chun I, Yu CB, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c+ dendritic cells drive the differentiation of CD103+ CD8+ mucosal effector T cells via the cytokine TGF-β. Immunity. 2013;**38**:818-830. DOI: 10.1016/j.immuni.2013.03.004

[35] Chiang M-C, Tullett KM, Lee YS, Idris A, Ding Y, McDonald KJ, et al. Differential uptake and cross-presentation of soluble and necrotic cell antigen by human DC subsets. European Journal of Immunology. 2016;**46**:329-339. DOI: 10.1002/eji.201546023

[36] Colonna M, Trinchieri G, Liu Y-J. Plasmacytoid dendritic cells in immunity. Nature Immunology. 2004;**5**:1219

[37] Facchetti F, Vermi W, Mason D, Colonna M. The plasmacytoid monocyte/interferon producing cells. Virchows Archiv. 2003;**443**:703-717

[38] Grouard G, Rissoan M-C, Filgueira L, Durand I, Banchereau J, Liu Y-J. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. The Journal of Experimental Medicine. 1997;**185**:1101-1112

[39] Coccia EM, Severa M, Giacomini E, Monneron D, Remoli ME, Julkunen I, et al. Viral infection and Toll-like receptor agonists induce a differential expression of type I and λ interferons in human plasmacytoid and monocyte-derived dendritic cells. European Journal of Immunology. 2004;**34**:796-805. DOI: 10.1002/eji.200324610

[40] Gilliet M, Cao W, Liu Y-J. Plasmacytoid dendritic cells: Sensing

nucleic acids in viral infection and autoimmune diseases. Nature Reviews Immunology. 2008;**8**:594. DOI: 10.1038/nri2358

[41] Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. Nature Reviews. 2015;**15**:471-485. DOI: 10.1038/nri3865

[42] Alculumbre S, Raieli S, Hoffmann C, Chelbi R, Danlos F-X, Soumelis V. Plasmacytoid pre-dendritic cells (pDC): From molecular pathways to function and disease association. Seminars in Cell & Developmental Biology. 2019;**86**:24-35. DOI: 10.1016/j.semcdb.2018.02.014

[43] Murphy TL, Grajales-Reyes GE, Wu X, Tussiwand R, Briseño CG, Iwata A, et al. Transcriptional control of dendritic cell development. Annual Review of Immunology. 2016;**34**:93-119. DOI: 10.1146/annurev-immunol-032713-120204

[44] Musumeci A, Lutz K, Winheim E, Krug AB. What makes a pDC: Recent advances in understanding plasmacytoid DC development and heterogeneity. Frontiers in Immunology. 2019;**10**:1222. DOI: 10.3389/fimmu.2019.01222 [Published: 29 May 2019]

[45] Wu X, Briseño CG, Grajales-Reyes GE, Haldar M, Iwata A, Kretzer NM, et al. Transcription factor Zeb2 regulates commitment to plasmacytoid dendritic cell and monocyte fate. Proceedings of the National Academy of Sciences. 2016;**113**:14775-14780. DOI: 10.1073/pnas.1611408114

[46] Cisse B, Caton ML, Lehner M, Maeda T, Scheu S, Locksley R, et al. Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. Cell;**135**:37-48. DOI: 10.1016/j.cell.2008.09.016

[47] Matsui T, Connolly JE, Michnevitz M, Chaussabel D, Yu C-I, Glaser C, et al. CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. The Journal of Immunology. 2009;**182**:6815-6823

[48] Owczarczyk-Saczonek A, Sokołowska-Wojdyło M, Olszewska B, Malek M, Znajewska-Pander A, Kowalczyk A, et al. Clinicopathologic retrospective analysis of blastic plasmacytoid dendritic cell neoplasms. Postepy Dermatologii i Alergologii. 2018;**35**:128-138. DOI: 10.5114/ada.2017.72269

[49] Kubota S, Tokunaga K, Umezu T, Yokomizo-Nakano T, Sun Y, Oshima M, et al. Lineage-specific RUNX2 super-enhancer activates MYC and promotes the development of blastic plasmacytoid dendritic cell neoplasm. Nature Communications. 2019;**10**:1653. DOI: 10.1038/s41467-019-09710-z

[50] Sukswai N, Aung PP, Yin CC, Li S, Wang W, Wang SA, et al. Dual expression of TCF4 and CD123 is highly sensitive and specific for blastic plasmacytoid dendritic cell neoplasm. The American Journal of Surgical Pathology. 2019;**43**:1429-1437. DOI: 10.1097/pas.0000000000001316

