Dendritic cells as orchestrators of anticancer immunity and immunotherapy

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Abstract
Dendritic cells (DCs) are a heterogeneous group of antigen-presenting innate immune cells that regulate adaptive immunity, including against cancer. Therefore, understanding the precise activities of DCs in tumours and patients with cancer is important. The classification of DC subsets has historically been based on ontogeny; however, single-cell analyses are now additionally revealing a diversity of functional states of DCs in cancer. DCs can promote the activation of potent antitumour T cells and immune responses via numerous mechanisms, although they can also be hijacked by tumour-mediated factors to contribute to immune tolerance and cancer progression. Consequently, DC activities are often key determinants of the efficacy of immunotherapies, including immune-checkpoint inhibitors. Potentiating the antitumour functions of DCs or using them as tools to orchestrate short-term and long-term anticancer immunity has immense but as-yet underexploited therapeutic potential. In this Review, we outline the nature and emerging complexity of DC states, as well as their functions in regulating adaptive immunity across different cancer types. We also describe how DCs are required for the success of current immunotherapies, and explore the inherent potential of targeting DCs for cancer therapy. We focus on novel insights on DCs derived from patients with different cancers, single-cell studies of DCs, and their relevance to therapeutic strategies.

[H1] Introduction
Unleashing or enhancing the antitumour activity of T cells is the basis of most currently approved cancer immunotherapies. A prime example is provided by immune-checkpoint inhibitors (ICIs), which are aimed at reverting tumour-induced exhaustion of anticancer T cells and have transformed the treatment landscape owing to their unparalleled efficacy across many cancer types[1,2]. However, increasing the proportion of patients who respond to immunotherapy and mitigating the associated toxicities are priority areas for ongoing clinical research.

The priming and sustained orchestration of antitumour immune responses depends on the presentation of tumour antigens to naive T cells in concert with functional cues by dendritic cells (DCs), a class of professional antigen-presenting cells (APCs) that link innate and adaptive immunity[3,4]. DC abundance strongly correlates with a good prognosis in patients with cancer and clinical benefit from ICIs[5,6]. Technological advances have provided fresh insight into the functional states of intratumoural DCs across a range of cancer types, thus increasing knowledge of the diversity of DC subtypes and their roles — both normal and impaired — in this disease. The accumulating knowledge illustrates the potential of DCs as therapeutic targets or agents and as predictors of patient responses to immunotherapies such as ICIs. Indeed, direct therapeutic modulation of DC functions in patients or ex vivo manipulation of DCs for use as cancer vaccines are fast-developing fields that have already produced their first clinical successes[7–13]. However,

despite the ability of DCs to elicit anticancer immunity[5,6], DC-based cancer therapies continue to fall short of their full potential.

Herein, we explore the emerging heterogeneity of DCs in human cancers that is being revealed through single-cell analyses. We further provide an overview on DC presence and phenotype in patients with different cancer types. Additionally, we discuss the relevance of DCs for the efficacy of immunotherapy and new approaches combining DC-based treatments with other anticancer therapies, as well as their sequencing and timing. Overall, we focus on novel concepts and developments related to DC diversity, functions and applications in cancer, outlining how the advances in our understanding of DC biology can be leveraged to improve immunotherapy.

[H1] Functional diversity of DCs in cancer

[H2] DC lineages and their prognostic associations

Conventional DCs (cDCs) develop from common DC precursors (CDPs) in the bone marrow and fall into two broad categories, cDC1s and cDC2s[3,14,15] (Fig. 1a). Whereas cDC1s are a transcriptionally homogenous and clearly identifiable subset, the annotation of various subclusters among cDC2s in single-cell analyses of human and mouse bone marrow, blood and/or spleen samples demonstrates the notable heterogeneity of this group[14–20]. Consistent evidence indicates a phenotypic and ontogenic distinction of cDC2s from a non-conventional DC3 subset. CDP-derived cDC2s are characterized by combinations of the cell-surface protein markers CD5 (in humans) and/or ESAM (in mice), in contrast with DC3s, which typically express varying levels of CD14, CD163 (in humans) and/or CD16/CD32 (in mice) and arise from monocyte–DC progenitors[15–19]. Historical classifications of DCs also include ontogenetically diverse cells such as monocyte-derived DCs (MoDCs), plasmacytoid DCs (pDCs) and Langerhans cells (LCs) (Fig. 1a). Furthermore, a population termed transitional DCs (tDCs), lying along a phenotypic continuum between pDCs and cDC2s, has been characterized in mouse and humans[15,20].

Higher expression of cDC1-related gene signatures in human tumours generally correlates with an increased abundance of $CD8^+$ T cells and natural killer (NK) cells, a better prognosis, and favourable responses to ICI[21–29]. The expression of cDC2-defining genes in the tumour microenvironment (TME) is similarly associated with better prognosis in patients with certain cancers, including breast cancer and head and neck squamous cell carcinoma (HNSCC)[24,29–31], but predicts a poor prognosis in others, such as non-small-cell lung cancer (NSCLC)[32]. However, the intratumoural presence of $CD5^+$ cDC2s generally correlates with improved overall survival (OS) across several cancer types[33].

MoDCs mostly differentiate within tissues from infiltrating monocytes in response to various insults, including cancer. They are generally considered to be less immunostimulatory than cDCs, but can induce anticancer T cell responses in certain settings[6]. Elevated intratumoural gene-expression signatures defined using in vitro-generated resting or activated MoDCs have no notable prognostic value in patients with cancer, but analyses vary across individual studies[34–37].

pDCs are an important source of type I interferons (IFNs) in cancer but are generally inefficient APCs[6]. However, upon appropriate ex vivo activation and reinfusion, pDCs can potently attract and stimulate tumour-specific T cells in patients with melanoma[38,39]. A high abundance of pDCs in tumours has been reported to predict a favourable (e.g. for some breast cancers)[24,29,40,41] or unfavourable patient prognosis, depending on the cancer type[24,42,43].

LCs are embryonic progenitor-derived macrophages with DC-like functionality and notable T cell-priming potential[3]. Human LC-derived IL-15 can induce cytotoxic $CD8^+$ T cell expansion in co-culture experiments[44], and LC tumour infiltration correlates with better outcomes in patient with breast cancer[24,45]. However, the role of LCs in cancer remains understudied.

[H2] Preclinical data on the roles of DC subsets in anticancer immunity

To induce anticancer adaptive CD8$^+$ T cell and CD4$^+$ T cell responses[6], DCs take up and process tumour material for presentation of tumour antigens on MHC molecules (signal 1). DCs also sense damage signals in the TME through specialized receptors, such as pattern recognition receptors (PRRs), and subsequently mature to express co-stimulatory or co-inhibitory molecules (signal 2), and secrete soluble factors and cytokines (signal 3) that guide T cell polarization. Upon maturation, DCs generally traffic from the TME along chemokine gradients to tumour-draining lymph nodes (TDLNs), where initial priming of naive T cells takes place. Moreover, DCs are actively recruited to the TME, where they can in turn attract T cells and other immune cells and locally modulate their function, mainly via signals 1, 2 and 3 (Fig. 2). However, cancer cells and various factors often present in the TME can inhibit these antitumour functions of DCs and even skew DC activity towards immunosuppression and tolerance. In this situation, DCs can actively hinder anticancer adaptive immunity and induce tumour-promoting T cell responses, thereby facilitating tumour progression[6,46,47].

cDC1s are potent inducers of anticancer immunity and transport more tumour antigens to TDLNs than other DC subsets in models of several cancer types[21–23,25,30,48]. Nevertheless, data from studies in various cancer contexts demonstrate the importance of cDC2s and MoDCs for antitumour immune responses[49–52]. The specific roles of cDC2s and MoDCs in cancer are, however, less studied than those of cDC1s[6,53], and knowledge of these roles is likely to improve with the development of tools to better identify and selectively target particular DC subsets in mice[54].

[H3] Ingestion of tumour material

Intratumoural DCs recognize and take up tumour material, tumour antigens and dead or dying cancer cells via multiple mechanisms[6] (Fig. 2), initiated by interactions involving receptors such as AXL–LRP-1–RANBP9 complexes[55], TIM-3[56] and TIM-4[57]. These processes are strongly influenced by the type of cancer cell death, which can promote or impede DC immunogenicity depending on the release of danger signals[58,59]. For example, sensing of necrotic cells can be decoded as immunogenic by dendritic cell natural killer lectin group receptor 1 (DNGR-1, also known as C-type lectin domain family 9 member A (CLEC9A)) on cDC1s, which favours antigen cross-presentation[60,61]. By contrast, sensing of cancer cells undergoing a programmed form of necrosis by the C-type lectin receptor CLEC1A limits the cross-presentation potential and maturation of human cDC1s, but not the maturation of cDC2s[62]. Moreover, CLEC1A absence or blockade reduces tumour growth in immunocompetent or humanized mouse models of cancer, respectively[62]. In mouse models of lung adenocarcinoma, tumour-mediated downregulation of the phosphatidylserine receptor TIM-4 on cDC1s at a late time point dampens tumour-antigen uptake and cross-presentation, resulting in impaired CD8$^+$ T cell responses[57].

[H3] Immunogenic maturation

To effectively prime antitumour T cells, DCs must undergo immunogenic maturation (Fig. 2). In mouse models of melanoma and colon carcinoma, the priming of tumour antigen-specific T cells by cDC1s requires the co-stimulatory molecules CD40 and CD80/CD86, but not CD70[63]. In mouse xenograft models of immunogenic melanoma, intratumoural maturation, cytokine production and tumour-specific CD8$^+$ T cell-priming potential of cDC1s are coordinated by NF-κB signalling and an IFNγ-responsive, interferon regulatory factor 1 (IRF1)-mediated programme[64]. These findings are supported by data from patients with melanoma demonstrating that IRF1/NF-κB-related chemokine and cytokine signatures correlate with cDC1 numbers, activated CD8$^+$ T cell abundance and favourable OS[64]. The maturation of mouse and human cDCs

in the TME is also promoted by endocytosis of tumour-derived extracellular DNA, which induces cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) signalling and thus triggers type I IFN and chemokine secretion by these cells, thereby activating cDC-driven antitumour immune responses[65]. Accordingly, an enhanced basal activation of intratumoural cDCs is observed in several human cancer types[27,28,48,66]; however, systemic and/or intratumoral DC maturation is largely dysfunctional and is repressed by multiple mechanisms[6,67,68]. These mechanism include TIM-3-mediated suppression of extracellular DNA uptake and thus downstream cGAS–STING signalling (Fig. 3)[65], and the action of sialic acid-binding immunoglobulin-like lectins (Siglecs), which induce suppressive signals via intracellular immunoreceptor tyrosine-based inhibitory motif (ITIMs). Intratumoural cDCs express high levels of certain Siglecs (such as Siglec-7 and Siglec-9 in patients and Siglec-E in mouse models), and genetic deletion of Siglec-E increases the immunogenic responsiveness of cDC1s and cDC2s from mice[69]. In the case of MoDC-like cells, maturation, migration and induction of antitumour immunity are limited by adhesion via β2-integrin, which induces epigenetic alterations and thereby changes chromatin accessibility, resulting in impaired expression of an Ikaros–NF-κB-mediated transcriptional programme in these cells[70].

[H3] Migration from the TME to TDLNs

The migration of cDC1s from the TME to TDLNs is key for the induction of anticancer T cell responses (Fig. 2). This migration requires C-C-motif chemokine receptor 7 (CCR7) expression by cDC1s[25] as well as CD40-mediated transcriptional upregulation of Bcl-xL, which promotes the survival of migratory cDC1s[71]. In a genetically engineered, autochthonous mouse model of lung adenocarcinoma, high tumour-antigen uptake by cDC1s and their trafficking to TDLNs maintains a pool of tumour-reactive CD8$^+$ T cells at early disease stages; however, the abundance of migrated cDC1s in TDLNs decreases with tumour progression and these cells upregulate co-inhibitory molecules (including PD-L1) and downregulate co-stimulatory ligands (such as CD86)[72]. cDC2s also efficiently carry tumour antigens from the TME to TDLNs and directly prime anticancer CD8$^+$ T cell responses[73]. Of note, both migratory cDC1s and cDC2s can 'hand off' tumour antigens to other DCs in the TDLNs (Fig. 2), preferentially to TDLN-resident cDC1s, via membrane-encapsulated vesicles transferred through direct synaptic contacts[73]. Nevertheless, the TLDN-resident cDC1s that acquire tumour antigens in this manner are poor primers of CD8$^+$ T cells[73], possibly owing to suboptimal co-transfer of contextual activation cues from the migratory DCs[74].

[H3] Antigen presentation, antigen transfer and T cell priming

In mice, cDC1s excel at cross-presentation of exogenous tumour antigens from dead cancer cells to cognate CD8$^+$ T cells in TDLNs[75]. This CD8$^+$ T cell cross-priming activity of cDC1s is mediated by a variety of factors[53], including WDFY4[76], and depends on CD40 signalling in migratory cDC1s, owing in part to upregulation of CD70 that promotes T cell expansion via the co-stimulatory receptor CD27[71]. Moreover, mouse and human cDC1s can acquire preformed, functional antigen–MHC I complexes from tumour cells in a process called 'cross-dressing', which stimulates anticancer T cell responses[77,78]. Cross-dressing has also been observed in an IFN-stimulated gene-positive (ISG$^+$) subset of cDC2s, which thereby acquire intact tumour-antigen–MHC-I complexes and induce potent T cell-mediated anticancer immunity in the absence of cDC1s[79]. cDC1s can also prime tumour-specific CD4$^+$ T cells in TDLNs[80], which in turn stimulate CD40 signalling in cDC1s to boost their ability to induce anticancer CD8$^+$ T cell responses[81]. However, conventional antitumour CD4$^+$ T cell responses seem to be more potently

activated by cDC2s; these responses are notably potentiated by ICIs and impaired by regulatory T (T_{reg}) cells[30,31].

[H3] T cell attraction and reactivation in the TME

DCs in the TME not only sample tumour antigens, but also locally sustain and shape immune networks (Fig. 2). NK cells attract cDC1s to the TME by secreting lymphotactin (XCL1) and C-C-motif chemokine 5 (CCL5) and promote their survival locally by providing FMS-related tyrosine kinase 3 ligand (FLT3L)[21]. In mice systemically overexpressing FLT3L (through plasmid-mediated gene transfer), intratumoural cDC1s are themselves a source of CCL5, and expression of this chemokine is dampened by CLEC9A signalling in these cells[82]. cDC1s within the TME also attract $CD8^+$ T cells via production of C-X-C-motif chemokine 9 (CXCL9) and CXCL10[83], and can re-stimulate tumour-infiltrating T cells through secretion of cytokines such as IL-12 and type III IFNs[24,84]. Indeed, cross-presenting $CCR7^-$ $CXCL9^+$ cDC1s in the tumour stroma can form clusters with $CD8^+$ T cells expressing CXCR3 (the receptor for CXCL9), leading to the activation of stem-like $TCF1^+CD8^+$ T cells and protective anticancer immunity in mouse models[85]. Consistent with these findings, intratumoural cDC1–$CD8^+$ T cell clustering and $CCR7^-CXCL9^+$ cDC1 gene-expression signatures correlate with improved OS in patients with HNSCC or various other cancer types[85]. Notably, the induction of anticancer immune responses in the TME by cDC1s, and to a lesser extent by cDC2s, is dependent on glutamine uptake via SLC38A2 and subsequent signalling via the folliculin (FLCN)–transcription factor EB (TFEB) nutrient-sensing axis; alleviating the glutamine-mediated metabolic crosstalk and competition for glutamine between cancer cells and cDC1s reduces tumour progression and ameliorates therapy resistance in mouse models[86].

Nevertheless, in mouse models of *Kras*-driven pancreatic adenocarcinoma (but not lung adenocarcinoma), decline in cDC1 and cDC2 numbers and function are evident even at preinvasive stages of neoplasia, and progressively worsen as tumours progress, despite the high basal activation of intratumoural cDC1s[48,66]. Furthermore, cDC1s can sustain precursors of exhausted T cells in the TME, preventing their overactivation that further aggravates exhaustion and facilitating their reinvigoration by ICIs to expand and exert potent antitumour responses[87]. Many of the activities of cDC1s in the TME that promote antitumour immunity are impeded by cancer cell-secreted prostaglandin E_2 (PGE_2), given that signals mediated by the PGE_2 receptor EP2 and EP4 induce loss of IRF8 expression and dysfunction in cDC1s[88]. T_{reg} cells in the TME and TDLNs also have upregulated expression of CXCR3 and are thereby attracted by and interact with $CXCL9^+$ cDC1s, thus impairing cross-presentation and $CD8^+$ T cell-mediated cancer immunity[89]. Cancer cells can also activate β-catenin signalling that suppresses cDC1 recruitment to the TME, and thereby evade immune surveillance and ICI susceptibility; however, this mechanism could be reversed through exogenous expression of CCL5 in cancer cells or intratumoural injection of bone marrow-derived DCs in mouse models[26,90]. Additionally, tumour-derived IL-6 can impede the development of cDCs (especially cDC1s) in the bone marrow by inducing C/EBPβ–ZEB2 signalling in CDPs[91].

[H2] Complexity of intratumoural DC functional states revealed by single-cell analyses

The unbiased characterization of human tumours at the single-cell level has revealed a complex immune landscape in which intratumoural DC heterogeneity extends beyond ontogeny to encompass distinct functional states[92] (Fig. 1b). Several single-cell transcriptomic analyses have revealed that many DC functional states are conserved across diverse cancer types (including lung, breast, liver, colorectal and ovarian cancers) and between mice and humans[93] (TABLE 1).

Notably, no comparable conservation of gene-expression patterns is found for other myeloid populations, such as tumour-infiltrating macrophages[92].

In line with earlier conceptions, cDC1s constitute a discrete and rare DC population in the TME[23]. Nevertheless, different activation states of intratumoural cDC1s have been described, such as an MHC-II^{high} CCR7^{-} state characteristic of CXCL9-expressing cDC1s with robust cross-presentation activity that are typically found in proximity to proliferating TCF1^{+} CD8^{+} T cells[85] (Fig. 1b). Intratumoural cDC2s are an abundant and heterogeneous group characterized by the expression of CD1c in humans[92]. Generally, this group can be transcriptionally subdivided into multiple populations that probably reflect different lineages and functional states, and the abundance of each subpopulation varies across different cancer types[16,92,94]. For example, DCs with a transcriptome similar to that associated with an IFN-induced cDC2 activation state (ISG^{+} cDC2s; Fig. 1b) have been found in the TME of regressing fibrosarcomas in mice and in tumours from patients with various cancer types[79,92] (TABLE 1). Cells that share the expression of characteristic genes from cDC2s (*CD1C*) and LCs (*CD1A* and *CD207*) are also frequently found in human tumours and often termed LC-like DC2s (Fig. 1 and TABLE 1). The TME of numerous cancers contains DCs exhibiting a transcriptomic state within the cDC2-to-monocyte continuum[79,85], including DCs that are distinct from CD14^{+} monocytes, cDC2s[94] and with features resembling DC3s[94] ; the inherent heterogeneity of these cells and the consequent complexity of their definition and analysis has resulted in various annotations, including 'DC3'[94] and 'MoDCs–cDC2'[92] (Fig. 1b and TABLE 1).

