Dendritic cells in cancer immunology and immunotherapy

*Stefanie K. Wculek[1], Francisco J. Cueto[1], Adriana M. Mujal[2,7], Ignacio Melero[3,4,5,6], Matthew F. Krummel[2] and David Sancho[1]**

Abstract | Dendritic cells (DCs) are a diverse group of specialized antigen-presenting cells with key roles in the initiation and regulation of innate and adaptive immune responses. As such, there is currently much interest in modulating DC function to improve cancer immunotherapy. Many strategies have been developed to target DCs in cancer, such as the administration of antigens with immunomodulators that mobilize and activate endogenous DCs, as well as the generation of DC-based vaccines. A better understanding of the diversity and functions of DC subsets and of how these are shaped by the tumour microenvironment could lead to improved therapies for cancer. Here we will outline how different DC subsets influence immunity and tolerance in cancer settings and discuss the implications for both established cancer treatments and novel immunotherapy strategies.

Cancers originate from the uncontrolled proliferative activity of the organism's cells and present characteristic hallmarks[1]. Despite their self-origin, tumours can induce immune responses. However, the incomplete elimination of tumour cells by the immune system can result in the persistence of 'immune-edited' tumours that are not efficiently cleared by the immune system[2]. The association of infections with spontaneous tumour regression and the capacity of the immune system to reject immunogenic tumours in preclinical models[1] support the role of the immune system in protection against cancers. Moreover, large-scale projects such as The Cancer Genome Atlas and the Immunoprofiler Initiative have identified tumour-infiltrating immune cells — either through gene-expression signatures[3–6] or by direct observation of these cell types[7] — as important correlates of cancer prognosis and treatment responsiveness.

Although dendritic cells (DCs) constitute a rare immune cell population within tumours and lymphoid organs, these cells are central for the initiation of antigen-specific immunity and tolerance[8]. Therefore, manipulation of DCs holds great potential for inducing efficient antitumour immunity[8]. DCs promote immunity or tolerance by sampling and presenting antigens to T cells and by providing immunomodulatory signals through cell–cell contacts and cytokines[9,10]. DC functions are determined by their integration of environmental signals, which are sensed via surface-expressed and intracellular receptors for cytokines, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs)[11]. Recent data highlight the specific roles of DC subsets in antitumour immunity, with key implications for therapy[12,13]. In that regard, most of our general understanding of DC subsets and functions is based on observations in mouse models, and, currently, increasing efforts aim to evaluate the biology of human DCs. In this Review, we will discuss the main functions of DCs in cancer immunology and consider the therapeutic potential of targeting DCs in patients with cancer.

DCs in cancer immunology

Diversity of DC subsets. Distinct DC subpopulations as categorized by developmental, phenotypical and functional criteria have been recognized in mice and humans (TABLE 1). Mouse conventional DCs (cDCs) derive from common DC precursors (CDPs) in the bone marrow and comprise two main subsets, the CD8α$^+$ and/or CD103$^+$ cDC1 subset and the more heterogeneous CD11b$^+$ cDC2 subset[10,14] (TABLE 1). B220$^+$ plasmacytoid DCs (pDCs) develop from both CDPs and lymphoid progenitors, yielding two functionally distinct pDC subsets[15]. Inflammatory conditions can lead to the CC-chemokine receptor 2 (CCR2)-dependent recruitment of monocytes from the blood that differentiate into monocyte-derived DCs (MoDCs) in peripheral tissues[9,11]. DCs can also exhibit distinct localization and trafficking properties. For example, tumour-draining lymph nodes (TDLNs) typically comprise distinct subsets of resident and migratory cDC1s and cDC2s as well as other migratory DCs, such as peripheral tissue-specific cDC subsets and MoDCs[10,11,16,17]. Notably, DC subsets in human blood (namely CD141$^+$ cDC1s, CD1c$^+$ cDC2s and CD123$^+$ pDCs) closely resemble their mouse

[1]*Immunobiology Laboratory, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.*

[2]*Department of Pathology, University of California, San Francisco, CA, USA.*

[3]*Division of Immunology and Immunotherapy, Center for Applied Medical Research, University of Navarra, Pamplona, Spain.*