[51] Sullivan JM, Rizzieri DA. Treatment of blastic plasmacytoid dendritic cell neoplasm. Hematology-American Society of Hematology Education Program. 2016;**2016**(1):16-23. DOI: 10.1182/asheducation-2016.1.16

[52] Jen EY, Gao X, Li L, Zhuang L, Simpson NE, Aryal B, et al. FDA approval summary: Tagraxofusp-erzs for treatment of blastic plasmacytoid dendritic cell neoplasm. Clinical Cancer Research. 2020;**26**:532-536. DOI: 10.1158/1078-0432.ccr-19-2329

[53] Cavanagh L, Saal R, Grimmet K, Thomas R. Proliferation in monocyte-derived dendritic cell cultures is caused by progenitor cells capable

of myeloid differentiation. Blood. 1998;**92**:1598-1607

[54] Reid CD, Stackpoole A, Meager A, Tikerpae J. Interactions of tumor necrosis factor with granulocyte-macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent CD34+ progenitors in human bone marrow. Journal of Immunology. 1992;**149**:2681-2688

[55] Santiago-Schwartz F, Belilos E, Diamond B, Carsons S. TNF in combination with GM-CSF enhances the differentiation of neonatal cord bloods cells into dendritic cells and macrophages. Journal of Leukocyte Biology. 1992;**52**:274-281

[56] Gabrilovich D, Nadaf S, Corak J, Berzofsky J, Carbone D. Dendritic cells in antitumor immune responses II. Dendritic cells grown from bone marrow precursors, but not mature DC from tumor-bearing mice, are effective antigen carriers in the therapy of established tumors. Cellular Immunology. 1996;**170**:111-119

[57] Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. The Journal of Experimental Medicine. 1994;**179**:1109-1118. DOI: 10.1084/jem.179.4.1109

[58] Helft J, Bottcher J, Chakravarty P, Zelenay S, Huotari J, Schraml BU, et al. GM-CSF mouse bone marrow cultures comprise a heterogeneous population of CD11c(+)MHCII(+) macrophages and dendritic cells. Immunity. 2015;**42**:1197-1211. DOI: 10.1016/j.immuni.2015.05.018

[59] Guilliams M, Malissen B. A death notice for in-vitro-generated GM-CSF dendritic cells? Immunity. 2015;**42**:988-990. DOI: 10.1016/j.immuni.2015.05.020

[60] Lutz MB, Inaba K, Schuler G, Romani N. Still alive and kicking: In-vitro-generated GM-CSF dendritic cells! Immunity. 2016;**44**:1-2. DOI: 10.1016/j.immuni.2015.12.013

[61] Helft J, Bottcher JP, Chakravarty P, Zelenay S, Huotari J, Schraml BU, et al. Alive but confused: Heterogeneity of CD11c(+) MHC class II(+) cells in GM-CSF mouse bone marrow cultures. Immunity. 2016;**44**:3-4. DOI: 10.1016/j.immuni.2015.12.014

[62] Xu Y, Zhan Y, Lew AM, Naik SH, Kershaw MH. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. The Journal of Immunology. 2007;**179**:7577-7584. DOI: 10.4049/jimmunol.179.11.7577

[63] Marigo I, Zilio S, Desantis G, Mlecnik B, Agnellini AHR, Ugel S, et al. T cell cancer therapy requires CD40-CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells. Cancer Cell. 2016;**30**:377-390. DOI: 10.1016/j.ccell.2016.08.004

[64] Kumar C, Kohli S, Bapsy PP, Vaid AK, Jain M, Attili VSS, et al. Immune modulation by dendritic-cell-based cancer vaccines. Journal of Biosciences. 2017;**42**:161-173. DOI: 10.1007/s12038-017-9665-x

[65] Murphy GP, Tjoa BA, Simmons SJ, Jarisch J, Bowes VA, Ragde H, et al. Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: A phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. Prostate. 1999;**38**:73-78

[66] Rodriguez J, Castanon E, Perez-Gracia JL, Rodriguez I, Viudez A,

Alfaro C, et al. A randomized phase II clinical trial of dendritic cell vaccination following complete resection of colon cancer liver metastasis. Journal for Immunotherapy of Cancer. 2018;**6**:96. DOI: 10.1186/s40425-018-0405-z

[67] Bol KF, Schreibelt G, Rabold K, Wculek SK, Schwarze JK, Dzionek A, et al. The clinical application of cancer immunotherapy based on naturally circulating dendritic cells. Journal for Immunotherapy of Cancer. 2019;**7**:109-109. DOI: 10.1186/s40425-019-0580-6