[H2] CCR7^{+} DCs — a distinct DC functional state in cancer

Intratumoral DCs characterized by a gene-expression signature enriched for maturation markers (such as *CD80* and *CD83*), immunoregulatory molecules including PD-L1 (*CD274*) and/or PD-L2 (*PDCD1LG2*), and factors involved in cell migration (for example, *CCR7* and *FSCN1*) have been ubiquitously identified in mouse and human tumours[93,95], and have been variably referred to as mature DCs enriched in immunoregulatory molecules (mregDCs)[67], DC3[92], LAMP3^{+} DCs[96] or CCR7^{+} DCs[97] (TABLE 1). Indeed, no consensus currently exists regarding a common term for these DCs, although their description as CCR7^{+} DCs that have not egressed the tumour implies that they constitute a DC subset with a unique functional state, as opposed to a distinct DC lineage with a differential ontogenic or developmental origin[67] (Fig. 1b). Supporting this hypothesis, CCR7^{+} DCs identified in mouse and human tumours do not express hallmark lineage genes of cDC1 or cDC2s (such as *XCR1, CLEC9A* and *CD1C*)[67,92,98], in line with previous descriptions of cells with characteristics of CCR7^{+} DCs as 'mature cDCs'[99]. Nonetheless, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) analyses have revealed that intratumoural CCR7^{+} DCs do express cDC1-specific or cDC2-specific protein markers, including XCR1, CD103 or CD11b on mouse CCR7^{+} DCs and CD141 or CD1c on human CCR7^{+} DCs[67], which suggests a potential dual origin of CCR7^{+} DCs. This hypothesis is reinforced by computational trajectory inference analyses of DCs from human tumours, which indicate that cDC1s and cDC2s can converge on a highly similar activation and/or maturation state[92,96]. Interestingly, whereas cDC2s are generally the most frequent cDC subset in the TME[96], the most abundant CCR7^{+} DC subset has been reported to be cDC1-derived[92], implying that cDC1s are either more susceptible to acquiring the CCR7^{+} state or more long-lived in this state than cDC2s. However, other studies involving computational trajectory analysis indicate a preferential cDC2 origin of CCR7^{+} DCs[100,101].

The triggering cause of this matured DC state remains unclear. Initiation of the CCR7^{+} DC programme does not seem to require extravasation through lymphatic vessels, although DCs that have ingested tumour antigens phenotypically resemble CCR7^{+} DCs[67]. The intratumoural

presence of CCR7$^+$ DCs is variable and tumour-context-specific. A clear enrichment of intratumoural CCR7$^+$ DCs has been reported in patients with oesophageal carcinoma, hepatocellular carcinoma (HCC), gastric adenocarcinoma and colorectal carcinoma (CRC) compared with adjacent noncancerous tissue[92,102], but no difference was observed in NSCLC or other tumour types[92,94]. However, the DCs with the expression signature of CCR7$^+$ DCs are not exclusive to DCs localized within the TME; as they have been identified in lymph nodes and nonmalignant tissues collected from tumour-bearing mice[67] and patients with cancer[92,103].

Functionally, the combined expression of activation/maturation and regulatory molecules confers CCR7$^+$ DCs with a dual activity that is the focus of ongoing research. In immunohistochemical analyses of human tumours, CCR7$^+$ DCs are often observed in close proximity to both T_{reg} cells and PD-1$^+$ CD8$^+$ T cells[92,96,100,104]. Data from a study of human NSCLCs also indicate that CCR7$^+$ DCs within tertiary lymphoid structures found in the TME also closely interact with a recently described CD4$^+$ PD-1$^+$ CXCL13$^+$ T helper tumour-specific (Tht) cell subset, which seems to be required for ICI efficacy[105]. In mouse models, intratumoural CCR7$^+$ DCs can enhance the generation of T_{reg} cells and prime tumour-specific CD8$^+$ T cell proliferation[67]. CITE-seq and correlation analyses have enabled investigation of the functional specialization of CCR7$^+$ DCs derived from cDC1s and cDC2s (referred to as CCR7$^+$ cDC1s and CCR7$^+$ cDC2s, respectively). These studies have revealed that *IL12B*, which stimulates the activity of antitumour CD8$^+$ T cells[84,106], is exclusively expressed by CCR7$^+$ cDC1s[67,92] (as it is in cDC1s versus cDC2s[6]), whereas expression of *CCL17* and *IDO1*, which induce the recruitment and proliferation of intratumoural T_{reg} cells, is specific to CCR7$^+$ cDC2s in human tumours[92,100].

Notably, mouse and human CCR7$^+$ DCs in the TME seem to be less immunogenic than those in nonmalignant tissues[67,92], as indicated by lower levels of IL-12 production and higher expression of PD-L1 at both the mRNA and protein levels as well as *CMTM6* (a key regulator of PD-L1 stability)[67,92]. This less immunogenic state of intratumoural CCR7$^+$ DCs is at least partially driven by increased IL-4 signalling and AXL-mediated uptake of tumour antigens[67]. The immunogenicity of intratumoural CCR7$^+$ DCs is further diminished by a hypoxic niche generated by quiescent cancer cells, which impairs CCR7$^+$ DC expression of MHC molecules, IL-12 and migration-related molecules[107].

An abundance of mature LAMP$^+$ DCs in the TME, determined through immunohistochemistry, is a long-known predictor of favourable clinical outcomes in patients with melanoma[108] or NSCLC[109]. Subsequently, single-cell transcriptomic analyses have confirmed these associations, revealing correlations between gene-expression signatures of CCR7$^+$ DCs and an improved prognosis in patients with CRC[102] or NSCLC[98]. The relative intratumoural abundance of CCR7$^+$ DCs, but not cDC1s and cDC2s, predicted clonal T cell expansion upon anti-PD-1 antibody treatment (indicating better prognosis) in patients with triple-negative breast cancer (TNBC)[110], and CCR7$^+$ DCs were found to be enriched in CRCs with high microsatellite instability (MSI-H), which are often highly responsive to ICIs (in stark contrast to microsatellite stable CRCs)[102].

Overall, despite the high expression of immunoregulatory molecules by CCR7$^+$ DCs in the TME, their activities seem to be highly context dependent and often rather 'suboptimally immunogenic' (as opposed to immunosuppressive per se). Full characterization of the roles that DCs in this functional state have in cancer will require in-depth functional testing as well as further correlative analyses integrating other tumour immune indicators and patient outcomes.

[H2] Presence and phenotype of DC subsets across different cancers

Despite the conserved gene-expression signatures of intratumoural DC states, emerging data are revealing tumour type-specific differences in the abundance and functional diversity of DC

subsets in patients with cancer. In patients with melanoma, increased co-stimulatory molecule and cytokine expression by intratumoural cDC1s and cDC2s indicates a more activated basal state than that of their circulating counterparts, and increased abundance of activated cDC1s correlates with improved clinical outcomes[27]. However, patients with advanced-stage melanoma have decreased numbers of circulating cDC1s compared with healthy volunteers, which are also less responsive to Toll-like receptor (TLR)-mediated activation ex vivo[111]. Conversely, blood and intratumoural cDC2s from patients with melanoma seem to have generally impaired responses to TLR stimulation, and the activation status of these cells predicts a marker-dependent worse or better prognosis[27]. Nevertheless, autologous cDC2s can induce T cell responses in patients with metastatic melanoma when applied as a therapeutic vaccine following brief ex vivo activation and loading with melanoma-associated antigen[112]. Moreover, cDC2 subsets can trigger antitumour $CD4^+$ T cell responses after migrating to TDLNs in a mouse model of melanoma, and a high intratumoural density of analogous cDC2s relative to cDC1s in patients with this cancer type that respond to anti-PD-1 antibodies correlates with increased abundance of antitumour $CD4^+$ T cells ($ICOS^+$ $PD\text{-}1^{low}$), but decreased levels of $CD8^+$ T cells in the TME[31]. An intratumoural $CD5^+$ cDC2 subset seems to be a particularly potent stimulator of antitumour $CD4^+$ and $CD8^+$ T cells in patients with melanoma, correlating with improved survival[33]. Patients with ICI-responsive metastatic melanoma also have higher frequencies of MoDCs in the TME than those with ICI-resistant disease, which implies a role for these cells in T cell-mediated anticancer immunity[113]. Indeed, higher circulating monocyte numbers have been reported to be predictive of improved responses to ICIs in patients with advanced-stage melanoma[114].

Circulating cDC1s and cDC2s are similarly hyperactivated in patients with ovarian and prostate cancer, as evidenced by co-stimulatory protein expression. Nevertheless, blood cDC1 frequencies are low in patients with ovarian cancer, and cDC1 responsiveness to TLR3 stimulation is impaired in both cancer types[28]. Human MoDCs differentiated in the presence of ovarian or prostate cancer cell-conditioned media in vitro seem to have tumour-promoting effects via a number of immunosuppressive mechanisms[115,116], which can be reverted by chemotherapy in a mouse model of ovarian cancer[117].

In patients with glioblastoma, the number of circulating cDC2s, but not cDC1s, is reduced compared with healthy donors, and the cDC2s from these patients express low levels of MHC, costimulatory molecules and T cell-activating cytokines[118]. In a mouse model of glioblastoma, enhancing DC activation by TLR3 stimulation improves survival and enhances the antitumour activity of ICIs[119].

Circulating cDC2s are also less abundant in patients with NSCLC than in healthy donors, although a lower abundance of this cell type correlates with improved prognosis[32]. By contrast, high cDC1 numbers and TIM-4 expression (which facilitates tumour antigen uptake) in the TME correlate with favourable OS and response to PD-1 therapy in patients with lung adenocarcinoma[57]. However, gene-expression profiles indicate that the fraction of intratumoural resting DCs and $CD8^+$ T cells is lower in advanced-stage human lung adenocarcinoma than in early stage disease[120]. Interestingly, the interaction of $CCR7^+$ DCs with $CD4^+$ Tht cells was first detected in the TME of human NSCLCs[105].

In patients with breast cancer, cDC1s and cDC2s are more abundant within the TME than in neighbouring nonmalignant tissue, and the transcriptomic changes in intratumoural cDCs seem to be breast cancer-subtype specific[29]. Nevertheless, in the TME of TNBC both cDC subsets consistently upregulate the type I IFN signalling pathway[29]. However, circulating and tumour-infiltrating cDC1s, but not cDC2s, from patients with breast cancer produce high levels of type III IFN (specifically IFN-λ1), which is further upregulated upon TLR3 stimulation and promotes a TME that favours antitumour T_H1 and $CD8^+$ T cell responses[24]. Conversely, cDC2s potently

stimulate anticancer immunity in poorly immunogenic breast cancers. Whereas cDC1s impair the antitumour activity of $CD4^+$ T cells in mouse models of breast cancer, cDC1 depletion results in enhanced activation and migration of cDC2s to TDLNs, resulting in enhanced $CD4^+$ T cell and $CD8^+$ T cell priming and improved tumour control[30]. The abundance of MoDCs ($CD14^+$ DCs) is also higher in breast cancers versus adjacent nonmalignant tissue, and, similar to cDCs, MoDCs in the TME of TNBC have higher expression of type I IFN pathway-associated genes than those cells in the TME of other breast cancer subtypes[29]. Nevertheless, MoDCs generated ex vivo from PBMCs of patients with breast cancer seem to induce immunosuppressive T_{reg} cells[121].

In patients with gastric cancer, the abundance of circulating cDC2s is increased compared with healthy donors, independently of cancer stage[122]. Nevertheless, evidence suggests that DC-mediated antitumour immunity in patients with gastric cancers is suppressed via overexpression of the mRNA N^6-methyladenosine (m^6A) modification reader protein YTHDF1[123]. Consistent with this notion, deletion of *YTHDF1* in gastric cancer cells enhances tumour infiltration by IL-12-producing $MHCII^{high}$ DCs in mouse models, in concert with local $CD4^+$ and $CD8^+$ T cell activation[123].

An increased abundance of cDCs is also detected in the TME of patients with HNSCC compared with nonmalignant tonsillar tissue; however, intratumoural cDC1s and cDC2s notably downregulate genes involved in immune activation and anticancer immunity[124]. Moreover, HNSCCs are characterized by a higher T_{reg} cell and $CCR7^+$ DC abundance than other cancers[104]. Nevertheless, in HNSCCs with a low proportion of T_{reg} cells among tumour-infiltrating T cells, a high density of intratumoural cDC2 is associated with an increase frequency of $CD4^+$ T cells with an antitumour phenotype ($ICOS^+$ PD-1^{low}) and improved progression-free survival (PFS)[31].

[H1] Importance of DCs for ICI efficacy

ICIs and other immunomodulatory antibodies that are used or being tested as treatments for patients with cancer (such as anti-PD-1, anti-PD-L1, anti-CD137 or anti-TIM-3 antibodies) principally work by reinvigorating or enhancing the activity of pre-existing tumour-reactive T cells[125]. Preclinical evidence indicates that DCs, and particularly cDC1s, are vital for priming and maintaining such antitumour T cell responses and hence for the therapeutic effect of ICIs. First, tumours in mice constitutively lacking CD11c-expressing cells[126] or cDC1s[127–131] are largely resistant to ICI. Second, cDC1 depletion before or during exposure to immunomodulatory antibodies also hampers cancer control in mouse models[84,132]. Emerging evidence also indicates that the efficacy of ICIs and other immunomodulatory antibodies is shaped, in addition to their effects on T cells, by direct or indirect effects on the antitumour functions of DCs (Fig. 3).

[H2] Crosstalk between DCs and immune-checkpoint inhibition

Upon interaction with its ligands PD-L1 and PD-L2, PD-1 induces signals in T cells that largely block co-stimulatory CD28 signalling; the resulting suppression of T cell activity can be prevented by treatment with anti-PD-1 or anti-PD-L1 antibodies[2]. PD-L1 and/or PD-L2 can be expressed by various cells in the TME, including cancer cells and APCs such as macrophages and DCs. Preclinical data indicate that the antitumour efficacy of anti-PD-1 antibodies is 'licensed' by crosstalk between T cells and cDCs (Fig. 3b). Upon PD-1 inhibition, activated $CD8^+$ T cells secrete IFNγ, which activates cDCs, particularly cDC1s, to produce IL-12 (Ref.[84]). In turn, cDC-secreted IL-12 further stimulates cytotoxic $CD8^+$ T cells, enhancing the anti-PD-(L)1 antibody-mediated rescue of T cell dysfunction in mouse models of cancer[84]. Additionally, anti-PD-1 antibodies (with or without anti-CD137 antibodies) increase the intratumoural abundance of cDC1s as well as the frequency and duration of T cell–DC interactions in the TME in mouse models[132]. Mechanistically, CXCL9 secretion by intratumoural cDC1s has been shown to foster

their interaction with CXCR3-expressing $CD8^+$ T cells, which is essential for T cell proliferation and cytotoxicity and, thus, anti-PD-1 antibody efficacy[133]. In line with these findings, expansion and stimulation of cDC1s in humanized mice with FLT3L and a TLR3 ligand renders human melanoma xenografts susceptible to anti-PD-1 antibodies[111]. In preclinical models of melanoma, anti-PD-1 antibodies also increase $CD5^+$ cDC numbers in the TME and TDLNs via reduced production of IL-6 (Ref.[33]). CD5 expression by cDCs, which is largely restricted to cDC2s in humans, drives the activation of $CD5^{high}$ $CD4^+$ and $CD8^+$ T cells and is, therefore, required for the efficacy of anti-PD-1 antibodies. Moreover, a $CD5^+$ DC signature correlates with responsiveness to ICIs in patients with melanoma[33]. Notably, a high number of circulating $CD14^+$ $CD16^-$ HLA-DR^{hi} monocytes prior to treatment with anti-PD-1 antibodies is a strong predictor of favourable PFS and OS in patients with advanced-stage melanoma[114]. Furthermore, anti-PD-1 antibody treatment of patients with melanoma increases intratumoural differentiation of monocytes to MoDCs, a cell type that expresses high levels of costimulatory molecules and is associated with increased T cell expansion in the TME mice exposed to anti-PD-1 antibodies[113]. In patients with TNBC, anti-PD-1 antibody-based therapy also expanded $CCR7^+$ DCs (characterized by expression of CCL19), which correlated with the presence of tertiary lymphoid structures and lymphoid aggregates and a favourable clinical response[134].

Emerging evidence indicates that DCs also underlie the therapeutic efficacy of anti-PD-L1 antibodies (Fig. 3b). First, PD-L1-deficient tumours remain susceptible to treatment with anti-PD-L1 or anti-PD1[135–137]. Second, despite macrophages being the predominant source of PD-L1 in the TME[94,138], the specific deletion of PD-L1 in cDCs or cDC1s leads to efficient tumour control and determines susceptibility to PD-L1 blockade in mouse models (with PD-L1 expression on DCs being required for a response)[130,138]. A likely explanation for these observations is that intratumoural $CCR7^+$ DCs express the highest levels of PD-L1[94,138]. Moreover, both anti-PD-1 and anti-PD-L1 antibodies lose their ability to control tumour growth in the absence of costimulatory signalling induced by DCs expressing CD80 and/or CD86, which engage with and activate CD28 in effector T cells[139,140]. Accordingly, increasing intratumoural DC (mainly cDC1) abundance and activation via FLT3L administration combined with triple in situ immunomodulation (radiotherapy, and TLR3–CD40 stimulation) induces stem-like $CD8^+$ T cells and overcomes resistance to anti-PD-L1 antibodies in multiple orthotopic tumour models[141]. These data, together with the lack of synergy upon combining anti-PD-L1 antibodies with PD-L1 deletion in DCs, suggest that DCs are a crucial source of immunosuppressive PD-L1 in the context of cancer[130,138]. Furthermore, anti-PD-(L)1 antibodies seem to promote de novo expansion of previously undetected stem-like T cells in patients with cancer, rather than reinvigorating exhausted intratumoural T cells (which might be unrecoverable)[142–144]. This finding suggests that anti-PD-(L)1 antibodies disrupt inhibitory interactions between DCs and tumour-antigen-specific T cells not only in the TME but also in TDLNs. In line with this hypothesis, selective targeting of anti-PD-L1 antibodies to TDLNs (where high PD-L1 expression is mostly found on cDC2s) through intrapleural administration results in effective cancer control in mice, indicating that ICIs can stimulate antitumour effector T cells in TDLNs[145].