[4]*Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain.*

[5]*University Clinic, University of Navarra, Pamplona, Spain.*

[6]*Centro de Investigación Biomédica en Red Cáncer, Madrid, Spain.*

[7]*Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA.*

**e-mail: dsancho@cnic.es*

https://doi.org/10.1038/s41577-019-0210-z

Table 1 | **Human and mouse DC subsets**

DC subset	Morphology	Presence in vivo	Development, growth and transcription factors	Main surface markers		Main PRRs		Main functional specialization	
				Mouse	Human	Mouse	Human	Mouse	Human
pDCs	Plasma cell-like	Resident in lymphoid tissues and found in blood, lung (mouse) and tonsil (human)	HSC, CDP/ depend on FLT3L/E2-2, IRF7	CD11c^{low}, MHC-II^{low}, B220^{+}, CD317^{+}, SIGLEC-H^{+}, CD172a^{+}, CD209^{+}, CCR2^{low}, CCR9^{+}, CXCR3^{+}	CD11c^{-}, HLA-DR^{low}, CD123^{+}, CD303^{+} (CLEC4C^{+}), CD304^{+}, CCR2^{+}, CXCR3^{+}	TLR7, TLR9, TLR12, RLR, STING, CLEC12A	TLR7, TLR9, RLR, STING, CLEC12A	Control of viral infections, type I interferon secretion. Generally poor antigen presentation, but can be stimulated to activate CD8^{+} T cells (cross-presentation). Implicated in cancer cell killing	Type I and type III interferon secretion upon acute or chronic viral infection. Can be stimulated to activate CD8^{+} T cells (cross-presentation). Implicated in progression of autoimmune diseases. Role in tolerogenic settings poorly described but correlate with poor prognosis in cancer
cDC1s	Irregular, stellate shape with extensive cell membrane processes	Resident in lymphoid tissues and found in blood. Migratory subsets are present in peripheral tissues and lymph nodes	HSC, CDP, pre-cDC/depend on FLT3L, GM-CSF/BATF3, IRF8, BCL-6, ID2, ZBTB46, NFIL3, Notch signalling	CD11c^{+}, MHC-II^{+}, CD8α^{+}, (resident) CD103^{+}, (migratory) CD24^{+}, XCR1^{+}, CLEC9A^{+}, DEC205^{+}	CD11c^{low}, HLA-DR^{+}, CD141^{+a}, XCR1^{+}, CLEC9A^{+}, DEC205^{+}	TLR2–TLR4, TLR11–TLR13, STING, CLEC12A	TLR1, TLR3, TLR6, TLR8, TLR10, STING, CLEC12A	Cellular immunity against tumours and intracellular pathogens, CD8^{+} T cell-type and T_H1 cell-type immunity. Specialized on cross-presentation. High secretion of IL-12 and type I and type III interferons. Implicated in self-tolerance in the steady state (via cross-presentation)	Cellular immunity against tumours and intracellular pathogens, CD8^{+} T cell-type and T_H1 cell-type immunity. Specialized on cross-presentation. Produce type I and type III interferons and IL-12 at lower levels. Correlate with beneficial prognosis in cancer. Role in tolerogenic settings poorly described
cDC2s	Irregular, stellate shape with extensive cell membrane processes	Resident in lymphoid tissues and found in blood. Migratory subsets are present in peripheral tissues and lymph nodes	HSC, CDP, pre-cDC/depend upon FLT3L, GM-CSF/IRF4, ID2, RBPJ, NOTCH2, KLF4, ZBTB46	CD11c^{+}, MHC-II^{+}, CD11b^{+} (high), CD172a^{+}	CD11c^{+}, HLA-DR^{+}, CD1c^{+a}, CD11b^{+}, CD172a^{+}, CD1a^{+} (migratory), CD14 and CD5 (subset)	TLR1, TLR2, TLR4–TLR9, TLR13, RLR, NLR, STING, CLEC4A, CLEC6A, CLEC7A, (CLEC12A)	TLR1–TLR9, RLR, NLR, STING, CLEC4A, CLEC6A, CLEC7A, CLEC10A, CLEC12A	Context dependent, large repertoire of PRRs and pro-inflammatory and anti-inflammatory cytokines. Humoral and cellular immunity against extracellular pathogens, T follicular helper cell activation, T_H2 cell-type and T_H17 cell-type immunity. Implicated in T_H17 cell homeostasis in gut and lung. Induction of CD4^{+} T cell immunity in cancer.	Context dependent, large repertoire of PRRs and pro-inflammatory and anti-inflammatory cytokines, including IL-12. Mainly induce T_H17 cell activation but also T_H1 cell, T_H2 cell, T_{reg} cell and CD8^{+} T cell (cross-presentation) activation, depending on the context and precise cDC2 subpopulation. Maintain T_{reg} cell–T_H17 cell homeostasis in gut (and lung)

Table 1 (cont.) | **Human and mouse DC subsets**

DC subset	Morphology	Presence in vivo	Development, growth and transcription factors	Main surface markers		Main PRRs		Main functional specialization	
				Mouse	Human	Mouse	Human	Mouse	Human
MoDCs	Context dependent	Differentiate from monocytes in peripheral tissues on inflammation. Resident in skin, lung and intestine	Monocytes/ mainly depend on CSF1R, in vitro GM-CSF and IL-4/MAFB, KLF4, express ZBTB46	$CD11c^+$, MHC-II^+, $CD11b^+$, $Ly6C^+$, $CD64^+$, $CD206^+$, $CD209^+$, $CD14^+$, $CCR2^+$	$CD11c^+$, $HLA\text{-}DR^+$, $CD1c^+$, $CD11b^+$, $CD14^+$, $CD64^+$, $CD206^+$, $CD209^+$, $CD172a^+$, $CD1a^+$, $CCR2^+$	Not well defined	Not well defined	Mainly generated during inflammation conditioning their functions: direct antimicrobial effector functions and induction of $CD8^+$ T cell-type, T_H1 cell-type, T_H2 cell-type and T_H17 cell-type immunity. Implicated in T_{reg} cell generation and immunosuppression in cancer as well as in autoimmune pathogenesis. Involved in regulatory functions in steady state skin	Mostly studied in vitro, functions depend on signals/ stimulation and can be skewed towards $CD8^+$ T cell-type, T_{reg} cell-type, T_H1 cell-type, T_H2 cell-type and T_H17 cell-type immunity. Implicated in regulatory functions in steady-state skin

Overview of key characteristics of the predominant dendritic cell (DC) subsets found in humans and mice: plasmacytoid DCs (pDCs), conventional type I DCs (cDC1s), conventional type 2 DCs (cDC2s) and monocyte-derived DCs (MoDCs). References are provided throughout the main text. BATF3, basic leucine zipper transcription factor ATF-like 3; BCL-6, B cell lymphoma 6 protein; CCR, CC-chemokine receptor; CDP, common DC progenitor; CSF1R, colony-stimulating factor 1 receptor; CXCR3, CXC-chemokine receptor 3; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; HSC, haematopoietic stem cell; ID2, inhibitor of DNA binding 2; IRF, interferon-regulatory factor; KLF4, Kruppel-like factor 4; MAFB, MAF BZIP transcription factor B; MHC-II, MHC class II; NFIL3, nuclear factor IL-3-regulated protein; NLR, NOD-like receptor; PRR, pattern recognition receptor; RBPJ, recombining binding protein suppressor of hairless; RLR, retinoic acid-inducible gene I-like receptor; T_H1 cell, type 1 $CD4^+$ T helper cell; T_H2 cell, type 2 $CD4^+$ T helper cell; T_H17 cell, IL-17-producing $CD4^+$ T helper cell; TLR, Toll-like receptor; T_{reg} cell, regulatory $CD4^+$ T cell; ZBTB46, zinc-finger and BTB domain-containing 46. [a]Classical surface markers used to define human cDC1s (for example, CD141) and cDC2s (for example, CD1c) can be induced on other DC subsets in culture and in tissues.

counterparts in transcriptional and main functional analyses[9,18] (TABLE 1); however, these comparisons have to be evaluated with caution. For example, single-cell RNA sequencing recently identified additional types of human DC subsets in blood or TDLNs[17,18], and the surface markers used to identify human DC subsets may be unreliable in different tissue microenvironments[9].

