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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	<i>BATF3, IRF8</i>	<i>IRF4, IRF8</i>	<i>TCF4, SPIB, ZEB2, IRF4, IRF8, IRF7</i>
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 anti-viral 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-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 *exogenous* 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 promoter 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 vivo* 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

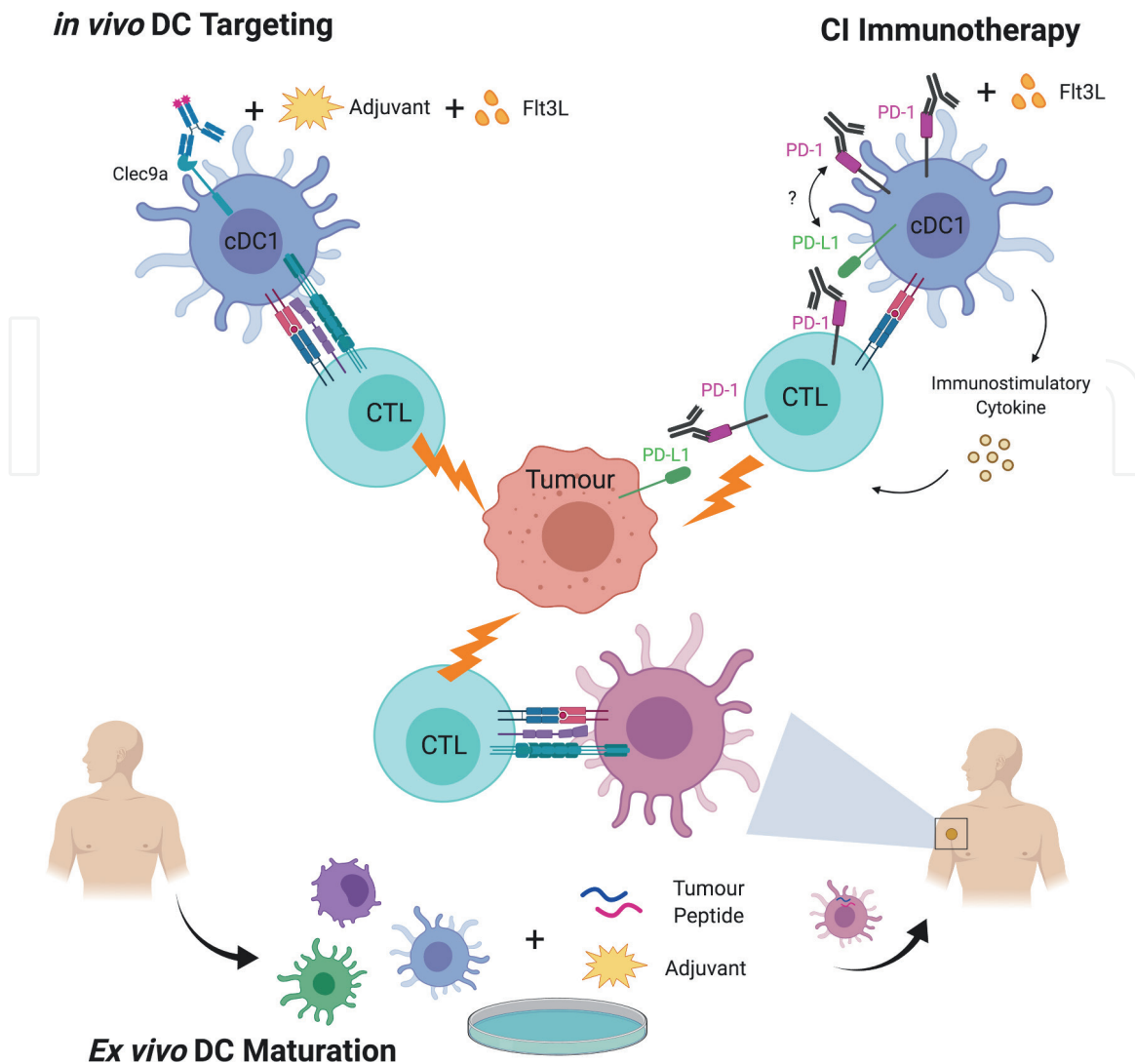


Figure 1. Overview of potential roles of DC in cancer therapies. To improve current cancer treatments and the activation of tumour-specific CTL, DC may be directly targeted in vivo (Section 6) or may themselves be the targets of checkpoint immunotherapies (Section 8). Ex vivo manipulation of DC (Section 6) may also be beneficial in some cancer patients. In vivo targeting strategies may also be combined with FIt3-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 TGF β 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 Pembrolizumab 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-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 through 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 through 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-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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