Paradoxically, PD-L1 expression by DCs can also support their ability to stimulate antitumour immunity. T cell-secreted IFNγ can upregulate PD-L1 expression on DCs during antigen presentation, protecting them against T cell-mediated cytotoxicity[130]. Consequently, PD-L1 blockade can trigger premature elimination of DCs, thus favouring the priming of $CD8^+$ effector T cells targeting dominant antigens over those targeting subdominant antigens (owing to the generally faster activation of the former, which subsequently induces DC lysis prior to activation of subdominant T cells), thereby promoting immune evasion of cancer cells harbouring subdominant antigens and thus tumour recurrence[146]. Moreover, PD-L1 can interact in cis with

CD80 on the surface of cDCs[138,147,148], and the resulting CD80–PD-L1 heterodimers block the inhibitory trans PD-L1–PD-1 interaction while maintaining the activating trans CD80–CD28 interaction of cDC1s and cDC2s with effector T cells (Fig. 3b). CD80–PD-L1 heterodimerization also decreases CD80 homodimerization on DCs, thereby reducing the transendocytosis of CD80 homodimers by T_{reg} cells via trans interaction with CTLA4[148] (Fig. 3c). In keeping with these findings, antibody-mediated disruption of CD80–PD-L1 heterodimers on DCs modestly increases tumour growth in preclinical models[138], and PD-L1 blockade can facilitate CD80 depletion from DCs[148], which might hamper the co-stimulation of antitumour T cells. These mechanisms might at least partially explain the synergistic effect observed in mice when combining anti-PD-L1 and anti-CTLA4 antibodies, with the latter preventing CD80 loss from intratumoural DCs and resulting in superior antitumour responses than those achieved with anti-PD-L1 antibody monotherapy[148,149] (Fig. 3c).

The presence and activation states of DCs are also emerging as potent predictors of clinical response to ICI therapy (BOX 1). The intratumoural gene-expression signatures of 'stimulatory DCs' or 'DC1s helped by $CD4^+$ T cells' predict the responsiveness of patients with metastatic melanoma to anti-PD-1 antibodies[22,150]. In addition, low expression of cDC1 transcriptional signatures in pretreatment tumour biopsy samples has been associated with disease recurrence after neoadjuvant or adjuvant treatment with anti-PD-L1 and anti-CTLA4 antibodies in patients with stage III melanoma[131], and an increased frequency of cDC1s and their interaction with $CD8^+$ T cells in the TME is characteristic of TNBCs, which are more responsive to ICIs than other subtypes of breast cancer[151]. Furthermore, the intratumoural presence of $CCL19^+$ DCs partially predicts the response of patients with TNBC to anti-PD-(L)1 antibodies, but not to chemotherapy[134]. Finally, a study using a proximity ligation assay revealed that a high density of PD-1–PD-L1 interactions in TDLNs, which occur mainly between T cells and DCs, is also associated with early disease relapse (within 48 months) after surgery in patients with stage II melanoma[145]. This finding might suggest a DC-mediated immunosuppressive axis in this setting that could be directly targeted using anti-PD-(L)1 antibodies.

[H2] DCs and TIM-3 blockade

TIM-3 is a co-inhibitory receptor expressed on exhausted intratumoural T cells, and combined inhibition of TIM-3 and PD-1 has shown antitumour activity in early phase clinical trials[152]. However, TIM-3 is also expressed by various other cell types in the TME, including cDC1s[153–155]. Moreover, conditional genetic deletion of TIM-3 in DCs, but not T cells, enhances antitumour immunity in mouse models of colon cancer and NSCLC[155]. Mechanistically, TIM-3 can interact with HMGB1 bound to extracellular DNA released from dying cancer cells, thereby interfering with sensing of this alarmin; TIM-3 loss or blockade disrupts this interaction and promotes endosomal internalization of extracellular DNA by tumour-associated cDC1s, which can activate the cGAS–STING pathway and trigger type I IFN-dependent expression of CXCL9[65,153,154] (Fig. 3c). TIM-3 inhibition also increases oxidative-stress in cDCs, thereby inducing inflammasome activation and IL-1β and IL-18 production[155]. Thus, disruption of TIM-3 ultimately enhances the ability of DCs to stimulate intratumoural T cell proliferation and effector functions[65,155]. Accordingly, anti-TIM-3 antibodies synergize with nucleic acid adjuvants and chemotherapy to stimulate antitumour immunity in multiple mouse models[153,154,156].

In contrast with these findings, TIM-3 inhibition might also impair DC-mediated anticancer immunity by preventing binding of TIM-3 to phosphatidylserine exposed by apoptotic cells, which is required for uptake of these cells by cDC1s[56]. However, in mouse models, TIM-3 blockade does not impair tumour antigen uptake by intratumoural cDCs, but does reduces the number of tumour-antigen-loaded cDCs in the TDLN[156]. Expression of CEACAM1, another

ligand that induces inhibitory signalling by TIM-3, has been found to be upregulated in exhausted T cells in the TME[157]. Notably, all clinically active antagonistic antibodies targeting TIM-3 impair its binding of both phosphatidylserine and CEACAM1 (Ref.[158]), although the resultant effects on DC function remain unexplored.

[H1] DC-based approaches to cancer immunotherapy

Antitumour immune responses have been successfully induced in patients with cancer using tools to enhance the immunogenic activities of DCs or to deliver tumour antigens to DCs and other APCs. These tools include the granulocyte–macrophage colony-stimulating factor (GM-CSF)-expressing oncolytic herpes virus talimogene laherparepvec[7], the TLR7 agonist imiquimod[8], the tuberculosis vaccine Bacillus Calmette-Guérin[9], the TLR4 agonists OK-432 (also known as picibanil; an inactivated form of *Streptococcus pyogenes* A3su)[10] and monophosphoryl lipid A[11], and the TLR3 agonist YS-ON-001 (polyinosinic-polycytidylic acid (poly I:C) plus an inactivated rabies virus vaccine)[12]. Moreover, adoptive transfer of DCs is being actively explored in clinical studies as an anticancer vaccination strategy, and the autologous, prostatic acid phosphatase antigen-loaded DC-based vaccine sipuleucel-T is approved for the treatment of patients with metastatic castration-resistant prostate cancer[13]. Here, we focus on the latest developments in the targeting and/or use of DCs for anticancer therapy as well as considerations regarding the timing of treatments.

[H2] New approaches to the in vivo activation or targeting of DCs

The most explored strategy for DC activation in vivo involves the local administration of PRR agonists, as reviewed in detail[6,159,160]. In mouse models, DC-dependent antitumour immunity is stimulated by the administration of TLR ligands in combination with ICIs, radiotherapy and/or growth factors or cytokines, such as DC-specific FLT3L or type I IFN[161–164]. Ex vivo treatment with poly I:C (a TLR3 ligand) and R848 (a TLR7/8 ligand) induces robust activation of human blood-derived DCs and enhances their ability to stimulate the expansion of antigen-specific $CD8^+$ T cells[165]. In patients with Merkel cell carcinoma, intratumoural administration of a TLR4 agonist (G100) has been shown to enhance antitumour immune responses and induce tumour regression[166]. Moreover, the addition of intralesional TLR9 agonists (such as SD-101 or vidutolimod) to systemic ICIs is under ongoing investigation in clinical trials involving patients with melanoma or HNSCC, with initial data demonstrating T cell activation, objective responses and reversal of ICI resistance[167,168]. In addition to systemic or intratumoural administration, longstanding research interest has been focused on approaches to deliver PRR agonists specifically to DCs[6]. For example, STING agonists encapsulated in liposomal nanoparticles targeting $CD103^+$ DCs via a CLEC9A-binding peptide have been shown to improve the antitumour activity of anti-PD-L1 antibodies in mouse models and to avoid the toxicity associated with systemic STING activation[169].

Agonistic anti-CD40 antibodies present an alternative strategy for activating DCs and other APCs. CD40, a member of the TNF receptor family, is upregulated on stimulated DCs and promotes DC activation and survival. Anti-CD40 antibodies have cDC1-dependent antitumour activity as single agents[170,171] and synergize with ICIs, adoptive DC transfer and FLT3L treatment in preclinical models[71,72,84,102,172,173]. Several combination therapies with CD40 agonists are currently under clinical evaluation across various cancer types (for example, NCT02376699 and NCT03123783), and results thus far indicate tolerability and partial clinical activity[174–176]. Novel strategies for targeted CD40 stimulation of DCs, such as bispecific antibodies (bsAbs) co-targeting CD40 and tumour antigens[177] or CD40 ligand-coated vesicles containing tumor antigens[178], are also being explored preclinically. In mice, bsAbs designed to activate CD40

signalling specifically in DCs or cDC1s (including CD40 × CD11c, CD40 × CLEC9A and CD40 × DEC205 bsAbs) improve antitumour immunity compared with mono-specific anti-CD40 antibodies while mitigating toxic effects, underscoring the relevance of DCs to the therapeutic activity of CD40 agonists[171].

Tumour-specific immune responses can also be promoted by the administration of tumour antigens, and data from preclinical models demonstrate that the resultant antitumour effects depend on endogenous DCs[179]. Accordingly, various delivery platforms have been developed to target tumour antigens directly to APCs (DCs and macrophages)[180–182], including antigens coupled to DC-targeted antibodies[183] and nanoparticles with[184,185] or without[179] functionalization for selective DC targeting. Systemically administered nonfunctionalized RNA lipoplexes encoding tumour-associated antigens or neoantigens can be ingested by APCs[179], and therapeutic vaccination with such complexes, alone or in combination with ICIs or ICIs and chemotherapy, have been shown to induce potent vaccine-specific T cell responses that correlate with durable objective responses in patients with advanced-stage melanoma and delayed disease recurrence in those with resectable pancreatic ductal adenocarcinoma[179,186,187]. This promising strategy is being further explored in a clinical trial involving a larger cohort of patients with advanced-stage melanoma refractory to anti-PD-1 antibodies (NCT04526899). Similarly, non-targeted multiepitope DNA-liposome vaccines are effectively taken up by DCs and limit melanoma growth and metastasis in mouse models[188]. In another approach, lipid nanoparticles containing DNA-encoded tumour antigens were functionalized with a mannose-mimicking moiety to enhance their uptake by mannose receptor-expressing APCs, including DCs; their administration induced a protective anticancer immune response in a mouse model of melanoma[189].

A novel strategy to enhance antitumour immunity relies on enhancing DC–T cell interactions, which correlate with patient prognosis[85]. Antagonistic anti-PD-1 and anti-TIM-3 antibodies promote intratumoural DC–T cell interactions by enhancing expression of CXCR3 on T cells and production of its ligand CXCL9 by DCs[133,156] (Fig. 3b and d). Increased CXCL9 production by intratumoural DCs (for example, through epigenetic modulation with 3-deazaneplanocin A plus 5-aza-2'-deoxycytidine) can alleviate resistance to anti-PD-1 treatment in mouse models, and increased plasma levels of CXCR3 ligands (CXCL9 as well as CXCL10) within a few months of initiating anti-PD-1 antibody-based therapy are associated with a favourable response in patients with melanoma[133]. Moreover, bsAbs that combine PD-L1 blockade with the potential for T cell recruitment to DCs (for example, CD3 × PD-L1 or LAG-3 × PD-L1) have improved antitumour activity compared with mono-specific anti-PD-L1 antibodies in mouse models[190,191].

[H2] DC-based vaccination

DC-based vaccination is a strategy to enhance antitumour immunity by circumventing immunosuppression of DCs[6,192,193]. This approach involves the use of widely varying protocols to culture patient-derived DCs with tumour antigens and stimulants, and prepare them for reinfusion into the patient[6]. Despite the robust clinical safety of DC-based vaccination[6,194], objective response rates rarely exceeded 15%[195], and to date only one such product has received FDA approval (sipuleucel-T)[13]. Hence, given the enormous potential of DCs to induce anticancer immunity, an urgent need exists to improve DC vaccination approaches[194]. Here, we review progress made over the past decade in developing anticancer DC vaccines, with a focus on advances in manufacturing protocols that enhance their clinical efficacy.

Most preclinical and clinical DC-based vaccination preparations are not based on naturally ocurring DCs (nDCs) directly purified from blood, but rather DC surrogates: for example, cells generated by culturing mouse bone marrow or human blood monocytes with GM-CSF[6,194] (TABLE 2) — products referred to as GM-CSF-induced bone marrow-derived DCs

(GMDCs) and MoDCs, respectively. The generation of effective antitumour immunity with such MoDCs requires their ex vivo activation, usually achieved through stimulation with a combination of cytokines and/or TLR ligands[195–200]. Other maturation strategies in development include culturing of DCs with mRNAs encoding stimulatory proteins (for example, CD40L)[201,202] and measures to modulate DC metabolism[203]. MoDCs can be loaded ex vivo with many types of tumour antigen, which are mostly classified as either anonymous or predefined. Anonymous antigens are unknown and generally obtained from tumour material, whereas predefined antigens are those that have been identified previously and can be patient-specific (mutated neoantigens) or shared by many patients (tumour-associated antigens). In both cases, loading with multiple tumour antigens that are presented on MHC I and MHC II molecules (to activate $CD8^+$ and $CD4^+$ T cells, respectively) improves the ability of MoDC vaccines to generate antitumour immune responses and overcome tumour antigen escape[193,204]. For example, successful phase I/II clinical trials in patients with glioblastoma used autologous cancer cell lysate-loaded MoDC vaccines[205,206], and a similar approach using the DCVax-L vaccine increased OS in a phase III trial when added to standard-of-care temozolomide-based therapy[207–209] (TABLE 2). These clinical data show that autologous tumour material is a feasible and efficient antigen source for DC vaccination against poorly immunogenic cancer types such as glioblastoma, for which predefined antigens are scarce[210]. Indeed, the efficacy of MoDC-based anticancer vaccination approaches has been assessed in multiple clinical trials[194,195]; however, only a handful of approaches have been tested in phase III trials (TABLE 2), and among them only sipuleucel-T has achieved regulatory approval.

DCs generated from mouse bone marrow by treatment with GM-CSF ex vivo do not in fact present tumour antigens to T cells after injection, but instead transfer them to endogenous natural cDCs, which are required for effective vaccination with GMDCs[211–213]. Furthermore, endogenous mouse and human cDCs are more efficient antigen presenters and inducers of T cell responses than DCs generated ex vivo with GM-CSF[214–217]. These findings led to the development of next-generation DC-based anticancer vaccines derived from nDCs, which comprise pDCs and cDCs[218]. Circulating nDCs are scarce, and their isolation in sufficient numbers became feasible only in the past decade[218,219]. Initial clinical studies of nDC-based vaccines used the more abundant cDC2 ($CD1c^+$) or pDC ($CD304^+$) subsets, both of which were found to induce antigen-specific T cell responses in patients with advanced-stage melanoma or castration-resistant prostate cancer, in some instances correlating with prolonged PFS[39,112,220,221]. An ongoing phase III trial is testing a cDC2 and pDC vaccine combination in patients with resected stage III melanoma (NCT02993315; TABLE 2); however, an interim analysis suggests no notable benefit over placebo[222]. The most potent vaccine-induced anticancer immunity is achieved with cDC1s[223]. Data from preclinical studies demonstrate the anticancer activity of natural cDC1-based vaccines[80], and vaccination with in vitro-generated cDC1s outperforms approaches using GMDCs[217]. Notably, cDC1 vaccines do not rely on antigen presentation by host cDCs for their antitumour activity[214]. An initial clinical trial of a cDC1 ($CD141^+$) vaccine demonstrated the feasibility of this approach in patients with cancer[224], and a dedicated phase I/II trial is further exploring cDC1-based vaccination in patients with ovarian cancer (NCT05773859).

[H2] Combination of DC vaccines with other cancer treatments

The mechanism of action of DC vaccines might complement those of other cancer treatments, and current research efforts therefore centre on unleashing the potential of DC-based vaccination through combinatorial approaches[225]. Here, we adopt a previously published categorization of candidate combination strategies including DC-based vaccination[195] to frame advances in this area and considerations for optimal treatment sequencing.

[H3] Interventions that enhance the strength of the immune effector response. Cancer therapies in this category include ICIs and adoptive T cell transfer (ACT). In mouse models, intratumoural injection of bone marrow-derived, FLT3L-activated DCs can revert ICI resistance[226], and DC-based vaccination synergizes with ICIs (anti-PD-1, anti-CTLA4 or anti-TIM-3 antibodies)[80,217,227–229] and with small interfering RNA-mediated silencing of LAG-3 and PD-1 or CTLA4 expression[230,231] to increase T cell activation, immune memory induction, clonal T cell diversity and host survival. The safety of this approach in patients with cancer has been demonstrated in phase I trials combining DC vaccines with anti-PD-1 antibodies[232,233] or anti-PD-(L)1 plus anti-CTLA4 antibodies[232,234]. Moreover, the combination of DC-based vaccination and anti-CTLA4 antibody therapy prolonged OS in patients with advanced-stage melanoma in a phase I/II clinical trial compared with previous data on anti-CTLA4 monotherapy[235]. The combination of DC-based vaccination with ACT also seemed to improve the clinical responses of patients with advanced-stage ICI-refractory melanoma compared with ACT monotherapy in a small-cohort phase I trial[197]. Ongoing clinical trials are testing alternative strategies combining DC-based vaccination with the administration of chimeric antigen receptor (CAR) T cells, cytokine-induced killer or NK cells, as well as recombinant cytokines or immune-stimulants[194].

These trials notably differ in the sequencing of treatments. DC-based vaccination before ICI accords with the notion that vaccine-driven T cell activation will benefit from subsequent ICI-mediated prevention of T cell exhaustion[232,235,236]. By contrast, delaying DC vaccination until after ICI or ACT aims to prolong the expansion of antigen-specific T cells[197,234]. Further research is needed to determine the optimal sequencing in each scenario.

[H3] Interventions that reverse tumour-associated immunosuppression. Cancer immunotherapies, including DC-based vaccination, can be impeded by T_{reg} cells and myeloid-derived suppressor cells (MDSCs) that induce T cell tolerance and attenuate effector T cell functions[195]. Clinical data indicate a negative correlation between pre-treatment circulating MDSC numbers and immunological responses to a DC vaccine[237], highlighting the need for adjunct therapies to control immunosuppression during DC-based vaccination. For example, administration of antigen-loaded DCs with silenced IDO, which drives tolerogenic functions of DCs, induces antitumour immunity in patients with cancer[238,239].

Small-molecule inhibitors or cytotoxic agents used to directly target cancer cells can also have indirect anticancer effects by relieving immunosuppression and/or potentiate the immunogenic functions of DCs[195]. For example, the tyrosine-kinase inhibitor sunitinib can decrease T_{reg} cell and MDSC abundance, and the combination of this agent with DC-based vaccination induces tumour-reactive T cell responses in patients with metastatic renal cell carcinoma (RCC)[240,241]. However, this effect did not translate into increased OS in patients with metastatic RCC receiving an autologous DC vaccine (rocapuldencel-T) plus sunitinib versus sunitinib alone in a subsequent phase III trial[202] (TABLE 2). Interestingly, high baseline circulating T_{reg} cell numbers predicted improved outcomes in patients receiving rocapuldencel-T plus sunitinib but, conversely, correlated with poor responses to sunitinib alone[202]. This finding provides proof-of-principle that combination therapy with sunitinib and DC-based vaccination can more effectively overcome immunosuppression in patients with RCC than sunitinib monotherapy and highlights the potential utility of circulating T_{reg} cells for selecting patients to receive sunitinib with versus without DC-based vaccination. In patients with recurrent ovarian cancer, the combination of low-dose cyclophosphamide with DC vaccination and bevacizumab (anti-VEGFA antibody) resulted in lower serum levels of immunosuppressive TGFβ, greater induction of tumour antigen-specific T cells and increased OS, as compared with bevacizumab

plus DC vaccination in the same pilot trial or with bevacizumab plus cyclophosphamide in a matched historical cohort[242].