Generally, functional specialization of DC subsets arises from their expression of different receptors, including pattern recognition receptors (PRRs)[9–11] (TABLE 1). Their T cell priming abilities may also differ, with pDCs showing relatively poor priming of naive T cells, although human and mouse pDCs can be stimulated to potently prime $CD8^+$ T cells[19–21]. In contrast, mouse and human cDC1s excel at inducing cellular immunity against intracellular pathogens and tumours due to their efficient processing and cross-presentation of exogenous antigens on MHC class I molecules to activate $CD8^+$ T cells and their ability to prime type 1 T helper cell (T_H1 cell) responses[10,11,14,21–23]. Analysis of interferon regulatory factor 4 (IRF4)-deficient mice (which lack cDC2s) suggests that cDC2s are potent inducers of $CD4^+$ T cell responses[24,25]. This notion is supported by a recent study demonstrating that cDC2s are crucial for inducing $CD4^+$ T cell-mediated immunity in cancer[17]. However, there is notable heterogeneity in human cDC2s, and 'DC-like' and 'monocyte-like' subtypes have been described that may differ in function[17,18,26]. Importantly, depending on the context, human cDC2s can induce the polarization of diverse subsets of $CD4^+$ T_H cells and activate $CD8^+$ T cells[21–23,26]. MoDCs are predominantly generated in response to inflammation and promote context-dependent differentiation of $CD4^+$ T cells towards a T_H1 cell, type 2 T helper cell (T_H2 cell) or IL-17-producing T helper cell (T_H17 cell) phenotype[27]. However, some human cDC2s can express MoDC markers and vice versa[9,18,26], and 'MoDC-like' cells generated during inflammation are now often classified as highly plastic or 'non-classical' monocytes rather than bona fide DCs[9,11]. Hence, further research is required to better define these DC and monocyte subsets and their behaviour across different tissues and inflammatory settings, especially in humans.

In the tumour microenvironment (TME), DCs acquire, process and present tumour-associated antigens (TAAs; TABLE 2) on MHC molecules (signal 1) and provide costimulation (signal 2) and soluble factors (signal 3) to shape T cell responses (FIG. 1). Next we discuss how these DCs function within the TME and TDLNs to promote immunity or tolerance to tumours.

Promotion of antitumour immunity by DCs. As $CD8^+$ T cells are often the main effectors of antitumour immunity, promoting cross-presentation of TAAs by DCs is considered paramount. cDC1s are often associated with superior cross-presentation of antigens, which results in stronger $CD8^+$ T cell immunity, and cDC1s can additionally support T_H1 cell polarization of $CD4^+$ T cells[3,28–32]. Basic leucine zipper transcription factor ATF-like 3 (BATF3)-dependent cDC1s are essential for the rejection of highly immunogenic tumours[28], and therapeutic vaccination with TAA-loaded natural cDC1s reduces tumour growth[32]. cDC1s can cross-present TAAs, which depends on the regulator of vesicular trafficking

Pathogen-associated molecular patterns (PAMPs). Conserved molecules expressed by microorganisms that activate host pattern recognition receptors to alert the immune system to the presence of infection.

Damage-associated molecular patterns (DAMPs). Endogenous molecular motifs associated with host cell death and tissue damage that can activate the immune system via pattern recognition receptors.

Pattern recognition receptors (PRRs). Germ line-encoded host sensors that detect pathogen-associated molecular patterns, although many of them have also been described to sense damage-associated molecular patterns. This interaction triggers signalling in the host cell.

Table 2 | **Frequently used tumour-associated antigens to load DCs**

TAA type	Examples of proteins/ source of TAAs	Cancer specificity	Advantages	Disadvantages
Differentiation antigens	MART1, GP100, tyrosinase, PAP, CEA	Low	High prevalence, cheap off-the-shelf products, allow conjugation	High probability of unspecificity and side effects
Overexpressed antigens	WT1, MUC1, ERBB2	Low	High prevalence, often cancer causative (oncogenes), cheap off-the-shelf products, allow conjugation	High probability of unspecificity and side effects
Viral antigens	HPV- and EBV-derived proteins	High	Very specific, often cancer causative (oncoviruses), allow conjugation	Limited prevalence of virus-associated tumours
Cancer–germ line/cancer–testis antigens	NY-ESO-1, MAGE (e.g. MAGEA3), GAGE and BAGE protein families	High	Specific, represent 50% of T cell-recognized TAAs, cheap off-the-shelf products, allow conjugation	Not exclusive to cancer (side effects possible, e.g. MAGEA3), limited prevalence
Mutated neoantigens	Mutated proteins specific to (individual) cancers	Highest	Very specific, high efficacy, often being unique to cancer/patient, might allow conjugation	Expensive, labour- and technology-intensive personalized product
Whole tumour antigens	Lysate of autologous or allogeneic dead cancer material (e.g. GVAX, Melacine, OncoVAX)	Variable	Complete cancer/patient-tailored TAA selection, no need for neoantigen identification. Contain additional DC-activating factors improving immunity, cheap	Limiting cancer material (autologous), suboptimal matching (allogeneic), uncontrolled TAA quality, some probability of side effects, more difficult to conjugate

Overview of types of tumour-associated antigens (TAAs) used in the clinic for dendritic cell (DC) loading in vivo or in vitro to elicit DC-mediated anticancer T cell activation. References are provided throughout the main text. BAGE, B melanoma antigen; CEA, carcinoembryonic antigen; EBV, Epstein–Barr virus; ERBB2, receptor tyrosine-protein kinase erbB-2; GAGE, G antigen; GP100, glycoprotein 100; HPV, human papillomavirus; MAGE, melanoma-associated antigen; MART1, melanoma antigen recognized by T cells 1; MUC1, mucin 1 cell surface associated; NY-ESO-1, New York oesophageal squamous cell carcinoma 1; PAP, prostatic acid phosphatase; WT1, Wilms tumour 1.

Tumour microenvironment (TME). Usually refers to the non-tumoural cells that surround tumour cells, including fibroblasts, blood vessels and immune cells as well as the milieu of extracellular factors such as cytokines, soluble molecules and extracellular matrix.