[68] Mody N, Dubey S, Sharma R, Agrawal U, Vyas SP. Dendritic cell-based vaccine research against cancer. Expert Review of Clinical Immunology. 2015;**11**:213-232. DOI: 10.1586/1744666X.2015.987663

[69] Reddy A, Sapp M, Feldman M, Subklewe M, Bhardwaj N. A monocyte conditioned medium is more effective than defined cytokines in mediating the terminal maturation of human dendritic cells. Blood. 1997;**90**:3640

[70] Wilgenhof S, Van Nuffel AMT, Benteyn D, Corthals J, Aerts C, Heirman C, et al. A phase IB study on intravenous synthetic mRNA electroporated dendritic cell immunotherapy in pretreated advanced melanoma patients. Annals of Oncology. 2013;**24**:2686-2693. DOI: 10.1093/annonc/mdt245

[71] Schreibelt G, Bol KF, Westdorp H, Wimmers F, Aarntzen EHJG, Boer T D-d, et al. Effective clinical responses in metastatic melanoma patients after vaccination with primary myeloid dendritic cells. Clinical Cancer Research. 2016;**22**:2155. DOI: 10.1158/1078-0432.CCR-15-2205

[72] Prue RL, Vari F, Radford KJ, Tong H, Hardy MY, D'Rozario R, et al. A phase I clinical trial of CD1c (BDCA-1)+ dendritic cells pulsed with HLA-A*0201 peptides for immunotherapy of metastatic hormone refractory prostate cancer. Journal of Immunotherapy. 2015;**38**:71-76. DOI: 10.1097/cji.0000000000000063

[73] Macri C, Dumont C, Johnston AP, Mintern JD. Targeting dendritic cells: A promising strategy to improve vaccine effectiveness. Clinical & Translational Immunology. 2016;**5**:e66-e66. DOI: 10.1038/cti.2016.6

[74] Caminschi I, Maraskovsky E, Heath W. Targeting dendritic cells in vivo for cancer therapy. Frontiers in Immunology. 2012;**3**:13

[75] Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S-I, Soares H, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. The Journal of Experimental Medicine. 2004;**199**:815-824. DOI: 10.1084/jem.20032220

[76] Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M, et al. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. The Journal of Experimental Medicine. 2001;**194**:769-779. DOI: 10.1084/jem.194.6.769

[77] Mahnke K, Ring S, Johnson TS, Schallenberg S, Schönfeld K, Storn V, et al. Induction of immunosuppressive functions of dendritic cells in vivo by CD4+CD25+ regulatory T cells: Role of B7-H3 expression and antigen presentation. European Journal of Immunology. 2007;**37**:2117-2126. DOI: 10.1002/eji.200636841

[78] Dhodapkar MV, Sznol M, Zhao B, Wang D, Carvajal RD, Keohan ML, et al. Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. Science Translational Medicine. 2014;**6**:232ra251. DOI: 10.1126/scitranslmed.3008068

[79] Tullett KM, Leal Rojas IM, Minoda Y, Tan PS, Zhang J-G, Smith C, et al. Targeting CLEC9A delivers antigen to human CD141(+) DC for CD4(+) and CD8(+)T cell recognition. JCI Insight. 2016;**1**:e87102-e87102. DOI: 10.1172/jci.insight.87102

[80] Kato M, McDonald KJ, Khan S, Ross IL, Vuckovic S, Chen K, et al. Expression of human DEC-205 (CD205) multilectin receptor on leukocytes. International Immunology. 2006;**18**:857-869. DOI: 10.1093/intimm/dxl022

[81] Schreibelt G, Klinkenberg LJJ, Cruz LJ, Tacken PJ, Tel J, Kreutz M, et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-) presentation by human blood BDCA3+ myeloid dendritic cells. Blood. 2012;**119**:2284. DOI: 10.1182/blood-2011-08-373944

[82] Li J, Ahmet F, Sullivan LC, Brooks AG, Kent SJ, De Rose R, et al. Caminschi, antibodies targeting Clec9A promote strong humoral immunity without adjuvant in mice and non-human primates. European Journal of Immunology. 2015;**45**:854-864. DOI: 10.1002/eji.201445127

[83] Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumour immune responses. Nature Reviews Cancer. 2018;**18**:139. DOI: 10.1038/nrc.2017.117