[H3] Interventions that primarily reduce tumour burden and increase cancer cell immune susceptibility. Chemotherapy, radiotherapy, targeted therapy and surgery are all aimed at shrinking or, ideally, eradicating tumours. Cancer-related immunosuppression is correlated with the extent of tumour burden[243]. Accordingly, the induction of a tumour-specific immune response by DCs is more profound in patients with resected stage III than unresectable stage IV melanoma[112,220,225]. Yet, DC vaccination has mostly been tested in patients with advanced-stage, unresectable cancers. Hence, adjunct and/or consolidative treatment of patients with DC vaccines at an earlier disease stage or in whom the tumour burden has been reduced using the aforementioned cancer therapies is a rational approach that is currently gaining relevance[194,225].

The low toxicity of autologous DC vaccines and their potential capacity to generate immune memory make them an ideal candidate adjuvant therapy after tumour resection to prevent disease recurrence[244,245]. Adjuvant DC-based vaccination has been tested in patients with glioblastoma[207,208,225], melanoma[220,225,246], RCC[225], HCC[247] or prostate cancer[248] and after resection of colon cancer liver metastases[249]. These clinical studies, including a completed phase III trial[207], provide evidence of the induction of anticancer immune responses and favourable clinical outcomes associated with adjuvant DC-based vaccination, and additional large-cohort trials of this therapeutic approach are ongoing (TABLE 2). Moreover, adjuvant DC-based vaccination seems to increase the likelihood that patients will respond to ICIs or chemotherapy upon disease recurrence[250,251]. The efficacy of neoadjuvant DC-based vaccination remains underexplored, but such treatment might synergize with approved neoadjuvant immunotherapies[252].

In addition to reducing the tumour burden, chemotherapy[253] and radiotherapy[254] trigger the immunogenic death of cancer cells, thereby promoting tumour antigen uptake by DCs and their activation[255]. These effects suggests a likely favourable outcome from adjunct DC-based vaccination, and a randomized phase II trial showed improved PFS of patients with stage III ovarian cancer who received chemotherapy followed sequentially by DC vaccination compared with patients who received chemotherapy alone or both treatments concomitantly (median not reached versus 21.4 months and 20.3 months, respectively; HR 0.39, 95% CI 0.16–0.96 for the comparison with chemotherapy alone; $P = 0.034$); a trend towards improved OS was also observed (4-year OS of 79% versus 63% and 71%, respectively)[256]. Nevertheless, concomitant DC-based vaccination and chemotherapy has been reported to prolong OS in patients with metastatic melanoma (median 18 months versus 11 months with chemotherapy alone; $P = 0.01$), suggesting that the optimal timing of vaccination is context specific[257].

[H1] Conclusions

The heterogeneity of DCs in cancer encompasses their ontogeny, functional states, responsiveness to stimulation, location, and systemic or local abundance. Gene-expression signatures clearly demonstrate that global features of intratumoural DC diversity are conserved across diverse cancer types. However, studies of individual cancers suggest the existence of tumour type-specific and stage-specific features of DC presence and functionality when comparing DCs in the blood, TDLNs, TME or nonmalignant tissues, and/or in comparisons with DCs from healthy donors. Future studies will need to address this complexity to advance understanding of DC biology in different human cancers and to harness the full therapeutic potential of DCs for the treatment of cancer. Hence, a community effort is required to standardize protocols for the study of intratumoural DC diversity (BOX 1), especially computational methods. This standardization will be essential to answer key questions on the biology of DCs in cancer that might be clinically

relevant. For example, do intratumoural and peripheral DCs adopt other activation states in patients with cancer and how do they relate to cancer progression? How do different TMEs and systemic effects of diverse cancer types influence the presence and phenotype of DCs? Are cancer type-specific DC functions relevant to the effectiveness of various treatments and/or the design of novel therapies? Or are DC functional states in cancer universal?

Moreover, the direct and indirect effects of immunotherapies such as ICIs on the activities of DCs (especially cDC1s) are emerging as key determinants of clinical efficacy. Given the importance of DC–T cell crosstalk for the success of ICI-based therapy, strategies to increase the abundance of DCs or enhance their antitumour functions have great potential for therapeutic synergy. Novel effective approaches have been developed to activate DCs, supply them with tumour antigens and enhance their T cell-priming abilities. However, how those interventions control the functional states of DCs in the TME remains largely unclear. For example, can the modulation of intratumoural DC functions be applied therapeutically to prevent immunosuppression? Is such modulation achievable through in vivo DC targeting? The adoptive transfer of activated DCs loaded with tumour antigens can induce tumour-specific T cell responses and favourable clinical outcomes, and this approach might be improved with next-generation DC vaccines based on potent nDCs. With regard to the latter, comparative efforts to identify the optimal and feasible combinations of antigen source, stimulating agent, administration route and DC subset for vaccinations are urgently needed.

A number of clinical advances have important implications for the use of DCs as a target or tool for cancer immunotherapy. First, the efficacy of DC-based treatments will be increased and costs reduced by patient pre-selection based on predictive biomarkers of clinical response (for example, cancer type or stage, and/or immune status). For example, clinical data suggest that DC-based vaccination might mostly benefit patients with immune 'cold' tumours[225,236]. Second, at this stage in which DC immunotherapies are tested in latest stages of the disease, it is relevant to quantify the immunogenic rather than the therapeutic efficacy of DC-based therapies. This will require immunomonitoring of patients as well as pharmacodynamic biomarkers, such as IL-12 production[202], to efficiently identify agents with relevant on-target immune-stimulating and, thus, potentially therapeutic activity. Third, DC-targeted treatments and DC vaccines will most probably form part of combination cancer therapies, and the priority will therefore be to identify the most synergistic treatments and the optimal sequence of their administration. The potential of DC-based therapies is likely to lie in the treatment of patients with a low tumour burden and, importantly in this regard, DC-based vaccination is characterized by a low incidence and severity of adverse events, as evidenced by accumulating clinical data[13,207]. Moreover, a poorly explored question is the potential of DCs in the generation of protective immune memory. If so, DC-based immunotherapy could provide a promising strategy to treat patients with a high risk of cancer relapse or metastasis. Therefore, we feel that testing the potential of DC-based therapies to induce anticancer immune memory in patients who are in complete remission, for example, following primary surgery, but are at risk of relapse should be a research priority.

References

1. Hellmann, M. D. *et al.* Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer. *N Engl J Med* **381**, 2020–2031 (2019).
2. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* **20**, 651–668 (2020).
3. Cabeza-Cabrerizo, M., Cardoso, A., Minutti, C. M., Pereira Da Costa, M. & Reis E Sousa, C. Dendritic Cells Revisited. *Annual Review of Immunology* **39**, 131–166 (2021).
4. Segura, E. Human dendritic cell subsets: An updated view of their ontogeny and

functional specialization. *European journal of immunology* **52**, 1759–1767 (2022).
5. Marciscano, A. E. & Anandasabapathy, N. The role of dendritic cells in cancer and anti-tumor immunity. *Seminars in Immunology* **52**, 101481 (2021).
6. Wculek, S. K. *et al.* Dendritic cells in cancer immunology and immunotherapy. *Nat Rev Immunol* **20**, 7–24 (2020).
7. Bommareddy, P. K., Patel, A., Hossain, S. & Kaufman, H. L. Talimogene Laherparepvec (T-VEC) and Other Oncolytic Viruses for the Treatment of Melanoma. *Am J Clin Dermatol* **18**, 1–15 (2017).
8. Kamath, P., Darwin, E., Arora, H. & Nouri, K. A Review on Imiquimod Therapy and Discussion on Optimal Management of Basal Cell Carcinomas. *Clin Drug Investig* **38**, 883–899 (2018).
9. Guallar-Garrido, S. & Julián, E. Bacillus Calmette-Guérin (BCG) Therapy for Bladder Cancer: An Update. *ITT* **Volume 9**, 1–11 (2020).
10. Fasching, G., Dollinger, C., Spendel, S. & Tepeneu, N. F. Treatment of lymphangiomas by means of sclerotherapy with OK-432 (Picibanil®) is safe and effective - A retrospective case series. *Ann Med Surg (Lond)* **81**, 104531 (2022).
11. Wang, Y.-Q., Bazin-Lee, H., Evans, J. T., Casella, C. R. & Mitchell, T. C. MPL Adjuvant Contains Competitive Antagonists of Human TLR4. *Front Immunol* **11**, 577823 (2020).
12. Goyvaerts, C. & Breckpot, K. The Journey of in vivo Virus Engineered Dendritic Cells From Bench to Bedside: A Bumpy Road. *Front Immunol* **9**, 2052 (2018).
13. Kantoff, P. W. *et al.* Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. *New England Journal of Medicine* **363**, 411–422 (2010).
14. Nakamizo, S. *et al.* Single-cell analysis of human skin identifies CD14+ type 3 dendritic cells co-producing IL1B and IL23A in psoriasis. *The Journal of experimental medicine* **218**, (2021).
15. Villani, A. C. *et al.* Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* **356**, (2017).
16. Brown, C. C. *et al.* Transcriptional Basis of Mouse and Human Dendritic Cell Heterogeneity. *Cell* **179**, 846-863.e24 (2019).
17. Liu, Z. *et al.* Dendritic cell type 3 arises from Ly6C+ monocyte-dendritic cell progenitors. *Immunity* **56**, 1761-1777.e6 (2023).
18. Dutertre, C.-A. *et al.* Single-Cell Analysis of Human Mononuclear Phagocytes Reveals Subset-Defining Markers and Identifies Circulating Inflammatory Dendritic Cells. *Immunity* **51**, 573-589.e8 (2019).
19. Cytlak, U. *et al.* Differential IRF8 Transcription Factor Requirement Defines Two Pathways of Dendritic Cell Development in Humans. *Immunity* **53**, 353-370.e8 (2020).
20. Sulczewski, F. B. *et al.* Transitional dendritic cells are distinct from conventional DC2 precursors and mediate proinflammatory antiviral responses. *Nat Immunol* **24**, 1265–1280 (2023).
21. Böttcher, J. P. *et al.* NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. *Cell* **172**, 1022-1037.e14 (2018).
22. Barry, K. C. *et al.* A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments. *Nat Med* **24**, 1178–1191 (2018).
23. Broz, M. L. *et al.* Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity. *Cancer Cell* **26**, 638–652 (2014).
24. Hubert, M. *et al.* IFN-III is selectively produced by cDC1 and predicts good clinical outcome in breast cancer. *Science immunology* **5**, 1–16 (2020).
25. Roberts, E. W. *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* **30**, 324–336 (2016).
26. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
27. Sosa Cuevas, E. *et al.* BDCA1+ cDC2s, BDCA2+ pDCs and BDCA3+ cDC1s reveal distinct pathophysiologic features and impact on clinical outcomes in melanoma patients. *Clinical & translational immunology* **9**, e1190 (2020).
28. Mastelic-Gavillet, B. *et al.* Quantitative and qualitative impairments in dendritic cell

subsets of patients with ovarian or prostate cancer. *European journal of cancer (Oxford, England : 1990)* **135**, 173–182 (2020).
29. Michea, P. *et al.* Adjustment of dendritic cells to the breast-cancer microenvironment is subset specific. *Nature immunology* **19**, 885–897 (2018).
30. Iwanowycz, S. *et al.* Type 2 dendritic cells mediate control of cytotoxic T cell resistant tumors. *JCI insight* **6**, (2021).
31. Binnewies, M. *et al.* Unleashing Type-2 Dendritic Cells to Drive Protective Antitumor CD4+ T Cell Immunity. *Cell* **177**, 556-571.e16 (2019).
32. Tabarkiewicz, J., Rybojad, P., Jablonka, A. & Rolinski, J. CD1c+ and CD303+ dendritic cells in peripheral blood, lymph nodes and tumor tissue of patients with non-small cell lung cancer. *Oncology reports* **19**, 237–43 (2008).
33. He, M. *et al.* CD5 expression by dendritic cells directs T cell immunity and sustains immunotherapy responses. *Science* **379**, eabg2752 (2023).
34. Gentles, A. J. *et al.* The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med* **21**, 938–945 (2015).
35. Charoentong, P. *et al.* Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep* **18**, 248–262 (2017).
36. Li, B. *et al.* Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol* **17**, 174 (2016).
37. Becht, E. *et al.* Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. *Genome Biol* **17**, 218 (2016).
38. van Beek, J. J. P. *et al.* Human pDCs Are Superior to cDC2s in Attracting Cytolytic Lymphocytes in Melanoma Patients Receiving DC Vaccination. *Cell Rep* **30**, 1027-1038.e4 (2020).
39. Tel, J. *et al.* Natural Human Plasmacytoid Dendritic Cells Induce Antigen-Specific T-Cell Responses in Melanoma Patients. *Cancer Research* **73**, 1063–1075 (2013).
40. Kießler, M. *et al.* Tumor-infiltrating plasmacytoid dendritic cells are associated with survival in human colon cancer. *Journal for immunotherapy of cancer* **9**, 1–10 (2021).
41. Tian, S. *et al.* A Comprehensive Investigation to Reveal the Relationship Between Plasmacytoid Dendritic Cells and Breast Cancer by Multiomics Data Analysis. *Frontiers in cell and developmental biology* **9**, 640476 (2021).
42. Aspord, C., Leccia, M.-T., Charles, J. & Plumas, J. Plasmacytoid Dendritic Cells Support Melanoma Progression by Promoting Th2 and Regulatory Immunity through OX40L and ICOSL. *Cancer Immunology Research* **1**, 402–415 (2013).
43. Han, N. *et al.* Increased tumor-infiltrating plasmacytoid dendritic cells promote cancer cell proliferation and invasion via TNF-α/NF-κB/CXCR-4 pathway in oral squamous cell carcinoma. *Journal of Cancer* **12**, 3045–3056 (2021).
44. Banchereau, J. *et al.* The differential production of cytokines by human Langerhans cells and dermal CD14(+) DCs controls CTL priming. *Blood* **119**, 5742–9 (2012).
45. La Rocca, G. *et al.* CD1a down-regulation in primary invasive ductal breast carcinoma may predict regional lymph node invasion and patient outcome. *Histopathology* **52**, 203–12 (2008).
46. Bandola-Simon, J. & Roche, P. A. Dysfunction of antigen processing and presentation by dendritic cells in cancer. *Molecular Immunology* 0–1 (2018) doi:10.1016/j.molimm.2018.03.025.
47. Xiao, Z. *et al.* Impaired function of dendritic cells within the tumor microenvironment. *Front. Immunol.* **14**, 1213629 (2023).
48. Hegde, S. *et al.* Dendritic Cell Paucity Leads to Dysfunctional Immune Surveillance in Pancreatic Cancer. *Cancer cell* **37**, 289-307.e9 (2020).
49. Segura, E., Durand, M. & Amigorena, S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. *J Exp Med* **210**, 1035–1047 (2013).
50. Di Blasio, S. *et al.* Human CD1c(+) DCs are critical cellular mediators of immune responses induced by immunogenic cell death. *Oncoimmunology* **5**, e1192739 (2016).

51. Kuhn, S., Yang, J. & Ronchese, F. Monocyte-Derived Dendritic Cells Are Essential for CD8(+) T Cell Activation and Antitumor Responses After Local Immunotherapy. *Frontiers in immunology* **6**, 584 (2015).
52. Ma, Y. *et al.* Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* **38**, 729–41 (2013).
53. Murphy, T. L. & Murphy, K. M. Dendritic cells in cancer immunology. *Cellular & molecular immunology* **19**, 3–13 (2022).
54. Liu, T.-T. *et al.* Ablation of cDC2 development by triple mutations within the Zeb2 enhancer. *Nature* **607**, 142–148 (2022).
55. Subramanian, M. *et al.* An AXL/LRP-1/RANBP9 complex mediates DC efferocytosis and antigen cross-presentation in vivo. *The Journal of clinical investigation* **124**, 1296–308 (2014).
56. Nakayama, M. *et al.* Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation. *Blood* **113**, 3821–3830 (2009).
57. Caronni, N. *et al.* TIM4 expression by dendritic cells mediates uptake of tumor-associated antigens and anti-tumor responses. *Nature communications* **12**, 2237 (2021).
58. Wiernicki, B. *et al.* Cancer cells dying from ferroptosis impede dendritic cell-mediated anti-tumor immunity. *Nature communications* **13**, 3676 (2022).
59. Fucikova, J. *et al.* Detection of immunogenic cell death and its relevance for cancer therapy. *Cell death & disease* **11**, 1013 (2020).
60. Sancho, D. *et al.* Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. *Nature* **458**, 899–903 (2009).
61. Giampazolias, E. *et al.* Secreted gelsolin inhibits DNGR-1-dependent cross-presentation and cancer immunity. *Cell* **184**, 4016-4031.e22 (2021).
62. Drouin, M. *et al.* CLEC-1 is a death sensor that limits antigen cross-presentation by dendritic cells and represents a target for cancer immunotherapy. *Science advances* **8**, eabo7621 (2022).
63. Yamauchi, T. *et al.* CD40 and CD80/86 signaling in cDC1s mediate effective neoantigen vaccination and generation of antigen-specific CX3CR1+ CD8+ T cells. *Cancer immunology, immunotherapy : CII* **71**, 137–151 (2022).
64. Ghislat, G. *et al.* NF-κB-dependent IRF1 activation programs cDC1 dendritic cells to drive antitumor immunity. *Science immunology* **6**, (2021).
65. De Mingo Pulido, Á. *et al.* The inhibitory receptor TIM-3 limits activation of the cGAS-STING pathway in intra-tumoral dendritic cells by suppressing extracellular DNA uptake. *Immunity* **54**, 1154-1167.e7 (2021).
66. Lin, J. H. *et al.* Type 1 conventional dendritic cells are systemically dysregulated early in pancreatic carcinogenesis. *The Journal of experimental medicine* **217**, (2020).
67. Maier, B. *et al.* A conserved dendritic-cell regulatory program limits antitumour immunity. *Nature* **580**, 257–262 (2020).
68. Lin, J. H. *et al.* Type 1 conventional dendritic cells are systemically dysregulated early in pancreatic carcinogenesis. *Journal of Experimental Medicine* **217**, e20190673 (2020).
69. Wang, J. *et al.* Siglec Receptors Modulate Dendritic Cell Activation and Antigen Presentation to T Cells in Cancer. *Frontiers in cell and developmental biology* **10**, 828916 (2022).
70. Guenther, C. *et al.* β2-Integrin Adhesion Regulates Dendritic Cell Epigenetic and Transcriptional Landscapes to Restrict Dendritic Cell Maturation and Tumor Rejection. *Cancer immunology research* **9**, 1354–1369 (2021).
71. Wu, R. *et al.* Mechanisms of CD40-dependent cDC1 licensing beyond costimulation. *Nature immunology* **23**, 1536–1550 (2022).
72. Schenkel, J. M. *et al.* Conventional type I dendritic cells maintain a reservoir of proliferative tumor-antigen specific TCF-1+ CD8+ T cells in tumor-draining lymph nodes. *Immunity* **54**, 2338-2353.e6 (2021).
73. Ruhland, M. K. *et al.* Visualizing Synaptic Transfer of Tumor Antigens among Dendritic Cells. *Cancer cell* **37**, 786-799.e5 (2020).
74. Pirillo, C. *et al.* Cotransfer of antigen and contextual information harmonizes peripheral and lymph node conventional dendritic cell activation. *Sci. Immunol.* **8**, eadg8249 (2023).