Tumour-associated antigens (TAAs). Autologous cellular antigens generated in tumour cells. They can be the product of mutated genes, antigens produced by oncogenic viruses, oncofetal antigens, altered glycolipids and glycoproteins, differentiation antigens specific for a cell type and overexpressed or aberrantly expressed cellular proteins.

WDFY4 (REF.[33]). DCs also require the soluble NSF attachment protein receptor (SNARE) protein SEC22B for efficient handling and cross-presentation of antigen, leading to protection against immunogenic tumours[34]. There is evidence that cDC2s and MoDCs may also cross-present antigen and cDC2s appear essential for priming of antitumour $CD4^+$ T cell responses[17]. Moreover, both cDC2s and MoDCs are fundamental for direct presentation or cross-presentation of TAAs following treatment with certain cancer chemotherapies, such as anthracyclines in mice[35–37]. In addition, both tumour-infiltrating DCs and DCs from TDLNs can directly present and cross-present TAAs to T cells, but their overall contribution to antitumour immunity remains unclear[16,38].

On sensing of appropriate cues, DCs mature and express chemokine receptors and costimulatory molecules. The best characterized chemokine receptor upregulated in maturing DCs is CCR7, which is necessary for the migration of tumour-infiltrating DCs into TDLNs[16]. However, CCR7 may also be involved in the recruitment of DCs into the TME[39–41], although its overall impact may be context specific. Among costimulatory molecules, DC-expressed CD80 and CD86 control the activation or suppression of T cells through interaction with CD28 or cytotoxic T lymphocyte antigen 4 (CTLA4), respectively[42]. Other costimulatory pathways involved in DC-mediated T cell priming and reactivation are a major focus of research to improve T cell-mediated immunity in cancer immunotherapy; these include the CD40–CD40L, CD137–CD137L, OX40–OX40L, GITR–GITRL and CD70–CD27 signalling axes (FIG. 1). CD137L (also known as 4-1BBL) is expressed on antigen-presenting cells (APCs) and promotes the activation and survival of $CD4^+$ and $CD8^+$ T cells through CD137 (REF.[43]). OX40L on DCs and macrophages also contributes to T cell survival, thereby favouring antitumour immunity[44]. GITRL on DCs promotes $CD8^+$ T cell immunity and the resistance of T cells to regulatory T cell (T_{reg} cell)-mediated immunosuppression[45]. Finally, CD70 on DCs supports $CD8^+$ T cell cross-priming, differentiation and antitumour immunity[46]. Reciprocal crosstalk signalling in DCs may also boost their function. For example, CD40 on DCs interacts with CD40L on T cells, leading to DC activation.

The effector activity of T cells depends on DC-derived cytokines, including IL-12 and type I interferons[47] (FIG. 1). In mice, although MoDCs can produce IL-12 after immunogenic stimulation[48], IL-12 is mainly generated by cDC1s and contributes to T_H1 cell and $CD8^+$ T cell priming[3,4,49]. In humans, both $CD141^+$ cDC1s and $CD1c^+$ cDC2s can produce IL-12 following Toll-like receptor (TLR) stimulation[29,50], but IL-12 levels within human cancers are also associated with increased cDC1 infiltration[4]. Type I interferons are in clinical use to treat patients with cancer[51], and the sensing of nucleic acids through the cGAS–STING pathway contributes to DC activation and type I interferon production in antitumour immunity[52,53]. DCs can also produce chemokines in the TME that attract T cells. For example, tumour-infiltrating cDC1s are the main producers of CXC-chemokine ligand 9 (CXCL9) and CXCL10 in the TME, which in turn promotes the recruitment of $CD8^+$ T cells into the TME[53]. cDC1s also support T cell reactivation in the TME[54,55]. In summary, DCs play a central role in antitumour immunity by conditioning the TME with soluble factors, as well as attracting and mediating priming of antitumour T cells.

DCs drive tolerance in the TME. Under the pressure of antitumour immunity, cancer cell variants can arise that exploit DCs to promote immune tolerance. Presentation of TAAs in the absence of costimulatory signals leads