[84] Karthaus N, Torensma R, Tel J. Deciphering the message broadcast by tumor-infiltrating dendritic cells. The American Journal of Pathology. 2012;**181**:733-742. DOI: 10.1016/j.ajpath.2012.05.012

[85] Ladányi A, Kiss J, Somlai B, Gilde K, Fejős Z, Mohos A, et al. Density of DC-LAMP+ mature dendritic cells in combination with activated T lymphocytes infiltrating primary cutaneous melanoma is a strong independent prognostic factor. Cancer Immunology, Immunotherapy. 2007;**56**:1459-1469. DOI: 10.1007/s00262-007-0286-3

[86] Coventry B, Heinzel S. CD1a in human cancers: A new role for an old molecule. Trends in Immunology. 2004;**25**:242-248. DOI: 10.1016/j.it.2004.03.002

[87] Saito H, Tsujitani S, Ikeguchi M, Maeta M, Kaibara N. Relationship between the expression of vascular endothelial growth factor and the density of dendritic cells in gastric adenocarcinoma tissue. British Journal of Cancer. 1998;**78**:1573-1577. DOI: 10.1038/bjc.1998.725

[88] Hilly O, Rath-Wolfson L, Koren R, Mizrachi A, Hamzany Y, Bachar G, et al. CD1a-positive dendritic cell density predicts disease-free survival in papillary thyroid carcinoma. Pathology—Research and Practice. 2015;**211**:652-656. DOI: 10.1016/j.prp.2015.05.009

[89] Coventry BJ, Lee PL, Gibbs D, Hart DNJ. Dendritic cell density and activation status in human breast cancer—CD1a, CMRF-44, CMRF-56 and CD-83 expression. British Journal of Cancer. 2002;**86**:546-551. DOI: 10.1038/sj.bjc.6600132

[90] Bell D, Chomarat P, Broyles D, Netto G, Harb GM, Lebecque S, et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. The Journal of Experimental Medicine. 1999;**190**:1417

[91] Truxova I, Kasikova L, Hensler M, Skapa P, Laco J, Pecen L, et al. Mature dendritic cells correlate with favorable immune infiltrate and improved prognosis in ovarian carcinoma patients. Journal for Immunotherapy of

Cancer. 2018;**6**:139-139. DOI: 10.1186/s40425-018-0446-3

[92] Lewko B, Zółtowska A, Stepinski J, Roszkiewicz A, Moszkowska G. Dendritic and cancer cells in the breat tumours—An immunohistochemical study: Short communication. Medical Science Monitor. 2000;**6**:892-895

[93] Lespagnard L, Gancberg D, Rouas G, Leclercq G, de Saint-Aubain Somerhausen N, Di Leo A, et al. Tumor-infiltrating dendritic cells in adenocarcinomas of the breast: A study of 143 neoplasms with a correlation to usual prognostic factors and to clinical outcome. International Journal of Cancer. 1999;**84**:309-314. DOI: 10.1002/(SICI)1097-0215(19990621)84:3<309::AID-IJC19>3.0.CO;2-3

[94] Hillenbrand EE, Neville AM, Coventry BJ. Immunohistochemical localization of CD1a-positive putative dendritic cells in human breast tumours. British Journal of Cancer. 1999;**79**:940-944. DOI: 10.1038/sj.bjc.6690150

[95] Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, 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**:324-336. DOI: 10.1016/j.ccell.2016.06.003

[96] 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**:711, e714-723. DOI: 10.1016/j.ccell.2017.04.003

[97] Böttcher JP, Bonavita E, Chakravarty P, Blees H, Cabeza-Cabrerizo M, Sammicheli S, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. Cell. 2018;**172**:1022, e1014-1037. DOI: 10.1016/j.cell.2018.01.004

[98] Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell. 2014;**26**:638-652. DOI: 10.1016/j.ccell.2014.09.007

[99] Daniel S, Chen IM. Oncology meets immunology: The cancer-immunity cycle. Immunity. 2013;**39**:1-10. DOI: 10.1016/j.immuni.2013.07.012

[100] Treilleux I, Blay J-Y, Bendriss-Vermare N, Ray-Coquard I, Bachelot T, Guastalla J-P, et al. Dendritic cell infiltration and prognosis of early stage breast cancer. Clinical Cancer Research. 2004;**10**:7466. DOI: 10.1158/1078-0432.CCR-04-0684

[101] Conrad C, Gregorio J, Wang Y-H, Ito T, Meller S, Hanabuchi S, et al. Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS Costimulation of Foxp3+ T-regulatory cells. Cancer Research. 2012;**72**:5240. DOI: 10.1158/0008-5472.CAN-12-2271