75. Hildner, K. *et al.* *Batf3* Deficiency Reveals a Critical Role for CD8α $^{+}$ Dendritic Cells in Cytotoxic T Cell Immunity. *Science* **322**, 1097–1100 (2008).
76. Theisen, D. J. *et al.* WDFY4 is required for cross-presentation in response to viral and tumor antigens. *Science (New York, N.Y.)* **362**, 694–699 (2018).
77. MacNabb, B. W. *et al.* Dendritic cells can prime anti-tumor CD8+ T cell responses through major histocompatibility complex cross-dressing. *Immunity* **55**, 982-997.e8 (2022).
78. Das Mohapatra, A. *et al.* Cross-dressing of CD8α+ Dendritic Cells with Antigens from Live Mouse Tumor Cells Is a Major Mechanism of Cross-priming. *Cancer immunology research* **8**, 1287–1299 (2020).
79. Duong, E. *et al.* Type I interferon activates MHC class I-dressed CD11b+ conventional dendritic cells to promote protective anti-tumor CD8+ T cell immunity. *Immunity* **55**, 308-323.e9 (2022).
80. Wculek, S. K. *et al.* Effective cancer immunotherapy by natural mouse conventional type-1 dendritic cells bearing dead tumor antigen. **5**, 1–16 (2019).
81. Ferris, S. T. *et al.* cDC1 prime and are licensed by CD4+ T cells to induce anti-tumour immunity. *Nature* **584**, 624–629 (2020).
82. Cueto, F. J. *et al.* DNGR-1 limits Flt3L-mediated antitumor immunity by restraining tumor-infiltrating type I conventional dendritic cells. *Journal for ImmunoTherapy of Cancer* **9**, e002054 (2021).
83. Spranger, S., Dai, D., Horton, B. & Gajewski, T. F. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer cell* **31**, 711-723.e4 (2017).
84. Garris, C. S. *et al.* Successful Anti-PD-1 Cancer Immunotherapy Requires T Cell-Dendritic Cell Crosstalk Involving the Cytokines IFN-γ and IL-12. *Immunity* **49**, 1148-1161.e7 (2018).
85. Meiser, P. *et al.* A distinct stimulatory cDC1 subpopulation amplifies CD8+ T cell responses in tumors for protective anti-cancer immunity. *Cancer Cell* S1535610823002180 (2023) doi:10.1016/j.ccell.2023.06.008.
86. Guo, C. *et al.* SLC38A2 and glutamine signalling in cDC1s dictate anti-tumour immunity. *Nature* **620**, 200–208 (2023).
87. Dähling, S. *et al.* Type 1 conventional dendritic cells maintain and guide the differentiation of precursors of exhausted T cells in distinct cellular niches. *Immunity* **55**, 656-670.e8 (2022).
88. Bayerl, F. *et al.* Tumor-derived prostaglandin E2 programs cDC1 dysfunction to impair intratumoral orchestration of anti-cancer T cell responses. *Immunity* **56**, 1341-1358.e11 (2023).
89. Moreno Ayala, M. A. *et al.* CXCR3 expression in regulatory T cells drives interactions with type I dendritic cells in tumors to restrict CD8+ T cell antitumor immunity. *Immunity* **56**, 1613-1630.e5 (2023).
90. Ruiz De Galarreta, M. *et al.* β-Catenin Activation Promotes Immune Escape and Resistance to Anti–PD-1 Therapy in Hepatocellular Carcinoma. *Cancer Discovery* **9**, 1124–1141 (2019).
91. Kim, S. *et al.* IL-6 selectively suppresses cDC1 specification via C/EBPβ. *Journal of Experimental Medicine* **220**, e20221757 (2023).
92. Cheng, S. *et al.* A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell* **184**, 792-809.e23 (2021).
93. Gerhard, G. M., Bill, R., Messemaker, M., Klein, A. M. & Pittet, M. J. Tumor-infiltrating dendritic cell states are conserved across solid human cancers. *Journal of Experimental Medicine* **218**, (2021).
94. Leader, A. M. *et al.* Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. *Cancer Cell* **39**, 1594-1609.e12 (2021).
95. Li, J. *et al.* Mature dendritic cells enriched in immunoregulatory molecules (mregDCs): A novel population in the tumour microenvironment and immunotherapy target. *Clinical & Translational Med* **13**, (2023).
96. Zhang, Q. *et al.* Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma. *Cell* **179**, 829-845.e20 (2019).

97. Pittet, M. J., Di Pilato, M., Garris, C. & Mempel, T. R. Dendritic cells as shepherds of T cell immunity in cancer. *Immunity* **56**, 2218–2230 (2023).
98. Zilionis, R. *et al.* Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species. *Immunity* **50**, 1317-1334.e10 (2019).
99. Ardouin, L. *et al.* Broad and Largely Concordant Molecular Changes Characterize Tolerogenic and Immunogenic Dendritic Cell Maturation in Thymus and Periphery. *Immunity* **45**, 305–318 (2016).
100. Liu, Y. *et al.* Tumour heterogeneity and intercellular networks of nasopharyngeal carcinoma at single cell resolution. *Nat Commun* **12**, 741 (2021).
101. Qian, J. *et al.* A pan-cancer blueprint of the heterogeneous tumor microenvironment revealed by single-cell profiling. *Cell Res* **30**, 745–762 (2020).
102. Zhang, L. *et al.* Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. *Cell* **181**, 442-459.e29 (2020).
103. Kim, N. *et al.* Single-cell RNA sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nat Commun* **11**, 2285 (2020).
104. Minohara, K. *et al.* Mature dendritic cells enriched in regulatory molecules may control regulatory T cells and the prognosis of head and neck cancer. *Cancer Science* **114**, 1256–1269 (2023).
105. Cohen, M. *et al.* The interaction of CD4+ helper T cells with dendritic cells shapes the tumor microenvironment and immune checkpoint blockade response. *Nature cancer* **3**, 303–317 (2022).
106. Ruffell, B. *et al.* Macrophage IL-10 Blocks CD8+ T Cell-Dependent Responses to Chemotherapy by Suppressing IL-12 Expression in Intratumoral Dendritic Cells. *Cancer Cell* **26**, 623–637 (2014).
107. Baldominos, P. *et al.* Quiescent cancer cells resist T cell attack by forming an immunosuppressive niche. *Cell* **185**, 1694-1708.e19 (2022).
108. Ladányi, 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 Immunol Immunother* **56**, 1459–1469 (2007).
109. Dieu-Nosjean, M.-C. *et al.* Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol* **26**, 4410–4417 (2008).
110. Bassez, A. *et al.* A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. *Nat Med* **27**, 820–832 (2021).
111. Lee, Y. S. *et al.* Human CD141+ dendritic cells (cDC1) are impaired in patients with advanced melanoma but can be targeted to enhance anti-PD-1 in a humanized mouse model. *Journal for immunotherapy of cancer* **9**, 1–12 (2021).
112. Schreibelt, G. *et al.* Effective Clinical Responses in Metastatic Melanoma Patients after Vaccination with Primary Myeloid Dendritic Cells. *Clinical Cancer Research* **22**, 2155–2166 (2016).
113. Schetters, S. T. T. *et al.* Monocyte-derived APCs are central to the response of PD1 checkpoint blockade and provide a therapeutic target for combination therapy. *Journal for immunotherapy of cancer* **8**, 1–16 (2020).
114. Krieg, C. *et al.* High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nature medicine* **24**, 144–153 (2018).
115. Surówka, J. *et al.* Influence of ovarian cancer type I and type II microenvironment on the phenotype and function of monocyte-derived dendritic cells. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* **19**, 1489–1497 (2017).
116. Spary, L. K. *et al.* Tumor stroma-derived factors skew monocyte to dendritic cell differentiation toward a suppressive CD14+ PD-L1+ phenotype in prostate cancer. *Oncoimmunology* **3**, e955331 (2014).
117. Hopkins, D., Sanchez, H., Berwin, B. & Wilkinson-Ryan, I. Cisplatin increases immune activity of monocytes and cytotoxic T-cells in a murine model of epithelial ovarian cancer. *Translational oncology* **14**, 101217 (2021).

118. Adhikaree, J. *et al.* Impaired circulating myeloid CD1c+ dendritic cell function in human glioblastoma is restored by p38 inhibition - implications for the next generation of DC vaccines. *Oncoimmunology* **8**, 1593803 (2019).
119. Garzon-Muvdi, T. *et al.* Dendritic cell activation enhances anti-PD-1 mediated immunotherapy against glioblastoma. *Oncotarget* **9**, 20681–20697 (2018).
120. Guo, J. *et al.* The miR-582/CD1B Axis Is Involved in Regulation of Dendritic Cells and Is Associated with Clinical Outcomes in Advanced Lung Adenocarcinoma. *BioMed research international* **2020**, 4360930 (2020).
121. Ramos, R. N. *et al.* Monocyte-derived dendritic cells from breast cancer patients are biased to induce CD4+CD25+Foxp3+ regulatory T cells. *Journal of leukocyte biology* **92**, 673–82 (2012).
122. Liu, W. *et al.* Gastric cancer patients have elevated plasmacytoid and CD1c+ dendritic cells in the peripheral blood. *Oncology letters* **15**, 5087–5092 (2018).
123. Bai, X. *et al.* Loss of YTHDF1 in gastric tumors restores sensitivity to antitumor immunity by recruiting mature dendritic cells. *Journal for immunotherapy of cancer* **10**, (2022).
124. Abolhalaj, M. *et al.* Profiling dendritic cell subsets in head and neck squamous cell tonsillar cancer and benign tonsils. *Scientific reports* **8**, 8030 (2018).
125. Tumeh, P. C. *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
126. Murillo, O. *et al.* In vivo depletion of DC impairs the anti-tumor effect of agonistic anti-CD137 mAb. *Eur. J. Immunol.* **39**, 2424–2436 (2009).
127. Gubin, M. M. *et al.* Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515**, 577–581 (2014).
128. Salmon, H. *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* **44**, 924–938 (2016).
129. Sánchez-Paulete, A. R. *et al.* Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy. *Annals of Oncology* **28**, xii44–xii55 (2017).
130. Peng, Q. *et al.* PD-L1 on dendritic cells attenuates T cell activation and regulates response to immune checkpoint blockade. *Nat Commun* **11**, 4835 (2020).
131. Liu, J. *et al.* Batf3 $^{+}$ DCs and type I IFN are critical for the efficacy of neoadjuvant cancer immunotherapy. *OncoImmunology* **8**, e1546068 (2019).
132. Teijeira, A. *et al.* Depletion of Conventional Type-1 Dendritic Cells in Established Tumors Suppresses Immunotherapy Efficacy. *Cancer Research* **82**, 4373–4385 (2022).
133. Chow, M. T. *et al.* Intratumoral Activity of the CXCR3 Chemokine System Is Required for the Efficacy of Anti-PD-1 Therapy. *Immunity* **50**, 1498-1512.e5 (2019).
134. Wu, S.-Y. *et al.* CCL19+ dendritic cells potentiate clinical benefit of anti-PD-(L)1 immunotherapy in triple-negative breast cancer. *Med* **4**, 373-393.e8 (2023).
135. Paz-Ares, L. *et al.* Pembrolizumab plus Chemotherapy for Squamous Non–Small-Cell Lung Cancer. *N Engl J Med* **379**, 2040–2051 (2018).
136. Tang, H. *et al.* PD-L1 on host cells is essential for PD-L1 blockade–mediated tumor regression. *Journal of Clinical Investigation* **128**, 580–588 (2018).
137. Powles, T. *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* **515**, 558–562 (2014).
138. Oh, S. A. *et al.* PD-L1 expression by dendritic cells is a key regulator of T-cell immunity in cancer. *Nat Cancer* **1**, 681–691 (2020).
139. Mayoux, M. *et al.* Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Sci. Transl. Med.* **12**, eaav7431 (2020).
140. Kamphorst, A. O. *et al.* Rescue of exhausted CD8 T cells by PD-1–targeted therapies is CD28-dependent. *Science* **355**, 1423–1427 (2017).
141. Oba, T. *et al.* Overcoming primary and acquired resistance to anti-PD-L1 therapy by induction and activation of tumor-residing cDC1s. *Nat Commun* **11**, 5415 (2020).
142. Pauken, K. E. *et al.* Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* **354**, 1160–1165 (2016).
143. Siddiqui, I. *et al.* Intratumoral Tcf1+PD-1+CD8+ T Cells with Stem-like Properties

Promote Tumor Control in Response to Vaccination and Checkpoint Blockade Immunotherapy. *Immunity* **50**, 195-211.e10 (2019).
144. Yost, K. E. *et al.* Clonal replacement of tumor-specific T cells following PD-1 blockade. *Nat Med* **25**, 1251–1259 (2019).
145. Dammeijer, F. *et al.* The PD-1/PD-L1-Checkpoint Restrains T cell Immunity in Tumor-Draining Lymph Nodes. *Cancer Cell* **38**, 685-700.e8 (2020).
146. Chen, L. *et al.* B7-H1 maintains the polyclonal T cell response by protecting dendritic cells from cytotoxic T lymphocyte destruction. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 3126–3131 (2018).
147. Sugiura, D. *et al.* Restriction of PD-1 function by *cis* -PD-L1/CD80 interactions is required for optimal T cell responses. *Science* **364**, 558–566 (2019).
148. Zhao, Y. *et al.* PD-L1:CD80 Cis-Heterodimer Triggers the Co-stimulatory Receptor CD28 While Repressing the Inhibitory PD-1 and CTLA-4 Pathways. *Immunity* **51**, 1059-1073.e9 (2019).
149. Duraiswamy, J., Kaluza, K. M., Freeman, G. J. & Coukos, G. Dual Blockade of PD-1 and CTLA-4 Combined with Tumor Vaccine Effectively Restores T-Cell Rejection Function in Tumors. *Cancer Research* **73**, 3591–3603 (2013).
150. Lei, X. *et al.* CD4+ helper T cells endow cDC1 with cancer-impeding functions in the human tumor micro-environment. *Nat Commun* **14**, 217 (2023).
151. Hammerl, D. *et al.* Spatial immunophenotypes predict response to anti-PD1 treatment and capture distinct paths of T cell evasion in triple negative breast cancer. *Nat Commun* **12**, 5668 (2021).
152. Wolf, Y., Anderson, A. C. & Kuchroo, V. K. TIM3 comes of age as an inhibitory receptor. *Nat Rev Immunol* **20**, 173–185 (2020).
153. Chiba, S. *et al.* Tumor-infiltrating DCs suppress nucleic acid–mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. *Nat Immunol* **13**, 832–842 (2012).
154. De Mingo Pulido, Á. *et al.* TIM-3 Regulates CD103+ Dendritic Cell Function and Response to Chemotherapy in Breast Cancer. *Cancer Cell* **33**, 60-74.e6 (2018).
155. Dixon, K. O. *et al.* TIM-3 restrains anti-tumour immunity by regulating inflammasome activation. *Nature* **595**, 101–106 (2021).
156. Gardner, A. *et al.* TIM-3 blockade enhances IL-12-dependent antitumor immunity by promoting CD8 $^{+}$ T cell and XCR1 $^{+}$ dendritic cell spatial co-localization. *J Immunother Cancer* **10**, e003571 (2022).
157. Huang, Y.-H. *et al.* CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. *Nature* **517**, 386–390 (2015).
158. Sabatos-Peyton, C. A. *et al.* Blockade of Tim-3 binding to phosphatidylserine and CEACAM1 is a shared feature of anti-Tim-3 antibodies that have functional efficacy. *OncoImmunology* **7**, e1385690 (2018).
159. Melero, I., Castanon, E., Alvarez, M., Champiat, S. & Marabelle, A. Intratumoural administration and tumour tissue targeting of cancer immunotherapies. *Nat Rev Clin Oncol* **18**, 558–576 (2021).
160. Rolfo, C., Giovannetti, E., Martinez, P., McCue, S. & Naing, A. Applications and clinical trial landscape using Toll-like receptor agonists to reduce the toll of cancer. *NPJ Precis Oncol* **7**, 26 (2023).
161. Cauwels, A. *et al.* Delivering Type I Interferon to Dendritic Cells Empowers Tumor Eradication and Immune Combination Treatments. *Cancer Research* **78**, 463–474 (2018).
162. Alvarez, M. *et al.* Intratumoral neoadjuvant immunotherapy based on the BO-112 viral RNA mimetic. *OncoImmunology* **12**, 2197370 (2023).
163. Cueto, F. J. & Sancho, D. The Flt3L/Flt3 Axis in Dendritic Cell Biology and Cancer Immunotherapy. *Cancers (Basel)* **13**, 1525 (2021).
164. Rodriguez-Ruiz, M. E. *et al.* Intratumoral BO-112 in combination with radiotherapy synergizes to achieve CD8 T-cell-mediated local tumor control. *J Immunother Cancer* **11**, e005011 (2023).
165. Hänel, G., Angerer, C., Petry, K., Lichtenegger, F. S. & Subklewe, M. Blood DCs