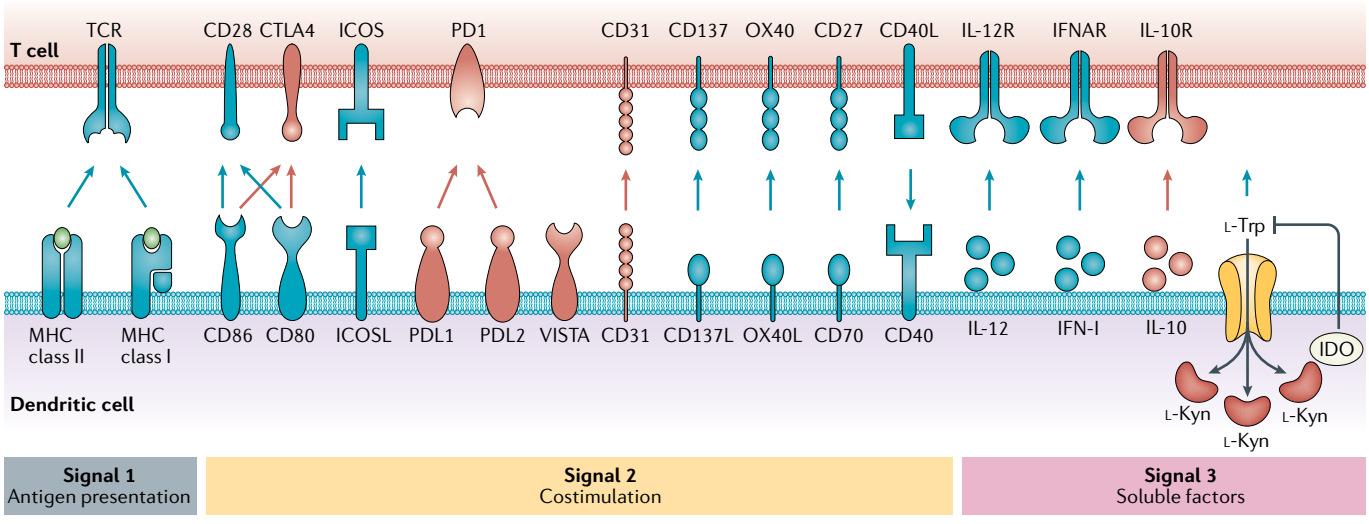

Fig. 1 | **Induction of T cell-mediated immunity or tolerance by DCs.** To control T cell activity, dendritic cells (DCs) can present tumour-associated antigens on MHC class I and MHC class II molecules. However, this in itself is not sufficient to prime effective antitumour immunity, which requires further positive signalling (blue arrows and receptors) through costimulatory molecules (belonging to the B7 and tumour necrosis factor (TNF) protein families) and soluble factors, such as IL-12 and type I interferon (IFN-I). Conversely, inhibitory mechanisms (red arrows and receptors) limit T cell activation. CTLA4, cytotoxic T lymphocyte antigen 4; ICOS, inducible T cell costimulator; IDO, indoleamine 2,3-dioxygenase; IL-10R, IL-10 receptor; IL-12R, IL-12 receptor; Kyn, kynurenine; PD1, programmed cell death protein 1; PDL1, programmed cell death 1 ligand 1; TCR, T cell receptor; VISTA, V-domain immunoglobulin suppressor of T cell activation.

to T cell anergy[8], and high engagement of inhibitory receptors can limit T cell effector activity (FIG. 1). CTLA4 expressed on T cells binds CD80 and CD86 on DCs with greater affinity than CD28, limiting costimulatory signalling and T cell activation[42]. Programmed cell death 1 ligand 1 (PDL1) and PDL2 on DCs and other cells in the TME also inhibit proliferation and cytokine production by programmed cell death 1 (PD1)-expressing activated T cells[56]. V-domain immunoglobulin suppressor of T cell activation (VISTA) is another inducible member of the PD1 family that is expressed by DCs and constrains T cell antitumour immunity[57]. CD31, a transhomophilic co-inhibitory molecule, induces a tolerogenic phenotype in DCs, skewing T cell priming towards T_{reg} cell generation instead of T_H1 cell differentiation[58].

DCs can also modulate T cell function by modifying the availability of metabolic substrates. L-Tryptophan is essential for T cell responses and is depleted through its conversion to L-kynurenine by the enzyme indoleamine 2,3-dioxygenase 1 (IDO1) (FIG. 1). IDO1 is induced in DCs following their recognition of apoptotic cells or following binding of CTLA4 by CD80 and CD86 (REF.[59]). Notably, increased IDO1 expression is observed in tumour-associated DCs[60], and DC-expressed IDO1 suppresses the proliferation and effector functions of $CD8^+$ T cells, natural killer (NK) cells and plasma cells and contributes to the differentiation of T_{reg} cells[60].

Modulation of DC function by tumours

In addition to TAAs and endogenous DAMPs, the TME also contains a network of immunosuppressive factors that can inhibit DC infiltration and subdue their antitumour activity; as such, the TME conditions the function of DCs in cancer immunology (FIG. 2a). Targeting these immunosuppressive pathways therapeutically may improve the recruitment, infiltration and effector activity of T cells in the TME.

Inhibition of cDC recruitment and differentiation. Few cDC1s are found in the TME owing to their suboptimal recruitment, differentiation or viability. However, an increased density of cDC1s within the TME is associated with improved prognosis and responsiveness to anti-PD1 immunotherapy in patients with cancer[3,6,7]. As an immune evasion mechanism, tumour cell-intrinsic factors can limit cDC1 recruitment. Both in humans and in mice, tumours with active β-catenin reduce CC-chemokine ligand 4 (CCL4) expression, resulting in lower cDC1 infiltration and increased tumour growth[5]. Conversely, tumour-infiltrating NK cells recruit cDC1s through production of CCL5 and XC-chemokine ligand 1 (XCL1)[6] and foster their survival with FMS-related tyrosine kinase 3 ligand (FLT3L)[7]. Yet, tumour cells can reduce NK cell viability and pro-inflammatory chemokine secretion by producing prostaglandin E_2 (PGE_2), and this in turn limits cDC1 density and favours tumour growth[6,61] (FIG. 2b).

The TME also curbs DC development and survival. Tumour-infiltrating lymphocytes, particularly NK cells, are the predominant producers of FLT3L in the TME[7], which is essential for cDC development and proliferation in situ[10,30] and fosters their survival[7]. Notably, tumour-derived vascular endothelial growth factor (VEGF) can inhibit FLT3L activity and negatively impact cDC differentiation in vitro[62]. Cancer cells and immune cells can also produce IL-6, a pro-inflammatory cytokine that impairs differentiation of cDCs and MoDCs[63–65]. Tumour-derived gangliosides and prostanoids (such as PGE_2) also inhibit cDC maturation and survival, as well as MoDC differentiation[64]. As cDC precursors are found

Cross-presentation
The presentation of exogenous antigens (which are typically presented on MHC class II antigens) on MHC class I molecules. It can occur through the vacuolar pathway, leading to loading of peptides onto MHC class I molecules in the phagosome. Alternatively, cross-presentation can involve the transfer of exogenously acquired antigens to the cytosol, where they are processed by the proteasome and degraded to peptides that are transported to the endoplasmic reticulum for loading on MHC class I molecules. The stimulation of naive cytotoxic $CD8^+$ T cells following cross-presentation is known as 'cross-priming' and is needed for antitumour immunity.

Immunogenic cell death
A form of cell death that induces an effective immune response through activation of dendritic cells. Hallmarks include the exposure of calreticulin on the cell surface and the active release of high mobility group protein B1 (HMGB1). This is in contrast to silent apoptosis, which is not immunogenic.

in the TME[66], tumour-derived factors could also locally affect pre-DC differentiation steps (FIG. 2b).