[102] Sisirak V, Faget J, Gobert M, Goutagny N, Vey N, Treilleux I, et al. Impaired IFN-α production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. Cancer Research. 2012;**72**:5188. DOI: 10.1158/0008-5472.CAN-11-3468

[103] Mitchell D, Chintala S, Dey M. Plasmacytoid dendritic cell in immunity and cancer. Journal of Neuroimmunology. 2018;**322**:63-73. DOI: 10.1016/j.jneuroim.2018.06.012

[104] Terra M, Oberkampf M, Fayolle C, Rosenbaum P, Guillerey C, Dadaglio G, et al. Tumor-derived TGFβ alters the ability of plasmacytoid dendritic cells to respond to innate immune signaling. Cancer Research.

2018;**78**:3014-3026. DOI: 10.1158/0008-5472.can-17-2719

[105] Takahashi A, Kono K, Ichihara F, Sugai H, Fujii H, Matsumoto Y. Vascular endothelial growth factor inhibits maturation of dendritic cells induced by lipopolysaccharide, but not by proinflammatory cytokines. Cancer Immunology, Immunotherapy. 2004;**53**:543-550. DOI: 10.1007/s00262-003-0466-8

[106] Della Porta M, Danova M, Rigolin GM, Brugnatelli S, Rovati B, Tronconi C, et al. Dendritic cells and vascular endothelial growth factor in colorectal cancer: Correlations with clinicobiological findings. Oncology. 2005;**68**:276-284. DOI: 10.1159/000086784

[107] H. B FXH, Dong QG, Sha HF, Bao GL, Liao ML. Ascular endothelial growth factor inhibits dendritic cells from patients with non-small cell lung carcinoma. Zhonghua Jie He He Hu Xi Za Zhi. 2003;**26**:539-543

[108] Fricke I, Mirza N, Dupont J, Lockhart C, Jackson A, Lee J-H, et al. Vascular endothelial growth factor-trap overcomes defects in dendritic cell differentiation but does not improve antigen-specific immune responses. Clinical Cancer Research. 2007;**13**:4840. DOI: 10.1158/1078-0432.CCR-07-0409

[109] Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nature Medicine. 1996;**2**:1096-1103. DOI: 10.1038/nm1096-1096

[110] Thepmalee C, Panya A, Junking M, Chieochansin T, Yenchitsomanus P-t. Inhibition of IL-10 and TGF-β receptors on dendritic cells enhances activation of effector T-cells to kill cholangiocarcinoma cells. Human Vaccines & Immunotherapeutics. 2018;**14**:1423-1431. DOI: 10.1080/21645515.2018.1431598

[111] Bharadwaj U, Li M, Zhang R, Chen C, Yao Q. Elevated Interleukin-6 and G-CSF in human pancreatic cancer cell conditioned medium suppress dendritic cell differentiation and activation. Cancer Research. 2007;**67**:5479. DOI: 10.1158/0008-5472.CAN-06-3963

[112] Fu C, Jiang A. Dendritic cells and CD8 T cell immunity in tumor microenvironment. Frontiers in Immunology. 2018;**9**:3059

[113] Zong J, Keskinov AA, Shurin GV, Shurin MR. Tumor-derived factors modulating dendritic cell function. Cancer Immunology, Immunotherapy. 2016;**65**:821-833. DOI: 10.1007/s00262-016-1820-y

[114] Kitamura H, Ohno Y, Toyoshima Y, Ohtake J, Homma S, Kawamura H, et al. Interleukin-6/STAT3 signaling as a promising target to improve the efficacy of cancer immunotherapy. Cancer Science. 2017;**108**:1947-1952. DOI: 10.1111/cas.13332

[115] Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature. 2015;**523**:231. DOI: 10.1038/nature14404

[116] Jiang L, Fang X, Wang H, Li D, Wang X. Ovarian cancer-intrinsic fatty acid synthase prevents anti-tumor immunity by disrupting tumor-infiltrating dendritic cells. Frontiers in Immunology. 2018;**9**:2927

[117] Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation.