activated with R848 and poly(I:C) induce antigen-specific immune responses against viral and tumor-associated antigens. *Cancer Immunol Immunother* **71**, 1705–1718 (2022).
166. Bhatia, S. *et al.* Intratumoral G100, a TLR4 Agonist, Induces Antitumor Immune Responses and Tumor Regression in Patients with Merkel Cell Carcinoma. *Clin Cancer Res* **25**, 1185–1195 (2019).
167. Cohen, E. E. W. *et al.* Intralesional SD-101 in Combination with Pembrolizumab in Anti-PD-1 Treatment-Naïve Head and Neck Squamous Cell Carcinoma: Results from a Multicenter, Phase II Trial. *Clin Cancer Res* **28**, 1157–1166 (2022).
168. Ribas, A. *et al.* Overcoming PD-1 Blockade Resistance with CpG-A Toll-Like Receptor 9 Agonist Vidutolimod in Patients with Metastatic Melanoma. *Cancer Discov* **11**, 2998–3007 (2021).
169. Doshi, A. S., Cantin, S., Prickett, L. B., Mele, D. A. & Amiji, M. Systemic nano-delivery of low-dose STING agonist targeted to CD103+ dendritic cells for cancer immunotherapy. *Journal of Controlled Release* **345**, 721–733 (2022).
170. Byrne, K. T. & Vonderheide, R. H. CD40 Stimulation Obviates Innate Sensors and Drives T Cell Immunity in Cancer. *Cell Reports* **15**, 2719–2732 (2016).
171. Salomon, R. *et al.* Bispecific antibodies increase the therapeutic window of CD40 agonists through selective dendritic cell targeting. *Nature Cancer* **3**, 287–302 (2022).
172. Deronic, A. *et al.* The human anti-CD40 agonist antibody mitazalimab (ADC-1013; JNJ-64457107) activates antigen-presenting cells, improves expansion of antigen-specific T cells, and enhances anti-tumor efficacy of a model cancer vaccine in vivo. *Cancer Immunology, Immunotherapy* **70**, 3629–3642 (2021).
173. Ngiow, S. F. *et al.* Agonistic CD40 mAb-Driven IL12 Reverses Resistance to Anti-PD1 in a T-cell–Rich Tumor. *Cancer Research* **76**, 6266–6277 (2016).
174. O'Hara, M. H. *et al.* CD40 agonistic monoclonal antibody APX005M (sotigalimab) and chemotherapy, with or without nivolumab, for the treatment of metastatic pancreatic adenocarcinoma: an open-label, multicentre, phase 1b study. *The Lancet Oncology* **22**, 118–131 (2021).
175. Weiss, S. A. *et al.* A Phase I Study of APX005M and Cabiralizumab with or without Nivolumab in Patients with Melanoma, Kidney Cancer, or Non-Small Cell Lung Cancer Resistant to Anti-PD-1/PD-L1. *Clin Cancer Res* **27**, 4757–4767 (2021).
176. Coveler, A. L. *et al.* Phase 1 dose-escalation study of SEA-CD40: a non-fucosylated CD40 agonist, in advanced solid tumors and lymphomas. *J Immunother Cancer* **11**, e005584 (2023).
177. Hägerbrand, K. *et al.* Bispecific antibodies targeting CD40 and tumor-associated antigens promote cross-priming of T cells resulting in an antitumor response superior to monospecific antibodies. *Journal for ImmunoTherapy of Cancer* **10**, 1–12 (2022).
178. Liu, W., Takahashi, Y., Morishita, M., Nishikawa, M. & Takakura, Y. Development of CD40L-modified tumor small extracellular vesicles for effective induction of antitumor immune response. *Nanomedicine* **15**, 1641–1652 (2020).
179. Kranz, L. M. *et al.* Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature* **534**, 396–401 (2016).
180. Pandya, A. *et al.* The future of cancer immunotherapy: DNA vaccines leading the way. *Med Oncol* **40**, 200 (2023).
181. Wang, Y. *et al.* mRNA vaccine: a potential therapeutic strategy. *Mol Cancer* **20**, 33 (2021).
182. Beck, J. D. *et al.* mRNA therapeutics in cancer immunotherapy. *Mol Cancer* **20**, 69 (2021).
183. Sancho, D. *et al.* Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *J. Clin. Invest.* **118**, 2098–2110 (2008).
184. Huang, J. *et al.* Targeted Co-delivery of Tumor Antigen and α-Galactosylceramide to CD141+ Dendritic Cells Induces a Potent Tumor Antigen-Specific Human CD8+ T Cell Response in Human Immune System Mice. *Front. Immunol.* **11**, 2043 (2020).
185. Cruz, L. J. *et al.* Targeted PLGA nano- but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN in vitro. *Journal of Controlled Release* **144**, 118–126

(2010).
186. Sahin, U. *et al.* An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* **585**, 107–112 (2020).
187. Rojas, L. A. *et al.* Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature* **618**, 144–150 (2023).
188. Yang, X. *et al.* Synthetic multiepitope neoantigen DNA vaccine for personalized cancer immunotherapy. *Nanomedicine: Nanotechnology, Biology, and Medicine* **37**, 102443 (2021).
189. Moku, G., Vangala, S., Gu, S. K. & Yakati, V. In vivo Targeting of DNA Vaccines to Dendritic Cells via the Mannose Receptor Induces Long-Lasting Immunity against Melanoma. *ChemBioChem* **22**, 523–531 (2021).
190. Liu, L. *et al.* Rejuvenation of tumour-specific T cells through bispecific antibodies targeting PD-L1 on dendritic cells. *Nat Biomed Eng* **5**, 1261–1273 (2021).
191. Jiang, H. *et al.* PD-L1/LAG-3 bispecific antibody enhances tumor-specific immunity. *OncoImmunology* **10**, 1943180 (2021).
192. Hsu, F. J. *et al.* Vaccination of patients with B–cell lymphoma using autologous antigen–pulsed dendritic cells. *Nature Medicine* **2**, 52–58 (1996).
193. Lin, M. J. *et al.* Cancer vaccines: the next immunotherapy frontier. *Nature Cancer* **3**, 911–926 (2022).
194. Laureano, R. S. *et al.* Trial watch: Dendritic cell (DC)-based immunotherapy for cancer. *OncoImmunology* **11**, 2096363 (2022).
195. Anguille, S., Smits, E. L., Lion, E., Van Tendeloo, V. F. & Berneman, Z. N. Clinical use of dendritic cells for cancer therapy. *The Lancet Oncology* **15**, e257–e267 (2014).
196. de Vries, I. J. M. *et al.* Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. *Clin Cancer Res* **9**, 5091–5100 (2003).
197. Lövgren, T. *et al.* Complete and long-lasting clinical responses in immune checkpoint inhibitor-resistant, metastasized melanoma treated with adoptive T cell transfer combined with DC vaccination. *OncoImmunology* **9**, 1792058 (2020).
198. Schreibelt, G. *et al.* Toll-like receptor expression and function in human dendritic cell subsets: implications for dendritic cell-based anti-cancer immunotherapy. *Cancer Immunol Immunother* **59**, 1573–1582 (2010).
199. Bol, K. F. *et al.* Prophylactic vaccines are potent activators of monocyte-derived dendritic cells and drive effective anti-tumor responses in melanoma patients at the cost of toxicity. *Cancer Immunol Immunother* **65**, 327–339 (2016).
200. Lövgren, T. *et al.* Enhanced stimulation of human tumor-specific T cells by dendritic cells matured in the presence of interferon-γ and multiple toll-like receptor agonists. *Cancer Immunol Immunother* **66**, 1333–1344 (2017).
201. Bonehill, A. *et al.* Single-Step Antigen Loading and Activation of Dendritic Cells by mRNA Electroporation for the Purpose of Therapeutic Vaccination in Melanoma Patients. *Clinical Cancer Research* **15**, 3366–3375 (2009).
202. Figlin, R. A. *et al.* Results of the ADAPT Phase 3 Study of Rocapuldencel-T in Combination with Sunitinib as First-Line Therapy in Patients with Metastatic Renal Cell Carcinoma. *Clinical Cancer Research* **26**, 2327–2336 (2020).
203. Currivan, E., Finlay, D. & Moreira, D. Dendritic cells metabolism: a strategic path to improve antitumoral DC vaccination. *Clinical and Experimental Immunology* **208**, 193–201 (2022).
204. Aarntzen, E. H. J. G. *et al.* Targeting CD4+ T-helper cells improves the induction of antitumor responses in dendritic cell -based vaccination. *Cancer Research* **73**, 19–29 (2013).
205. Chang, C.-N. *et al.* A phase I/II clinical trial investigating the adverse and therapeutic effects of a postoperative autologous dendritic cell tumor vaccine in patients with malignant glioma. *Journal of Clinical Neuroscience* **18**, 1048–1054 (2011).
206. Bota, D. A. *et al.* Phase 2 study of AV-GBM-1 (a tumor-initiating cell targeted dendritic cell vaccine) in newly diagnosed Glioblastoma patients: safety and efficacy assessment. *J Exp Clin Cancer Res* **41**, 344 (2022).
207. Liau, L. M. *et al.* Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent

Glioblastoma: A Phase 3 Prospective Externally Controlled Cohort Trial. *JAMA Oncol* **9**, 112 (2023).
208. Liau, L. M. *et al.* First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J Transl Med* **16**, 142 (2018).
209. Gatto, L. *et al.* DCVax-L Vaccination in Patients with Glioblastoma: Real Promise or Negative Trial? The Debate Is Open. *Cancers* **15**, 3251 (2023).
210. Jackson, C. M., Choi, J. & Lim, M. Mechanisms of immunotherapy resistance: lessons from glioblastoma. *Nat Immunol* **20**, 1100–1109 (2019).
211. Ashour, D. *et al.* IL-12 from endogenous cDC1, and not vaccine DC, is required for Th1 induction. *JCI Insight* **5**, e135143 (2020).
212. Kleindienst, P. & Brocker, T. Endogenous Dendritic Cells Are Required for Amplification of T Cell Responses Induced by Dendritic Cell Vaccines In Vivo. *The Journal of Immunology* **170**, 2817–2823 (2003).
213. Yewdall, A. W., Drutman, S. B., Jinwala, F., Bahjat, K. S. & Bhardwaj, N. CD8+ T Cell Priming by Dendritic Cell Vaccines Requires Antigen Transfer to Endogenous Antigen Presenting Cells. *PLoS ONE* **5**, e11144 (2010).
214. Ferris, S. T. *et al.* cDC1 Vaccines Drive Tumor Rejection by Direct Presentation Independently of Host cDC1. *Cancer Immunology Research* **10**, 920–931 (2022).
215. Freeman, J. L., Vari, F. & Hart, D. N. J. CMRF-56 Immunoselected Blood Dendritic Cell Preparations Activated With GM-CSF Induce Potent Antimyeloma Cytotoxic T-cell Responses. *Journal of Immunotherapy* **30**, 740–748 (2007).
216. Osugi, Y., Vuckovic, S. & Hart, D. N. J. Myeloid blood CD11c+ dendritic cells and monocyte-derived dendritic cells differ in their ability to stimulate T lymphocytes. *Blood* **100**, 2858–2866 (2002).
217. Zhou, Y. *et al.* Vaccine efficacy against primary and metastatic cancer with in vitro-generated CD103 + conventional dendritic cells. *Journal for ImmunoTherapy of Cancer* **8**, 1–13 (2020).
218. Bol, K. F. *et al.* The clinical application of cancer immunotherapy based on naturally circulating dendritic cells. *j. immunotherapy cancer* **7**, 109 (2019).
219. Wimmers, F., Schreibelt, G., Sköld, Annette E., A. E., Figdor, C. G. & De Vries, I. J. M. Paradigm Shift in Dendritic Cell-Based Immunotherapy: From in vitro Generated Monocyte-Derived DCs to Naturally Circulating DC Subsets. *Front. Immunol.* **5**, (2014).
220. Bloemendal, M. *et al.* Immunological responses to adjuvant vaccination with combined CD1c $^{+}$ myeloid and plasmacytoid dendritic cells in stage III melanoma patients. *OncoImmunology* **11**, 2015113 (2022).
221. Westdorp, H. *et al.* Blood-derived dendritic cell vaccinations induce immune responses that correlate with clinical outcome in patients with chemo-naive castration-resistant prostate cancer. *j. immunotherapy cancer* **7**, 302 (2019).
222. Bol, K. *et al.* 1078MO MIND-DC: A randomized phase III trial to assess the efficacy of adjuvant dendritic cell vaccination in comparison to placebo in stage IIIB and IIIC melanoma patients. *Annals of Oncology* **31**, S732 (2020).
223. Johnson, P., Rosendahl, N. & Radford, K. J. Conventional type 1 dendritic cells (cDC1) as cancer therapeutics: challenges and opportunities. *Expert Opinion on Biological Therapy* **22**, 465–472 (2022).
224. Schwarze, J. K. *et al.* Intratumoral administration of CD1c (BDCA-1) $^{+}$ and CD141 (BDCA-3) $^{+}$ myeloid dendritic cells in combination with talimogene laherparepvec in immune checkpoint blockade refractory advanced melanoma patients: a phase I clinical trial. *J Immunother Cancer* **10**, e005141 (2022).
225. Van Willigen, W. W. *et al.* Dendritic Cell Cancer Therapy: Vaccinating the Right Patient at the Right Time. *Front. Immunol.* **9**, 2265 (2018).
226. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
227. Chang, H. *et al.* Co-delivery of dendritic cell vaccine and ANTI-PD -1 antibody with cryomicroneedles for combinational immunotherapy. *Bioengineering & Transla Med* e10457 (2022) doi:10.1002/btm2.10457.

228. Stroopinsky, D. *et al.* Leukemia vaccine overcomes limitations of checkpoint blockade by evoking clonal T cell responses in a murine acute myeloid leukemia model. *haematol* **106**, 1330–1342 (2021).
229. Zhu, S. *et al.* An effective dendritic cell-based vaccine containing glioma stem-like cell lysate and CpG adjuvant for an orthotopic mouse model of glioma. *International Journal of Cancer* **144**, 2867–2879 (2019).
230. Esmaily, M. *et al.* Blockade of CTLA-4 increases anti-tumor response inducing potential of dendritic cell vaccine. *Journal of Controlled Release* **326**, 63–74 (2020).
231. Barshidi, A. *et al.* Dual Blockade of PD-1 and LAG3 Immune Checkpoints Increases Dendritic Cell Vaccine Mediated T Cell Responses in Breast Cancer Model. *Pharm Res* **39**, 1851–1866 (2022).
232. Van Willigen, W. W. *et al.* Response and survival of metastatic melanoma patients treated with immune checkpoint inhibition for recurrent disease on adjuvant dendritic cell vaccination. *OncoImmunology* **9**, 1738814 (2020).
233. Guo, Z. *et al.* Durable complete response to neoantigen-loaded dendritic-cell vaccine following anti-PD-1 therapy in metastatic gastric cancer. *npj Precis. Onc.* **6**, 34 (2022).
234. Schwarze, J. K. *et al.* Intratumoral Combinatorial Administration of CD1c (BDCA-1)+ Myeloid Dendritic Cells Plus Ipilimumab and Avelumab in Combination with Intravenous Low-Dose Nivolumab in Patients with Advanced Solid Tumors: A Phase IB Clinical Trial. *Vaccines* **8**, 670 (2020).
235. De Keersmaecker, B. *et al.* TriMix and tumor antigen mRNA electroporated dendritic cell vaccination plus ipilimumab: link between T-cell activation and clinical responses in advanced melanoma. *J Immunother Cancer* **8**, e000329 (2020).
236. Fucikova, J. *et al.* An Autologous Dendritic Cell Vaccine Promotes Anticancer Immunity in Patients with Ovarian Cancer with Low Mutational Burden and Cold Tumors. *Clinical Cancer Research* **28**, 3053–3065 (2022).
237. Zhang, W. *et al.* Phase I/II clinical trial of a Wilms' tumor 1-targeted dendritic cell vaccination-based immunotherapy in patients with advanced cancer. *Cancer Immunol Immunother* **68**, 121–130 (2019).
238. Sioud, M. *et al.* Diversification of Antitumour Immunity in a Patient with Metastatic Melanoma Treated with Ipilimumab and an IDO-Silenced Dendritic Cell Vaccine. *Case Reports in Medicine* **2016**, 1–7 (2016).
239. Sioud, M. *et al.* Silencing of indoleamine 2,3-dioxygenase enhances dendritic cell immunogenicity and antitumour immunity in cancer patients. *International Journal of Oncology* **43**, 280–288 (2013).
240. Matsushita, H. *et al.* A pilot study of autologous tumor lysate-loaded dendritic cell vaccination combined with sunitinib for metastatic renal cell carcinoma. *j. immunotherapy cancer* **2**, 30 (2014).
241. Amin, A. *et al.* Survival with AGS-003, an autologous dendritic cell–based immunotherapy, in combination with sunitinib in unfavorable risk patients with advanced renal cell carcinoma (RCC): Phase 2 study results. *j. immunotherapy cancer* **3**, 14 (2015).
242. Tanyi, J. L. *et al.* Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. *Sci. Transl. Med.* **10**, eaao5931 (2018).
243. Widén, K., Mozaffari, F., Choudhury, A. & Mellstedt, H. Overcoming immunosuppressive mechanisms. *Annals of Oncology* **19**, vii241–vii247 (2008).
244. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **168**, 707–723 (2017).
245. Finn, O. J. The dawn of vaccines for cancer prevention. *Nature Reviews Immunology* (2017).
246. Jansen, Y. *et al.* A randomized controlled phase II clinical trial on mRNA electroporated autologous monocyte-derived dendritic cells (TriMixDC-MEL) as adjuvant treatment for stage III/IV melanoma patients who are disease-free following the resection of macrometastases. *Cancer Immunol Immunother* **69**, 2589–2598 (2020).
247. Matsui, H. M. *et al.* Novel adjuvant dendritic cell therapy with transfection of heat-shock protein 70 messenger RNA for patients with hepatocellular carcinoma: a phase I/II prospective

randomized controlled clinical trial. *Cancer Immunol Immunother* **70**, 945–957 (2021).
248. Tryggestad, A. M. A. *et al.* Long-term first-in-man Phase I/II study of an adjuvant dendritic cell vaccine in patients with high-risk prostate cancer after radical prostatectomy. *The Prostate* **82**, 245–253 (2022).
249. Rodriguez, J. *et al.* A randomized phase II clinical trial of dendritic cell vaccination following complete resection of colon cancer liver metastasis. *j. immunotherapy cancer* **6**, 96 (2018).
250. Boudewijns, S. *et al.* Adjuvant dendritic cell vaccination induces tumor-specific immune responses in the majority of stage III melanoma patients. *OncoImmunology* **5**, e1191732 (2016).
251. Wheeler, C. J., Das, A., Liu, G., Yu, J. S. & Black, K. L. Clinical Responsiveness of Glioblastoma Multiforme to Chemotherapy after Vaccination. *Clinical Cancer Research* **10**, 5316–5326 (2004).
252. Mittendorf, E. A., Burgers, F., Haanen, J. & Cascone, T. Neoadjuvant Immunotherapy: Leveraging the Immune System to Treat Early-Stage Disease. *American Society of Clinical Oncology Educational Book* 189–203 (2022).
253. Truxova, I. *et al.* Rationale for the Combination of Dendritic Cell-Based Vaccination Approaches With Chemotherapy Agents. in *International Review of Cell and Molecular Biology* vol. 330 115–156 (Elsevier, 2017).
254. Rodríguez-Ruiz, M. E. *et al.* Combined immunotherapy encompassing intratumoral poly-ICLC, dendritic-cell vaccination and radiotherapy in advanced cancer patients. *Annals of Oncology* **29**, 1312–1319 (2018).
255. Galluzzi, L., Humeau, J., Buqué, A., Zitvogel, L. & Kroemer, G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. *Nat Rev Clin Oncol* **17**, 725–741 (2020).
256. Rob, L. *et al.* Safety and efficacy of dendritic cell-based immunotherapy DCVAC/OvCa added to first-line chemotherapy (carboplatin plus paclitaxel) for epithelial ovarian cancer: a phase 2, open-label, multicenter, randomized trial. *J Immunother Cancer* **10**, e003190 (2022).
257. Eisendle, K. *et al.* Combining chemotherapy and autologous peptide-pulsed dendritic cells provides survival benefit in stage IV melanoma patients. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* **18**, 1270–1277 (2020).
258. He, D. *et al.* Single-cell RNA sequencing reveals heterogeneous tumor and immune cell populations in early-stage lung adenocarcinomas harboring EGFR mutations. *Oncogene* **40**, 355–368 (2021).
259. Wu, S. Z. *et al.* A single-cell and spatially resolved atlas of human breast cancers. *Nature Genetics* **53**, 1334–1347 (2021).
260. Bassez, A. *et al.* A single-cell map of intratumoral changes during anti-PD1 treatment of patients with breast cancer. *Nat Med* **27**, 820–832 (2021).
261. Cillo, A. R. *et al.* Immune Landscape of Viral- and Carcinogen-Driven Head and Neck Cancer. *Immunity* **52**, 183-199.e9 (2020).
262. Ji, A. L. *et al.* Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma. *Cell* **182**, 497-514.e22 (2020).
263. Higano, C. S. *et al.* Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. *Cancer* **115**, 3670–3679 (2009).
264. Small, E. J. *et al.* Placebo-Controlled Phase III Trial of Immunologic Therapy with Sipuleucel-T (APC8015) in Patients with Metastatic, Asymptomatic Hormone Refractory Prostate Cancer. *JCO* **24**, 3089–3094 (2006).
265. Beer, T. M. *et al.* Randomized Trial of Autologous Cellular Immunotherapy with Sipuleucel-T in Androgen-Dependent Prostate Cancer. *Clinical Cancer Research* **17**, 4558–4567 (2011).
266. Beer, T. M. *et al.* Quality of Life After Sipuleucel-T Therapy: Results From a Randomized, Double-blind Study in Patients With Androgen-dependent Prostate Cancer. *Urology* **82**, 410–415 (2013).
267. Kantoff, P. W. *et al.* Sipuleucel-T Immunotherapy for Castration-Resistant Prostate Cancer. *N Engl J Med* **363**, 411–422 (2010).