Impairment of DC activation and antigen presentation. A number of active mechanisms in the TME perturb DC functions, resulting in insufficient T cell activation and, potentially, the induction of T cell tolerance to TAAs (FIG. 2a). Usually, phagocytosis of cells that have undergone immunogenic cell death induces activation of cDCs and effector T cell priming, but these processes are often inhibited in tumours. For instance, immunogenic cell death and immune activation in response to chemotherapy relies on the alarmin high mobility group protein B1 (HMGB1)[37]. HMGB1 recruits nucleic acids into DC endosomes, mediating the innate sensing of nucleic acids from dead tumour cells[67]. This process is prevented in tumour-infiltrating cDCs through high expression of T cell immunoglobulin mucin receptor 3 (TIM3), which sequesters HMGB1 (REF.[68]). Tumour cell expression of CD47 inhibits the detection of cancer cell-released mitochondrial DNA by signal-regulatory protein-α (SIRPα) on cDC2s, which otherwise would induce type I interferons[69]. The tumour also enforces immune-regulatory transcriptional programmes and limits DC-mediated production of pro-inflammatory cytokines. Versican, a tumour-derived TLR2 ligand, induces IL-10 and IL-6 and overexpression of their receptors, which facilitates signal transducer and activator of transcription 3 (STAT3) hyperphosphorylation in DCs and immunosuppression[63]. In addition, macrophages within tumours are a primary source of IL-10 that can abolish IL-12 production by cDC1s[4]. Long-term exposure of tumour-infiltrating mononuclear phagocytes to IFNγ promotes a

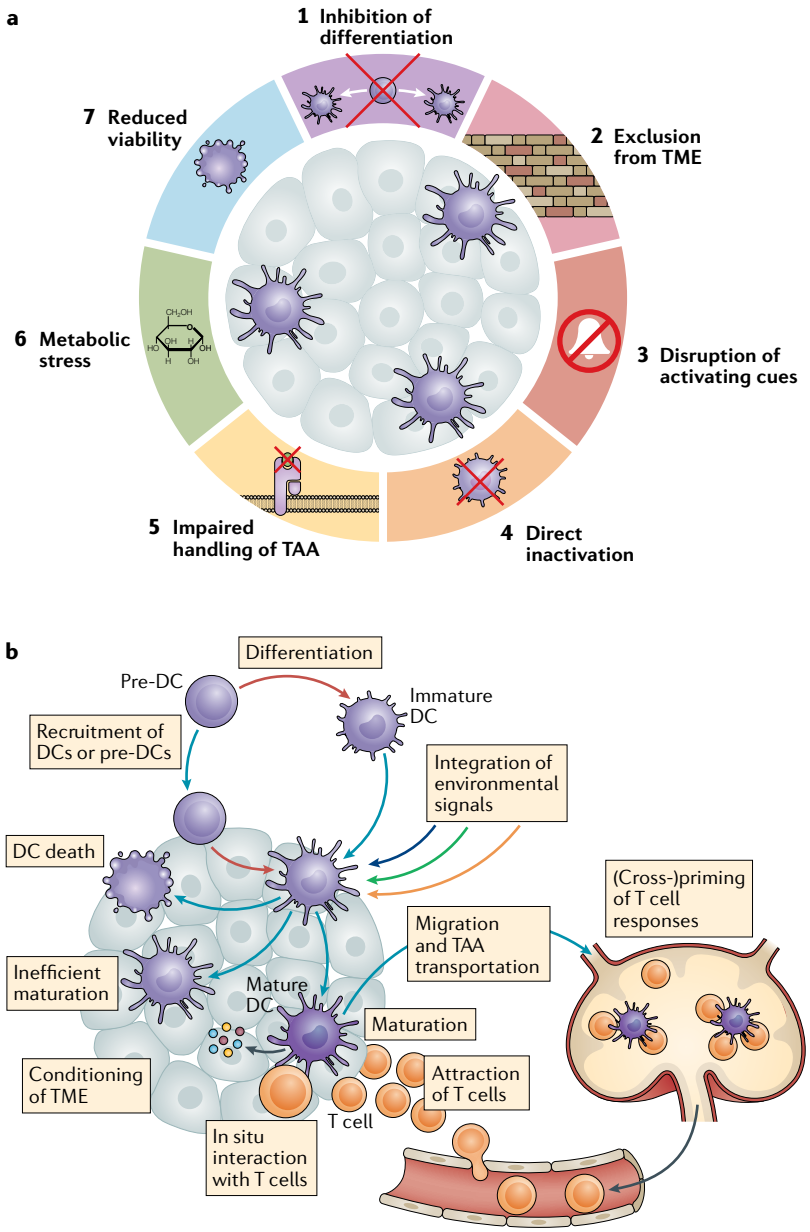

Fig. 2 | **Regulation of DC function by tumours.** The main aspects of dendritic cell (DC) biology that can be impaired by tumours are illustrated. **a** | The key features of DC biology that tumours target to suppress DC-mediated antitumour immunity. (**1**) Decreased availability of FMS-like tyrosine kinase 3 ligand (FLT3L) in the tumour microenvironment (TME) can reduce the terminal differentiation of pre-DCs, and cytokines (such as IL-6, IL-10 and transforming growth factor-β (TGFβ)) and tumour-derived prostanoids and gangliosides can affect both in situ and bone marrow generation of DCs. (**2**) Tumours can block the infiltration of DCs by reducing the expression of DC chemoattractants such as CC-chemokine ligand 4 (CCL4) or by preventing other cells such as natural killer cells from producing chemoattractants. (**3**) Tumours avoid detection by DCs by limiting the release of activating molecular cues. For example, three-prime repair exonuclease 1 (TREX1) degrades the alarmin ATP and prevents the recruitment of monocyte-derived dendritic cells into the TME, and T cell immunoglobulin mucin receptor 3 (TIM3) prevents high mobility group protein B1 (HMGB1)-mediated detection of dying cancer cells. (**4**) Tumours can influence DC maturation by the direct production of soluble mediators, such as IL-10, TGFβ, IL-6 or vascular endothelial growth factor (VEGF), which interfere with activating signalling pathways, for instance, by inducing the hyperphosphorylation of signal transducer and activator of transcription 3 (STAT3). Tumours can also indirectly affect DC maturation; for example, by producing colony-stimulating factor 1 (CSF1) to recruit tumour-associated macrophages that inhibit DC maturation. (**5**) The handling, presentation and cross-presentation of tumour-associated antigens (TAAs) by DCs is impaired by tumours, which promote the accumulation of half-degraded lipids that interfere with cargo trafficking within DCs. (**6**) Tumours modify DC metabolism to impair their functionality by increasing the accumulation of truncated fatty acids and by decreasing the availability of nutrients and oxygen. (**7**) Tumours can compromise DC viability by targeting factors such as the hypoxia response, endoplasmic reticulum stress or the BCL-2 protein family. **b** | Actions of DCs in tumours. DCs or their precursors can be recruited into the TME, where the latter can differentiate into DCs. Within the TME, DCs can sense different molecular cues that determine their fate, which can include cell death, inefficient activation and successful maturation. While immature DCs lack the capacity to prime T cell responses against tumours, or may even induce tolerance, mature DCs can migrate to tumour-draining lymph nodes to prime T cell responses, recruit T cells into the TME and produce immunostimulatory cytokines that condition the TME.

transcriptional programme that contributes to immune evasion in a suppressor of cytokine signalling 2 (SOCS2)-dependent manner[70]. CCR7-mediated migration of cDCs from tumours to TDLNs is restrained by tumour-derived agonists of liver X receptor-α (LXRα), and LXRα inhibition results in increased protection against tumour growth[41].