The Journal of Experimental Medicine. 2000;**192**:1027-1034. DOI: 10.1084/jem.192.7.1027

[118] Seidel JA, Otsuka A, Kabashima K. Anti-PD-1 and anti-CTLA-4 therapies in cancer: Mechanisms of action, efficacy, and limitations. Frontiers in Oncology. 2018;**8**:86. DOI: 10.3389/fonc.2018.00086

[119] Pulko V, Liu X, Krco CJ, Harris KJ, Frigola X, Kwon ED, et al. TLR3-stimulated dendritic cells up-regulate B7-H1 expression and influence the magnitude of CD8 T cell responses to tumor vaccination. Journal of Immunology. 2009;**183**:3634-3641. DOI: 10.4049/jimmunol.0900974

[120] Ray A, Das DS, Song Y, Richardson P, Munshi NC, Chauhan D, et al. Targeting PD1-PDL1 immune checkpoint in plasmacytoid dendritic cell interactions with T cells, natural killer cells and multiple myeloma cells. Leukemia. 2015;**29**:1441-1444. DOI: 10.1038/leu.2015.11

[121] Brown JA, Dorfman DM, Ma FR, Sullivan EL, Munoz O, Wood CR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. Journal of Immunology. 2003;**170**:1257-1266. DOI: 10.4049/jimmunol.170.3.1257

[122] Krieg C, Nowicka M, Guglietta S, Schindler S, Hartmann FJ, Weber LM, et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nature Medicine. 2018;**24**:144-153. DOI: 10.1038/nm.4466

[123] Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, 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**:924-938. DOI: 10.1016/j.immuni.2016.03.012

[124] Beavis PA, Henderson MA, Giuffrida L, Davenport AJ, Petley EV, House IG, et al. Dual PD-1 and CTLA-4 checkpoint blockade promotes antitumor immune responses through CD4(+) Foxp3(−) cell-mediated modulation of CD103(+) dendritic cells. Cancer Immunology Research. 2018;**6**:1069-1081. DOI: 10.1158/2326-6066.CIR-18-0291

[125] Alloatti A, Rookhuizen DC, Joannas L, Carpier JM, Iborra S, Magalhaes JG, et al. Critical role for Sec22b-dependent antigen cross-presentation in antitumor immunity. The Journal of Experimental Medicine. 2017;**214**:2231-2241. DOI: 10.1084/jem.20170229

[126] Garris CS, Arlauckas SP, Kohler RH, Trefny MP, Garren S, Piot C, 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**:1148-1161e1147. DOI: 10.1016/j.immuni.2018.09.024

[127] Bjoern J, Iversen TZ, Nitschke NJ, Andersen MH, Svane IM. Safety, immune and clinical responses in metastatic melanoma patients vaccinated with a long peptide derived from indoleamine 2,3-dioxygenase in combination with ipilimumab. Cytotherapy. 2016;**18**:1043-1055. DOI: 10.1016/j.jcyt.2016.05.010

[128] He Y, Cao J, Zhao C, Li X, Zhou C, Hirsch FR. TIM-3, a promising target for cancer immunotherapy. Oncotargets and Therapy. 2018;**11**:7005-7009. DOI: 10.2147/OTT.S170385

[129] Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 2007;**27**:111-122. DOI: 10.1016/j.immuni.2007.05.016

[130] Chaudhri A, Xiao Y, Klee AN, Wang X, Zhu B, Freeman GJ. PD-L1

binds to B7-1 only In Cis on the same cell surface. Cancer Immunology Research. 2018;**6**:921-929. DOI: 10.1158/2326-6066.CIR-17-0316

[131] Sugiura D, Maruhashi T, Okazaki IM, Shimizu K, Maeda TK, Takemoto T, et al. Restriction of PD-1 function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science. 2019;**364**: 558-566. DOI: 10.1126/science.aav7062

[132] Sierro SR, Donda A, Perret R, Guillaume P, Yagita H, Levy F, et al. Combination of lentivector immunization and low-dose chemotherapy or PD-1/PD-L1 blocking primes self-reactive T cells and induces anti-tumor immunity. European Journal of Immunology. 2011;**41**:2217-2228. DOI: 10.1002/eji.201041235

[133] Li B, VanRoey M, Wang C, Chen TH, Korman A, Jooss K. Anti-programmed death-1 synergizes with granulocyte macrophage colony-stimulating factor—Secreting tumor cell immunotherapy providing therapeutic benefit to mice with established tumors. Clinical Cancer Research. 2009;**15**:1623-1634. DOI: 10.1158/1078-0432.CCR-08-1825

[134] Rice AE, Latchman YE, Balint JP, Lee JH, Gabitzsch ES, Jones FR. An HPV-E6/E7 immunotherapy plus PD-1 checkpoint inhibition results in tumor regression and reduction in PD-L1 expression. Cancer Gene Therapy. 2015;**22**:454-462. DOI: 10.1038/cgt.2015.40