268. Javed, A., Sato, S. & Sato, T. Autologous melanoma cell vaccine using monocyte-derived dendritic cells (NBS20/eltrapuldencel-T). *Future Oncology* **12**, 751–762 (2016).
269. Vogelzang, N. J. *et al.* Efficacy and Safety of Autologous Dendritic Cell–Based Immunotherapy, Docetaxel, and Prednisone vs Placebo in Patients With Metastatic Castration-Resistant Prostate Cancer: The VIABLE Phase 3 Randomized Clinical Trial. *JAMA Oncol* **8**, 546 (2022).
270. Podrazil, M. *et al.* Phase I/II clinical trial of dendritic-cell based immunotherapy (DCVAC/PCa) combined with chemotherapy in patients with metastatic, castration-resistant prostate cancer. *Oncotarget* **6**, 18192–18205 (2015).
271. Schuler-Thurner, B. *et al.* Immuntherapie beim Aderhautmelanom: Vakzination gegen Krebs: Multizentrische adjuvante Phase-III-Impfstudie mit Tumor-RNA-beladenen dendritischen Zellen bei neu diagnostizierten, großen Uveamelanomen. *Ophthalmologe* **112**, 1017–1021 (2015).
272. Nutt, S. L. & Chopin, M. Transcriptional Networks Driving Dendritic Cell Differentiation and Function. *Immunity* **52**, 942–956 (2020).
273. Cheng, S. *et al.* A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell* **184**, 792-809.e23 (2021).
274. Im, Y. & Kim, Y. A Comprehensive Overview of RNA Deconvolution Methods and Their Application. *Mol Cells* **46**, 99–105 (2023).
275. Cancel, J.-C., Crozat, K., Dalod, M. & Mattiuz, R. Are Conventional Type 1 Dendritic Cells Critical for Protective Antitumor Immunity and How? *Front Immunol* **10**, 9 (2019).
276. Cheema, A. S., Duan, K., Dalod, M. & Vu Manh, T.-P. Harnessing Single-Cell RNA Sequencing to Identify Dendritic Cell Types, Characterize Their Biological States, and Infer Their Activation Trajectory. *Methods Mol Biol* **2618**, 319–373 (2023).
277. Bayerl, F. *et al.* Guidelines for visualization and analysis of DC in tissues using multiparameter fluorescence microscopy imaging methods. *Eur J Immunol* e2249923 (2023) doi:10.1002/eji.202249923.

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Author contributions

I.H.-M., S.K.W. and D.S. conceptualized the Review as well as the display items. All authors researched data for the article, contributed substantially to discussion of the content, wrote the article, and reviewed and/or edited the manuscript before submission.

Competing interests

I.H.M. serves as a scientific advisor for Pulmobiotics. S.K.W. serves as a scientific advisor for ONA therapeutics. D.S. serves as a scientific advisor for Pulmobiotics and has research

collaboration agreements with Inmunotek and Adendra Therapeutics. The other authors declare no competing interests.

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Table 1 │ Intratumoural DC heterogeneity revealed by scRNA-seq and CITE-seq studies involving patients with 16 different cancer types

Cancer type	DC lineages					DC states			
	cDC1	cDC2	tDC	pDC	MoDC-cDC2 (DC3)[a]	CCR7^{+} DCs	ISG^{+} cDC2	CXCL9^{+} cDC2	LC-like DC
Lung cancer	hDC1[98] CD141^{+} DCs[103,258] DC1[67] cDC1[92,94,101]	hDC2[98] CD1c^{+} DCs[103,258] DC2[67] cDC2[92,94,101]	—	pDC[92,94,98,101,103]	CD163^{+}CD14^{+} DCs[103] DC3[94] MoDC[98] cDC2_FCN1[92]	hDC3[98] mregDC[67,94] migDC[101] LAMP3^{+} DCs[92] Activated DCs[103]	cDC2_ISG15[92]	cDC2_CXCL9[92]	CD207^{+}CD1a^{+} LCs[103] cDC2_CD1A[92] LHC-like[94]
Breast cancer	cDC1:CLEC9A[259] cDC1[92,134,260]	cDC2:CD1C[259] cDC2[92,134,260]	ASDCs[260]	pDC[92,134]	cDC2_FCN1/IL1B[92]	DC:LAMP3[259] migDC[260] LAMP3^{+}[92] CCL19^{+}[134]	cDC2_ISG15[92]	cDC2_CXCL9[92]	LanghDC[260] Langerhans-like[101] LC-like[134] cDC2_CD1A[92]
HCC	cDC1[92,96]	cDC2[92,96]	—	—	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92,96]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
CRC	cDC1[92,101,102]	cDC2[92,101,102]	—	pDC[92,101,102]	cDC2_FCN1/IL1B[92]	migDC[101] LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	Langerhans-like[101] cDC2_CD1A[92]
NPC	cDC1[92]	cDC2[92,100]	—	DC_JCHAIN[100] pDC[92]	Monocyte-like DCs[100] cDC2_IL1B[92]	LAMP3^{+} DCs[92,100]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
Ovarian or fallopian tube carcinoma	cDC1[92,101]	cDC2[92,101]	—	pDC[92,101]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	Langerhans-like[101] cDC2_CD1A[92]
UCEC	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
PDAC	cDC1[92]	cDC2[92]	—		cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
RCC	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
Melanoma	cDC1[33,92]	CD1c^{+} cDC2[16] cDC2[92] CD5/cDC2	Pre-DCs[33]09/01/2024 16:20:00	pDC[92]	cDC2_FCN1[92]	LAMP3^{+} DCs[33,92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2-mLCs[33] cDC2_CD1A[92]
Thyroid carcinoma	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
Oesophageal cancer	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
Gastric cancer	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
Multiple myeloma	cDC1[92]	cDC2[92]	—	pDC[92]	cDC2_FCN1/IL1B[92]	LAMP3^{+} DCs[92]	cDC2_ISG15[92]	cDC2_CXCL9[92]	cDC2_CD1A[92]
HNSCC	cDC1[104]	cDC2[104] DCs[261]	—	—	—	IDO^{+} DCs[261] mregDCs[104]	—	—	—
CSCC	CLEC9A DCs[262]	CD1c DCs[262]	ASDCs[262]	pDCs[262]	—	migDCs[262]	—	—	LCs[262]

DC clusters identified in each of the cancer types are divided into subsets according to their ontogenic lineage (left) and functional state (right), based on expression of the following genes that were used for their annotation in the referenced single-cell transcriptomics studies: cDC1: *CLEC9A, XCR1, CD141, WDFY4* and *IRF8*; cDC2: *CD1C, CLEC10A, CLEC12A* and *FCER1A*; DC3

(MoDC-cDC2): *CD163, CD14, S100A8, FCGR3A* and *HLA-DQA1*; tDC: *AXL* and *SIGLEC6*; pDC: *LILRA4, GZMB, IL3RA* and *CXCR3*; CCR7^{+} DCs: *LAMP3, CCR7, CD274* and *FSCN1*; ISG^{+} DC2: *CD1C, ISG15, IFI6* and *IFIT1*; CXCL9^{+} DC2: *CD1C* and *CXCL9*; LC-like DC: *CD1C, CD1A* and *CD207*. ASDC, AXL^{+}SIGLEC6^{+} DC; cDC1, conventional type 1 dendritic cells; cDC2, conventional type 2 dendritic cells; CITE-seq, cellular indexing of transcriptomes and epitopes sequencing; CLEC9A, C-type lectin domain family 9 member A; CRC, colorectal carcinoma; CSCC, cutaneous squamous cell carcinoma; CXCL9, C-X-C motif chemokine 9; DC, dendritic cell; DC3, dendritic cells type 3; FCN1, ficolin-1; HCC, hepatocellular carcinoma; hDC, human dendritic cell; HNSCC, head and neck squamous cell carcinoma; IDO, indoleamine 2,3-dioxygenase; IL1B, IL-1β; ISG, interferon-stimulated gene; JCHAIN, immunoglobulin J chain; LAMP3, lysosome-associated membrane glycoprotein 3; LC, Langerhans cell; migDC, migratory dendritic cell; MoDC, monocyte-derived dendritic cells; NPC, nasopharyngeal cancer; PDAC, pancreatic ductal adenocarcinoma; pDC, plasmacytoid dendritic cells; RCC, renal cell carcinoma; scRNA-seq, single-cell RNA sequencing; tDC, tolerogenic dendritic cells; UCEC, uterine corpus endometrial carcinoma. [a]DC clusters annotated as ‘MoDC-cDC2 (DC3)’ probably constitute a continuum of cDC2, DC3 and MoDCs that is challenging to annotate owing to its complexity.

Table 2 | Representative completed or ongoing phase III trials of cancer treatments involving DC vaccination

Tumour type	DC product	Comparison	Trial status (ClinicalTrial.gov identifier)	Results	Ref.
Prostate cancer (metastatic, castration-resistant)	Sipuleucel T: autologous PBMCs (including DCs) pulsed with PAP/GM-CSF (PA2024)	Sipuleucel T vs PBMCs not pulsed with PA2024	Completed, 2003 (NCT01133704)	No significant differences in the treatment group compared to the control group (mOS 19.0 vs 15.7 months (HR 1.27, 95% CI 0.78–2.07; $P = 0.33$)[a]	263
Prostate cancer (metastatic, castration-resistant)	Sipuleucel T	Sipuleucel T vs PBMCs not pulsed with PA2024	Completed, 2004 (NCT00005947)	Improved mOS (25.9 vs 21.4 months with Sipuleucel T vs placebo (HR 1.71, 95% CI 1.13–2.58; $P = 0.01$)[a]	263,264
Prostate cancer (nonmetastatic, biochemically recurrent)	Sipuleucel-T	Sipuleucel T vs autologous PBMCs not pulsed with PA2024	Completed, 2006 (NCT00779402)	No significant differences in the treatment group compared to control group (primary end point of median time to biochemical failure was 18.0 vs 15.4 months (HR 0.936; $P = 0.737$)	265,266
Prostate cancer (metastatic, castration-resistant)	Sipuleucel T	Sipuleucel T vs autologous PBMCs not pulsed with PA2024	Completed, 2009 (NCT00065442)	Improved mOS (25.8 vs 21.7 months with sipuleucel T vs placebo; HR 0.78; 95% CI 0.62–0.98; $P = 0.03$))	267
Melanoma (advanced-stage; patients with more than one distant metastasis must have previously received ≥1 SOC therapy)	Eltrapuldencel-T: autologous MoDCs pulsed with irradiated autologous tumour cell line from patient's melanoma, plus GM-CSF	Eltrapuldencel-T vs autologous PBMCs and GM-CSF	Completed, 2016 (NCT01875653)	NA (Insufficient enrollment)	268
Renal cell carcinoma (advanced-stage)	Rocapuldencel-T: autologous MoDCs coelectroporated with mRNA amplified from autologous tumour, and CD40L-encoding mRNA	First-line sunitinib (SOC) with vs without rocapuldencel-T	Completed, 2017 (NCT01582672)	No significant differences in the treatment group compared to the control group (mOS 27.7 vs 32.4 months; HR 1.10, 95% CI 0.83–1.40)	202
Colorectal cancer (advanced-stage)	APDC: autologous MoDCs pulsed with autologous tumour lysate	First-line mFOLFOX6 chemotherapy with vs without APDC	Completed, 2020 (NCT02503150)	NA	NA
Prostate cancer (metastatic, castration-resistant)	DCVAC/PCa: autologous MoDCs pulsed with autologous UV-irradiated prostate tumour cell line (LNCaP) and matured with poly I:C	DCVAC/PCa vs placebo, both in combination with SOC and as maintenance therapy after SOC (docetaxel plus prednisone)	Completed, 2020 (NCT02111577)	No significant differences in the treatment group compared to the control group (mOS 23.9 vs 24.3 months HR 1.04, 95% CI 0.90–1.21; $P = 0.60$)	269,270
Ovarian, fallopian tube or peritoneal carcinoma (relapsed, platinum-sensitive)	DCVAC/OvCa: autologous MoDCs pulsed with lysed allogenic ovarian cancer cell lines and matured with poly I:C	DCVAC/OvCa vs placebo, both during platinum-based chemotherapy (with or without bevacizumab) and maintenance treatment with bevacizumab, a PARP inhibitor or BSC only	Withdrawn, 2021 (NCT03905902)	NA	NA
Glioblastoma multiforme(newly diagnosed or recurrent)	DCVax-L: Autologous MoDCs pulsed with surgically resected and enzymatically digested autologous tumour	Newly diagnosed disease: SOC surgery and RT/TMZ→TMZ plus either DCVax-L or placebo Recurrent disease: DCVax-L plus TMZ vs SOC therapies[b]	Completed, 2022 (NCT00045968)	Newly diagnosed disease: improved mOS (19.3 vs 16.5 months with DCVax-L vs control patients (external control population, ECP); HR 0.80, 98% CI 0.00–0.94; $P = 0.002$) Recurrent disease: improve mOS (13.2 vs 7.8 months with DCVax-L vs ECP (HR 0.58, 98% CI 0.00–0.76; $P < 0.001$)	207,208

Glioblastoma multiforme (recurrent)	ADCTA: autologous MoDCs pulsed with autologous tumour cell lysate	Bevacizumab with vs without ADCTA	Ongoing, unknown (NCT04277221)	NA	NA
Mesothelioma (newly diagnosed)	MesoPher: autologous MoDCs pulsed with allogenic tumour cell lysate (PheraLys)	MesoPher plus BSC vs BSC as maintenance treatment after chemotherapy	Ongoing, unknown (NCT03610360)	NA	NA
Glioblastoma (*MGMT*-promoter methylated, newly diagnosed)	Autologous MoDCs pulsed with mRNA from autologous glioma stem cells and TAAs (survivin and TERT)	Surgery and RT/TMZ→TMZ with vs without the DC vaccine	Active, not recruiting (NCT03548571)	NA	NA
Prostate cancer (low-risk, nonmetastatic)	Sipuleucel-T	Sipuleucel-T vs active surveillance	Active, not recruiting (NCT03686683)	NA	NA
Uveal melanoma (positive for monosomy 3 and non-metastatic)	Autologous MoDCs pulsed with autologous tumour mRNA	DC vaccine vs observation in adjuvant setting	Active, not recruiting (NCT01983748)	NA	[271]
Melanoma (stage IIIB and IIIC)	Autologous nDCs (cDC2s and pDCs and pulsed with TAA peptides	DC vaccine vs placebo in the adjuvant setting	Active, not recruiting (NCT02993315)	An interim analysis performed in 2020 showed no difference between the two-year RFS rate in treatment arm (21.4%) vs control arm (25%), HR 1.05, 95% CI 0.47-3.23); *P*=0.67.	[222]
Glioblastoma (newly diagnosed)	ICT-107: autologous MoDCs pulsed with six TAA peptides	Surgery and RT/TMZ→TMZ plus either ICT-107 vs control (autologous monocyte-enriched PBMCs)	Suspended, pending discussions with FDA to reinitiate the trial (NCT02546102)	NA	NA
Glioblastoma (newly diagnosed)	AV-GBM-1: autologous MoDCs pulsed with autologous tumour cell lines, plus GM-CSF	Surgery and RT/TMZ→TMZ plus either AV-GBM-1 or autologous monocytes and GM-CSF	Not yet recruiting (NCT05100641)	NA	NA

Some trials were excluded owing to limited available information, including NCT03905902, NCT04292769, NCT00006434, NCT01782274, NCT01782287 and NCT01759810. BSC, best supportive care; cDC2, conventional type 2 dendritic cell; DC, dendritic cell; MoDC, monocyte-derived dendritic cell; mOS, median overall survival; NA, not available; nDC, natural dendritic cell; PAP/GM-CSF, chimeric antigen consisting of the prostate tumour antigen prostatic acid phosphatase (PAP) linked to granulocyte–macrophage colony-stimulating factor (GM-CSF); PARP, poly (ADP-ribose) polymerase; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cells; poly I:C, polyinosinic–polycytidylic acid; RT/TMZ→TMZ, temozolomide-based chemoradiotherapy followed by maintenance temozolomide; SOC, standard of care; TAA, tumour-associated antigen. [a]Hazard ratio indicates the risk in patients treated with placebo divided by the risk in patients treated with sipuleucel-T, with a hazard ratio >1 indicating a greater risk with placebo vs sipuleucel-T. [b]In this trial, the study had a crossover design (patients were allowed to switch to the other arm upon disease progression). As a result, the placebo group was depleted and the control groups consisted of contemporaneous, matched external control patients treated with SOC therapies in other formal randomized trials.