Other TME components can also impair cross-presentation of TAAs. For instance, lipid peroxidation by-products promote endoplasmic reticulum stress in tumour-associated cDCs, and constitutive activation of the endoplasmic reticulum stress sensor IRE1α leads to lipid accumulation and reduced T cell activation[71]. Indeed, lipid-laden cDCs show defective processing of exogenous antigen and impaired cross-presentation in cancer[72]. Incorporation of oxidized lipids into cDC lipid bodies inhibits trafficking of peptide–MHC class I complexes to the cell surface[73]. Other metabolites in the TME can dampen DC function as well; for example, lactic acid is a metabolic product of tumour cells that impairs MoDC differentiation and activation[74].

Notably, the ability of pDCs to promote antitumour immunity through production of type I interferon is also inhibited by immunosuppressive factors in the TME[13]. Infiltration of tumours by pDCs correlates with poor patient prognosis in several cancers, and this seems to be due to the ability of pDCs to promote the expansion of T_{reg} cell populations in an inducible T cell costimulator ligand (ICOSL)-dependent manner[75]. Tumour-associated pDCs also fail to produce type I interferon in response to TLR9 ligands due to the relocation of TLR9 to late endosomal compartments[76]; nevertheless, this can be reversed by costimulation with TLR7 ligands[19,20]. Moreover, intratumoural administration of TLR9 ligands has shown very promising results[77], but their efficacy may rely on the generation of local inflammation and its immunostimulatory activity on other cells, such as cDCs[77].

In summary, DCs have the potential to promote efficient antitumour immunity by recruiting and activating different immune cells, but the TME is rich in immunosuppresive factors that limit the immunostimulatory capacity of DCs and instead skew DCs to an anti-inflammatory phenotype (FIG. 2a). In the following section, we consider how different cancer therapies can modulate DC functions to boost antitumour immunity.

DCs in the context of cancer therapy

Cancer therapies currently used in the clinic can affect or even depend on DCs. Here, we discuss how DCs can influence responsiveness to these treatments (FIG. 3).

Chemotherapy and DCs. Certain chemotherapeutics and targeted therapies used in oncology — including bortezomib, doxorubicin, epirubicin, idarubicin and mitoxantrone, and oxaliplatin — trigger immunogenic cell death that promotes antitumour immunity[78], and these responses depend on DCs[36] (FIG. 3a). Calreticulin is a well-known opsonin (or 'eat-me') signal, and its exposure on the cell surface is one of the first hallmarks of immunogenic cell death that favours the uptake of dying tumour cells by DCs[79]. Immunogenic death of tumour cells also leads to the release of ATP, which promotes DC recruitment (through P2RY2) and activation of the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome (through P2RX7)[80], leading to IL-1β production. ATP also initiates a cell-intrinsic type I interferon response that leads to the secretion of annexin A1 and HMGB1 from dying tumour cells. Annexin A1 binds formyl peptide receptor 1 (FPR1) on DCs to attract them to dying cancer cells[81]. HMGB1 can be sensed by both human and mouse DCs through TLR4, thereby promoting efficient processing and cross-presentation of TAAs derived from dying cancer cells[37]. Indeed, anthracycline-induced cell death promotes MoDC recruitment into the TME, and these cells cross-present TAAs to $CD8^+$ T cells[35] (FIG. 3a). Thus, chemotherapy-induced immunogenic death of cancer cells leads to the release of stimulatory factors collectively known as 'alarmins' that enhance DC activation and cross-presentation of TAAs, thereby improving antitumour $CD8^+$ T cell responses[38].

However, not all chemotherapies act via DCs by inducing immunogenic cell death, and there are additional effects that can influence antitumour immunity. Chemotherapy with platinum-based drugs reduces PDL2 expression by DCs and cancer cells, which skews T cell responses towards T_H1 cell differentiation and increases TAA-specific T cells[82]. The therapeutic efficacy of paclitaxel, however, is restricted by IL-10 production by tumour-associated macrophages, which inhibits IL-12 production by DCs[4]. Thus, different chemotherapeutic agents seem to act via specific DC subsets and perhaps their efficacy may be potentiated accordingly.

Radiation therapy and DCs. Radiation therapy preferentially targets highly proliferative cells. Direct killing of cancer cells by radiation therapy does not, however, entirely account for its overall effect on tumour progression. The antitumour activity of radiation therapy also includes local bystander effects, such as in situ reactive oxygen species production, release of DAMPs and cytotoxic mediators and modification of the immune TME. Moreover, radiation therapy can mediate long-range effects (out-of-field or abscopal effects) associated with efficient systemic cancer-specific immune responses mediated by immunogenic cell death induction[78] that rely on cDC1 priming of $CD8^+$ T cells[83] (FIG. 3b). Cytosolic DNA released by cancer cells after radiation therapy acts as a DAMP and signals through cGAS–STING to induce the production of type I interferon by DCs, contributing to antitumour immunity[84]. However, high non-fractionated radiation doses induce the expression of the DNase TREX1, which degrades cytosolic DNA and limits the production of type I interferon and the immunostimulatory effect on cDC1s[85]. Additionally, although canonical nuclear factor-κB (NF-κB) signalling is necessary for the antitumour immune responses induced by radiation therapy, non-canonical NF-κB signalling dampens antitumour immunity by inhibiting STING-mediated induction of type I interferons[86].