[135] Chung V, Kos FJ, Hardwick N, Yuan Y, Chao J, Li D, et al. Evaluation of safety and efficacy of p53MVA vaccine combined with pembrolizumab in patients with advanced solid cancers. Clinical & Translational Oncology. 2019;**21**:363-372. DOI: 10.1007/s12094-018-1932-2

[136] Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, et al. Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16-related cancer: A phase 2 clinical trial. JAMA Oncology. 2019;**5**:67-73. DOI: 10.1001/jamaoncol.2018.4051

[137] Weber JS, Kudchadkar RR, Yu B, Gallenstein D, Horak CE, Inzunza HD, et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. Journal of Clinical Oncology. 2013;**31**:4311-4318. DOI: 10.1200/JCO.2013.51.4802

[138] Gibney GT, Kudchadkar RR, DeConti RC, Thebeau MS, Czupryn MP, Tetteh L, et al. Safety, correlative markers, and clinical results of adjuvant nivolumab in combination with vaccine in resected high-risk metastatic melanoma. Clinical Cancer Research. 2015;**21**:712-720. DOI: 10.1158/1078-0432.CCR-14-2468

[139] Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: A molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011;**332**:600-603. DOI: 10.1126/science.1202947

[140] Hou TZ, Qureshi OS, Wang CJ, Baker J, Young SP, Walker LS, et al. A transendocytosis model of CTLA-4 function predicts its suppressive behavior on regulatory T cells. Journal of Immunology. 2015;**194**:2148-2159. DOI: 10.4049/jimmunol.1401876

[141] Ovcinnikovs V, Ross EM, Petersone L, et al. CTLA-4-mediated transendocytosis of costimulatory molecules primarily targets migratory dendritic cells. Science Immunology. 2019;**4**(35):eaaw0902. DOI: 10.1126/sciimmunol.aaw0902

[142] Munn DH, Sharma MD, Mellor AL. Ligation of B7-1/B7-2 by human CD4+ T cells triggers

indoleamine 2,3-dioxygenase activity in dendritic cells. Journal of Immunology. 2004;**172**:4100-4110. DOI: 10.4049/jimmunol.172.7.4100

[143] Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. Journal of Immunology. 2008;**181**:5396-5404. DOI: 10.4049/jimmunol.181.8.5396

[144] Mellor AL, Chandler P, Baban B, Hansen AM, Marshall B, Pihkala J, et al. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. International Immunology. 2004;**16**:1391-1401. DOI: 10.1093/intimm/dxh140

[145] Laurent S, Carrega P, Saverino D, Piccioli P, Camoriano M, Morabito A, et al. CTLA-4 is expressed by human monocyte-derived dendritic cells and regulates their functions. Human Immunology. 2010;**71**:934-941. DOI: 10.1016/j.humimm.2010.07.007

[146] Halpert MM, Konduri V, Liang D, Chen Y, Wing JB, Paust S, et al. Dendritic cell-secreted cytotoxic T-lymphocyte-associated Protein-4 regulates the T-cell response by Downmodulating bystander surface B7. Stem Cells and Development. 2016;**25**:774-787. DOI: 10.1089/scd.2016.0009

[147] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. The New England Journal of Medicine. 2010;**363**:711-723. DOI: 10.1056/NEJMoa1003466

[148] Sarnaik AA, Yu B, Yu D, Morelli D, Hall M, Bogle D, et al. Extended dose ipilimumab with a peptide vaccine: Immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. Clinical Cancer Research. 2011;**17**:896-906. DOI: 10.1158/1078-0432.CCR-10-2463

[149] Ribas A, Comin-Anduix B, Chmielowski B, Jalil J, de la Rocha P, McCannel TA, et al. Dendritic cell vaccination combined with CTLA4 blockade in patients with metastatic melanoma. Clinical Cancer Research. 2009;**15**:6267-6276. DOI: 10.1158/1078-0432.CCR-09-1254

[150] Wilgenhof S, Corthals J, Heirman C, van Baren N, Lucas S, Kvistborg P, et al. Phase II study of autologous monocyte-derived mRNA electroporated dendritic cells (TriMixDC-MEL) plus Ipilimumab in patients with pretreated advanced melanoma. Journal of Clinical Oncology. 2016;**34**:1330-1338. DOI: 10.1200/JCO.2015.63.4121