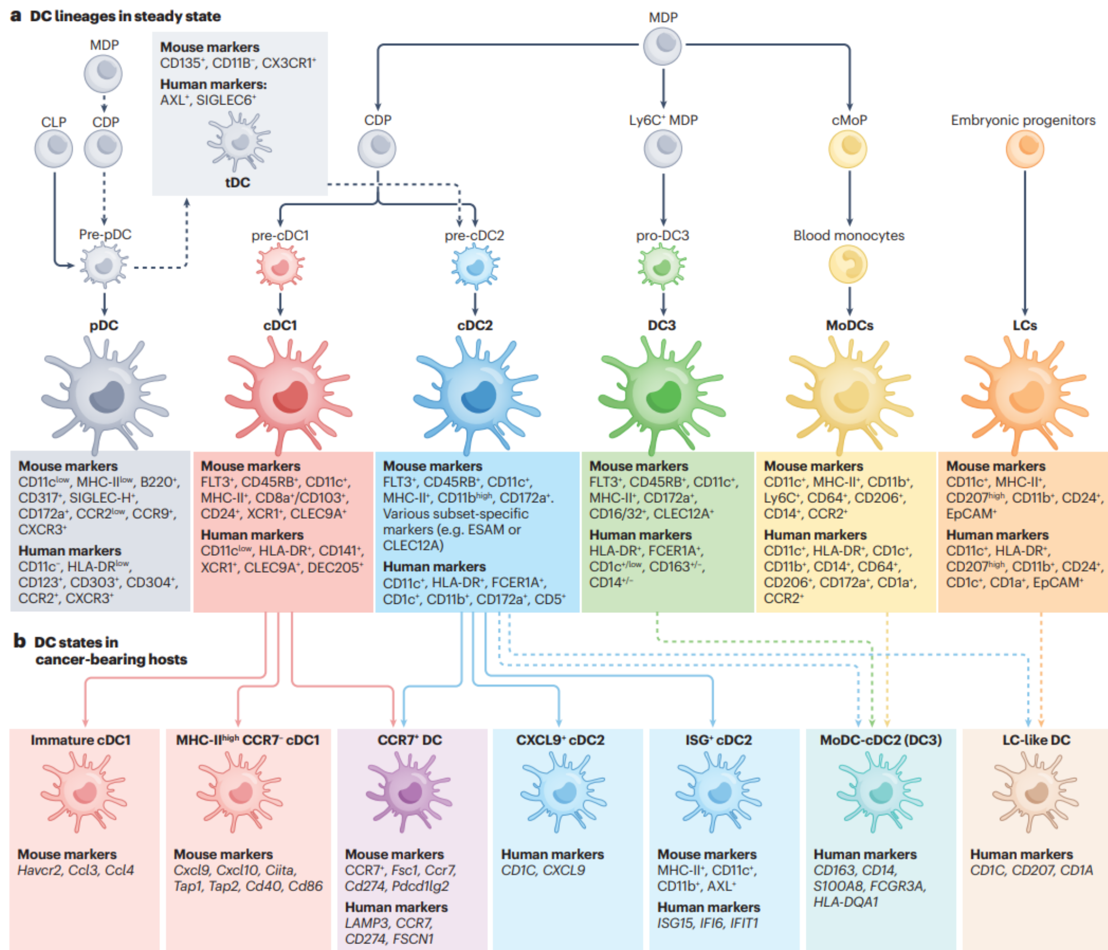

Fig. 1 | Dendritic cell heterogeneity. a, Normal physiological dendritic cell (DC) types, their cellular origins and the specific cell-surface markers used for their identification in mice and humans are indicated. Plasmacytoid dendritic cells (pDCs) derive from common lymphoid progenitors (CLPs), or otherwise differentiate from common DC progenitors (CDPs) — an origin shared with type 1 and type 2 conventional DCs (cDC1s and cDC2, respectively)[272]. Transitional DCs (tDCs) also share the latter origin with pDCs, have a phenotype spanning a pDC–cDC2 continuum and, in mice, can differentiate to ESAM$^+$ cDC2[20]. cDC1s and cDC2s develop from pre-cDC1 and pre-cDC2, respectively, which differentiate from CDPs before leaving the bone marrow. Type 3 DCs (DC3s) differentiate from pro-DC3s that, like CDPs, derive from monocyte-DC progenitors (MDPs). Mouse DC3s seem equivalent to the 'inflammatory' or 'monocyte-like' DC2 population identified in human blood[15]. However, classical blood monocyte-derived DCs (MoDCs), which also originate from MDPs but via common monocyte progenitors (cMoPs), become prevalent under inflammatory conditions[272]. Langerhans cells (LCs) reside in the epidermis and are self-maintaining macrophages but have traditionally been included in the DC family owing to functional similarity. b, DC heterogeneity expands within tumours, where DCs from different ontogenic lineages adopt distinct transcriptional profiles that are associated with specialized functional states. Mature DCs enriched in activation and immunoregulatory molecules (CCR7$^+$ DCs) are ubiquitously found in many human cancer types (see TABLE 1), and most studies suggest that this functional state can be adopted by both cDC1s and cDC2s. Immature cDC1s, which lack the expression of genes associated with DC activation, and MHC-II^{high} CCR7$^-$ cDC1s that are characterized by high *Cxcl9* expression and a prominent immunostimulatory function have been identified in mouse and human tumours[85]. cDC2s can also adopt particular functional states in the tumour microenvironment, such as those characterized by high expression of interferon-inducible genes (ISG$^+$ cDC2s) or *CXCL9* (CXCL9$^+$ DCs, which have been proposed to be CCR7$^+$ cDC2 precursors)[273]. DCs that co-express cDC2-associated, DC3-associated and

MoDC-associated genes are often present in human tumours; however, whether this 'MoDC-cDC2 (DC3)' state reflects transcriptional convergence of multiple DCs subsets requires further research[93]. Finally, a DC population sharing cDC2 and LC markers is commonly found in human tumours, but the origin and lineage of these LC-like DCs remains unclear.

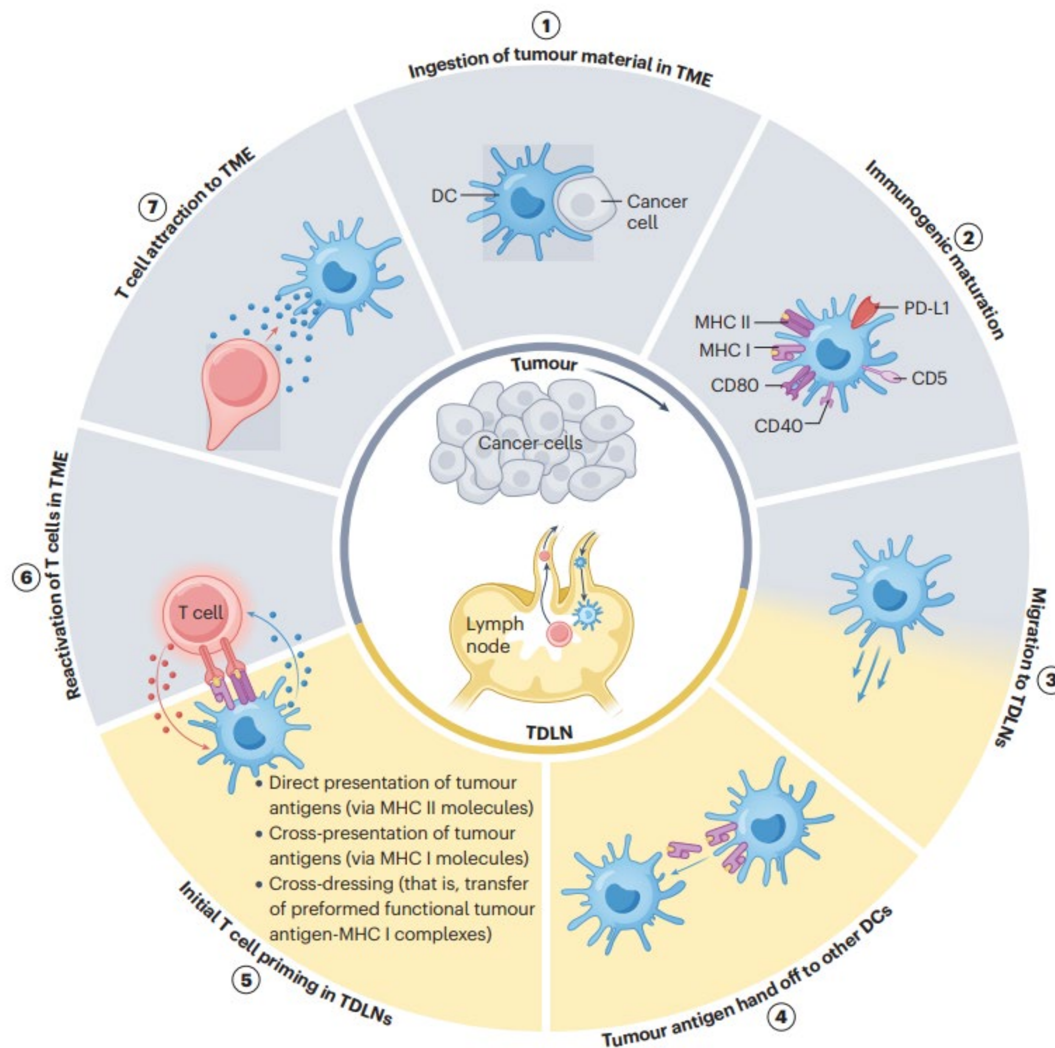

Fig. 2 | Hallmark functions of dendritic cells that induce antitumour T cell responses. Dendritic cells (DCs) in the tumour microenvironment (TME) can take up tumour antigens (**1**), sense danger signals, process the tumour antigens for presentation, undergo immunogenic activation (**2**), and migrate to the tumour-draining lymph nodes (TDLNs) (**3**). In the TDLNs, the migrated DCs transmit tumour antigens and maturation cues to resident DCs (**4**) and/or present tumour antigens together with co-stimulatory and soluble cytokine signals to naive T cells themselves for T cell priming (**5**). DCs in the TME can also produce factors that attract T cells (**6**), as well as fostering immunopermissive niches and re-stimulating tumour-reactive T cells (**7**). $CD4^+$ T cells are primed or re-activated by DCs via direct presentation of tumour antigens on MHC class II (MHC II) molecules, and DCs stimulate $CD8^+$ T cells via cross-presentation of exogenous tumour antigens on MHC I molecules or via cross-dressing with preformed antigen–MHC I complexes acquired directly from cancer cells. The primed T cells cooperate to kill cancer cells. Many of the anticancer functions of DCs are often impaired or suppressed in patients with cancer.

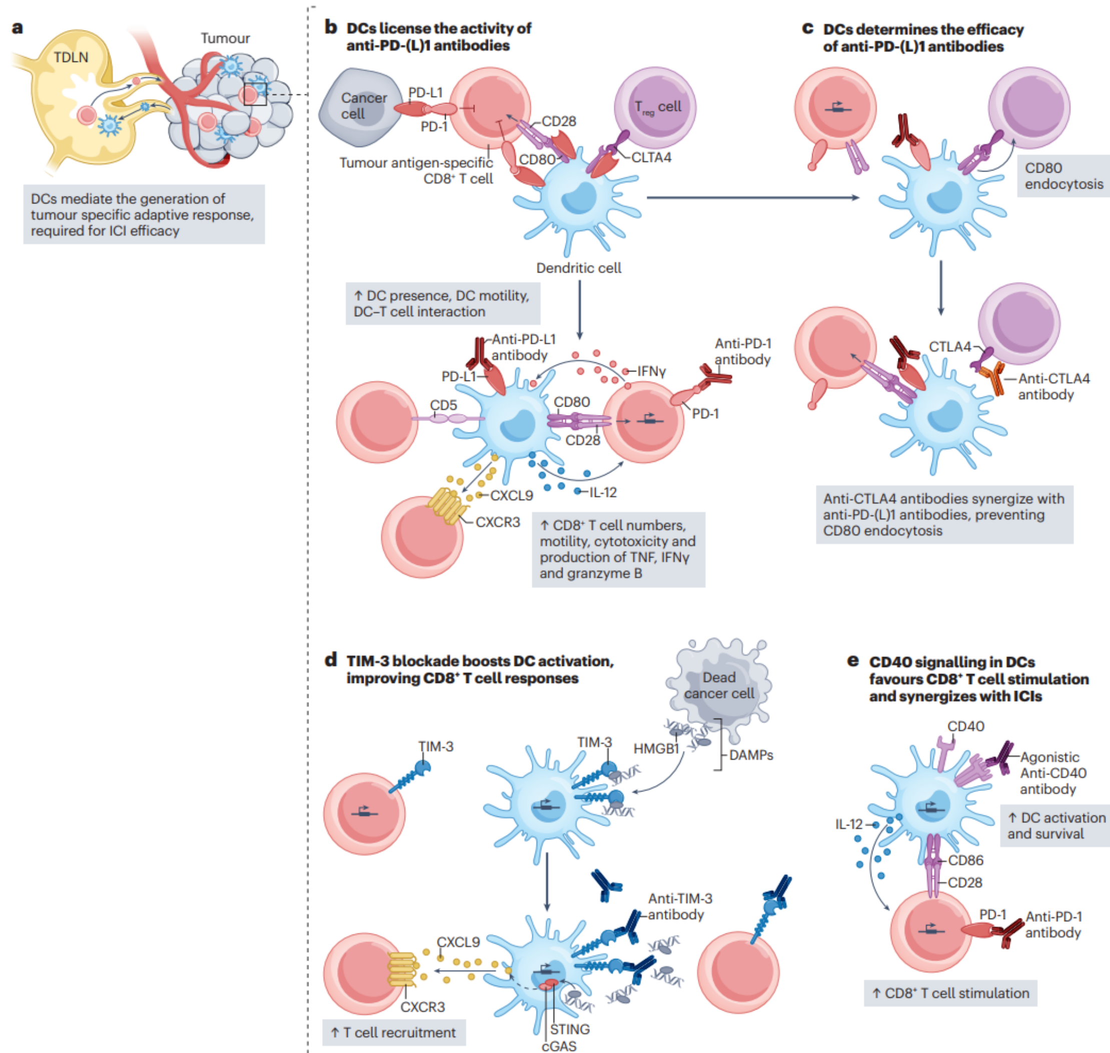

Fig. 3 | The importance of dendritic cells for the efficacy of immune-checkpoint inhibitors. Dendritic cells (DCs) have an essential role in the anticancer activity of immune-checkpoint inhibitors (ICIs) such as anti-PD-(L)1 and anti-TIM3 antibodies via the generation of tumour-specific T cell responses (**1**), which are required for the therapeutic efficacy of these agents. Furthermore, ICIs can reciprocally affect the antitumour function of DCs through multiple direct or indirect mechanisms (**2–5**). Anti-PD-(L)1 antibodies relieve $CD8^+$ T cell inhibition mediated by PD-L1–PD-1 signalling, but their efficacy is licensed by DC–T cell crosstalk (**2**). For example, IL-12, CXCL9 and/or CD5 expression by DCs enhances the anticancer activity of T cells and is crucial for the efficacy of ICIs in mouse models. Although anti-PD-L1 antibodies alleviate the inhibition of T cells by PD-L1 expressed on DC in the TME as well as in tumour-draining lymph nodes (TDLNs), these antibodies also disrupt the cis interaction between PD-L1 and CD80 on DCs[148] (**2, top**). Disruption of this interaction favours the formation of CD80 homodimers, which can be removed from DCs through CTLA4-mediated trogocytosis and transendocytosis by regulatory T (T_{reg} cells), thereby limiting interactions between CD80 homodimers on DCs and the co-stimulatory receptor CD28 on $CD8^+$ T cells (**3, top**). This effect can be suppressed by combining anti-PD-L1 antibodies with anti-CTLA4 antibodies, thus enhancing $CD8^+$ T cell (re-)activation and therapeutic efficacy[148] (**3, bottom**). TIM-3 on DCs can sequester danger-associated molecular patterns (DAMPs) such as HMGB1–DNA complexes released from dying

cancer cells (**4, top**). Thus, anti-TIM-3 antibodies facilitate DAMP sensing via the cGAS-STING pathway in DCs and thereby induce DAMP-mediated activation of an anticancer T cell response[65] (**4, bottom**). The anticancer effects of agonistic anti-CD40 antibodies, which synergize with ICIs, depend on DCs[171], with CD40-induced signalling promoting DC activation, survival and T cell-priming[71] (**5**).

Key points

- Dendritic cells (DCs) can induce adaptive anticancer immunity, mainly via uptake and presentation of tumour-associated antigens, migration to lymph nodes and T cell priming, and recruitment and activation of tumour-infiltrating T cells.
- Intratumoural DCs originate from distinct ontogenic lineages and adopt heterogeneous functional states.
- The diversity of intratumoural DCs is generally conserved across species and cancer types; however, cancer-type-specific features of DCs in the blood, lymph nodes and tumours of patients are emerging.
- The efficacy of many immune-checkpoint inhibitors in patients with cancer is dependent on anticancer functions of DCs, either directly or indirectly.
- The development of improved DC-based anticancer treatments, including agents targeting DCs in vivo and natural DC-based vaccines produced ex vivo, has the potential to improve patient outcomes, and such treatments are generally safe.
- Combining DC-based anticancer treatments with other (immuno)therapies has shown promise in preclinical studies; however, patient selection, treatment sequencing and immunomonitoring require optimization in clinical studies to identify the most synergistic combinations.

Box 1 │ Key technologies for the study of intratumoural DCs — advantages and unmet needs

Deconvolution of whole-tumour transcriptomic data

Computational deconvolution technologies enable the estimation of dendritic cell (DC) abundance in numerous bulk RNA datasets to establish correlations with other features of cancers and patient prognosis[21,22,24,34]. However, unified guidelines are needed to increase the robustness of this approach, including efforts aiming to 1) establish consensus signatures for DC lineages and activation states, for example, DC cluster-specific genes identified through whole-tumour single-cell RNA sequencing (scRNA-seq); 2) explore the efficacy of probabilistic models compared with gene-enrichment approaches[274]; and 3) corroborate in silico correlations through complementary experimental analyses[275].

scRNA-seq combined with CiTE-seq

scRNA-seq and cellular indexing of transcriptomes and epitopes sequencing (CITE-seq) enable the study of gene and/or cell-surface protein expression at single-cell resolution and can provide unparalleled insights into DC diversity in cancer. A community effort is needed to streamline scRNA-seq and CITE-seq analyses between studies. This efforts should include the development of robust strategies for a common annotation of DC clusters that overarches studies, for example, through cross-labelling of clusters versus reference gene signatures or signatures of previously described scRNA-seq clusters[276]. Notably, caution is needed when interpreting computational modelling of (activation) trajectories of DC clusters and experimental demonstration of in silico-generated hypotheses is recommended.

Flow cytometry and CyTOF

Flow cytometry and cytometry by time of flight (CyTOF) are among the most common tools used to analyse intratumoural DCs and rely on specific molecular tags labelled with fluorophores or metal ions, respectively, to detect the expression of particular target proteins on individual cells. Therefore, these technologies do not permit unbiased screening, but instead require the availability and previous knowledge of specific cell-surface proteins to identify DC populations of interest, which is increasingly challenging as DC diversity expands. For example, we recommend avoiding the use of CD14 to distinguish monocytes from DCs, as a subset of human type 3 DCs (DC3s) also expresses CD14 (Ref.[18]).

Microscopy and spatial transcriptomics

These technologies maintain the spatial organization of DCs in tumour tissues and preclude artefacts and/or misrepresentations related to tissue digestion[85]. Furthermore, intravital microscopy enables DC behaviour to be investigated in vivo, in real time[277]. Thus, these approaches might provide novel insights into intratumoural DC biology, but can be limited in terms of resolution and/or the availability antibody-based or other labelling tools.