[151] Anderson AC, Anderson DE, Bregoli L, Hastings WD, Kassam N, Lei C, et al. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. Science. 2007;**318**:1141-1143. DOI: 10.1126/science.1148536

[152] de Mingo Pulido A, Gardner A, Hiebler S, Soliman H, Rugo HS, Krummel MF, et al. TIM-3 regulates CD103(+) dendritic cell function and response to chemotherapy in breast cancer. Cancer Cell. 2018;**33**:60-74e66. DOI: 10.1016/j.ccell.2017.11.019

[153] Fromm PD, Kupresanin F, Brooks AE, Dunbar PR, Haniffa M, Hart DN, et al. A multi-laboratory comparison of blood dendritic cell populations. Clinical & Translational Immunology. 2016;**5**:e68. DOI: 10.1038/cti.2016.5

[154] Long L, Zhang X, Chen F, Pan Q, Phiphatwatchara P, Zeng Y, et al. The promising immune checkpoint

LAG-3: From tumor microenvironment to cancer immunotherapy. Genes & Cancer. 2018;**9**:176-189. DOI: 10.18632/genesandcancer.180

[155] Andreae S, Piras F, Burdin N, Triebel F. Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). Journal of Immunology. 2002;**168**:3874-3880. DOI: 10.4049/jimmunol.168.8.3874

[156] Avice MN, Sarfati M, Triebel F, Delespesse G, Demeure CE. Lymphocyte activation gene-3, a MHC class II ligand expressed on activated T cells, stimulates TNF-alpha and IL-12 production by monocytes and dendritic cells. Journal of Immunology. 1999;**162**:2748-2753

[157] Buisson S, Triebel F. MHC class II engagement by its ligand LAG-3 (CD223) leads to a distinct pattern of chemokine and chemokine receptor expression by human dendritic cells. Vaccine. 2003;**21**:862-868

[158] Brignone C, Gutierrez M, Mefti F, Brain E, Jarcau R, Cvitkovic F, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: Combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. Journal of Translational Medicine. 2010;**8**:71. DOI: 10.1186/1479-5876-8-71

[159] Camisaschi C, De Filippo A, Beretta V, Vergani B, Villa A, Vergani E, et al. Alternative activation of human plasmacytoid DCs in vitro and in melanoma lesions: Involvement of LAG-3. The Journal of Investigative Dermatology. 2014;**134**:1893-1902. DOI: 10.1038/jid.2014.29

[160] Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, et al. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Immunity. 2008;**28**:870-880. DOI: 10.1016/j.immuni.2008.03.018

[161] Marinelli O, Nabissi M, Morelli MB, Torquati L, Amantini C, Santoni G. ICOS-L as a potential therapeutic target for cancer immunotherapy. Current Protein & Peptide Science. 2018;**19**:1107-1113. DOI: 10.2174/1389203719666180608093913

[162] Ogata M, Ito T, Shimamoto K, Nakanishi T, Satsutani N, Miyamoto R, et al. Plasmacytoid dendritic cells have a cytokine-producing capacity to enhance ICOS ligand-mediated IL-10 production during T-cell priming. International Immunology. 2013;**25**:171-182. DOI: 10.1093/intimm/dxs103

[163] Wei S, Kryczek I, Zou L, Daniel B, Cheng P, Mottram P, et al. Plasmacytoid dendritic cells induce CD8+ regulatory T cells in human ovarian carcinoma. Cancer Research. 2005;**65**:5020-5026. DOI: 10.1158/0008-5472.CAN-04-4043

[164] Faget J, Bendriss-Vermare N, Gobert M, Durand I, Olive D, Biota C, et al. ICOS-ligand expression on plasmacytoid dendritic cells supports breast cancer progression by promoting the accumulation of immunosuppressive CD4+ T cells. Cancer Research. 2012;**72**:6130-6141. DOI: 10.1158/0008-5472.CAN-12-2409

[165] Huang XM, Liu XS, Lin XK, Yu H, Sun JY, Liu XK, et al. Role of plasmacytoid dendritic cells and inducible costimulator-positive regulatory T cells in the immunosuppression microenvironment of gastric cancer. Cancer Science. 2014;**105**:150-158. DOI: 10.1111/cas.12327

[166] Pedroza-Gonzalez A, Zhou G, Vargas-Mendez E, Boor PP, Mancham S, Verhoef C, et al. Tumor-infiltrating plasmacytoid dendritic cells promote immunosuppression by Tr1 cells in human liver tumors. Oncoimmunology. 2015;**4**:e1008355. DOI: 10.1080/2162402X.2015.1008355