Small-molecule inhibitors and DCs. Small-molecule inhibitors target key oncogenic signalling pathways — such as the STAT3, mitogen-activated protein

Out-of-field or abscopal effects
The ability of localized irradiation or treatment of a tumour to trigger a systemic antitumour effect that can lead to rejection of distant tumours or metastases.

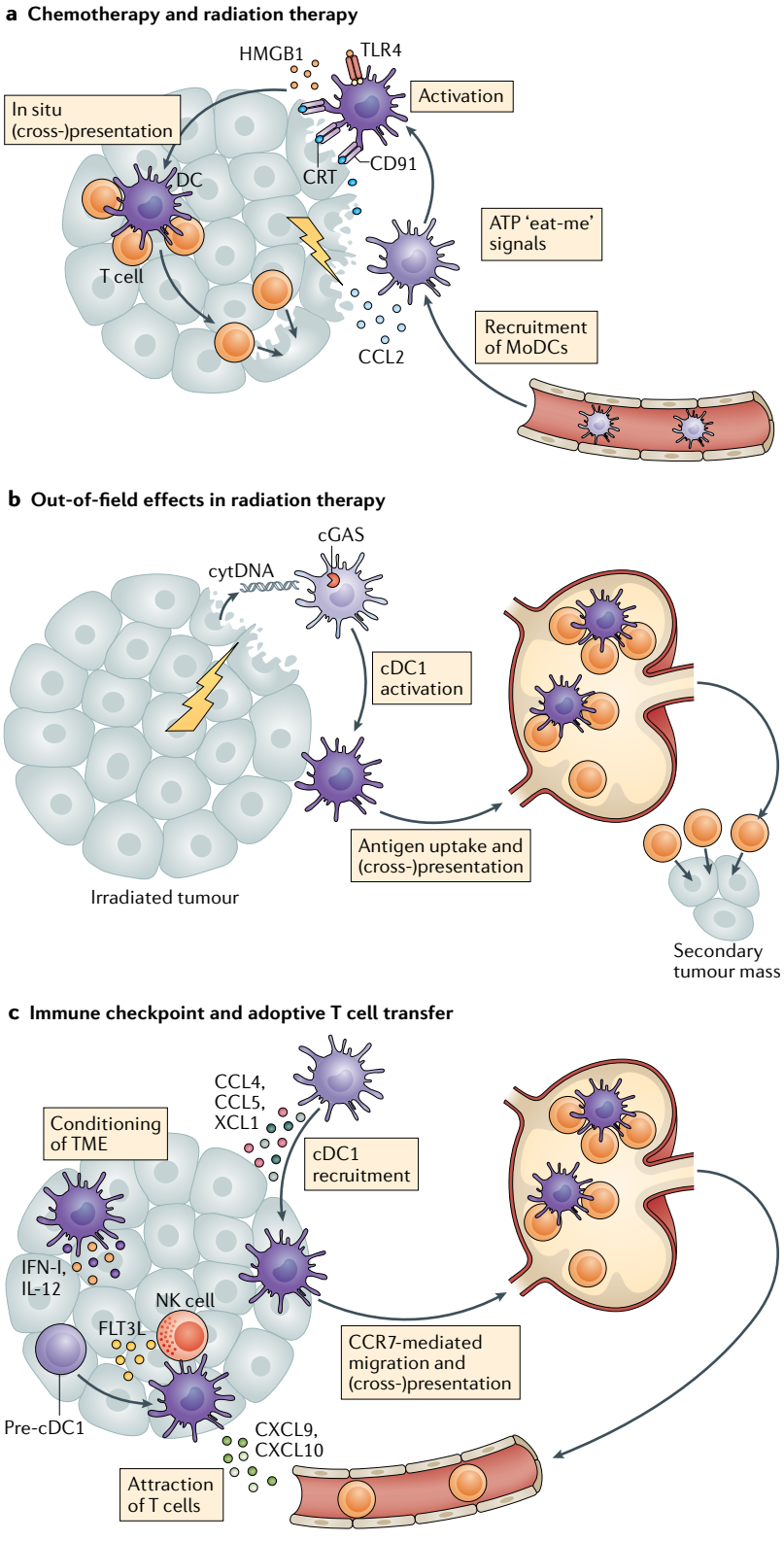

Fig. 3 | **DCs in the context of cancer therapy.** Dendritic cells (DCs) play an essential role in the generation of efficient antitumour immune responses triggered by different therapeutic strategies against cancer. **a** | Monocyte-derived DCs (MoDCs) mediate antitumour immunity triggered by chemotherapy- and local radiation therapy-induced immunogenic cell death. MoDCs are strongly recruited into the tumour microenvironment (TME) following treatment with immunogenic cell death inducers, and they prime robust $CD8^+$ T cell responses. **b** | Conventional type 1 DCs (cDC1s) contribute to the out-of-field (abscopal) effects of in situ radiation therapy, another inducer of immunogenic cell death. This response relies on the recognition of cancer cell-derived cytosolic DNA (cytDNA) by the cGAS–STING pathway. **c** | cDC1s strongly associate with the efficacy of immune checkpoint therapy and adoptive T cell transfer, due to their capacity to prime T cell responses locally and in the tumour-draining lymph nodes, to recruit T cells into the TME and to condition the TME by producing soluble factors. CCL2, CC-chemokine ligand 2; CCR7, CC-chemokine receptor 7; CRT, calreticulin; CXCL, CXC-chemokine ligand; FLT3L, FMS-like tyrosine kinase 3 ligand; IFN-I, type I interferon; NK, natural killer; TLR4, Toll-like receptor 4; XCL1, XC-chemokine ligand 1.

kinase (MAPK) and phosphoinositide 3-kinase–AKT–mechanistic target of rapamycin (mTOR) pathways — in tumour cells, but can also affect immune cells. The STAT3 and MAPK pathways are both involved in the signalling of IL-10, IL-6 and VEGF, which impair DC differentiation and inhibit IL-12 production by human MoDCs[87–89]. Activation of STAT3 generates a type of inflammation that promotes tumour growth and also inhibits DC-mediated antitumour immune responses[90]. The STAT3 inhibitor JSI-124 can reverse abnormal DC function in cancer[88], and, accordingly, mice with a STAT3 deficiency restricted to CD11c-expressing cells show resistance to tumour growth[91]. Compounds targeting the signalling upstream of STAT3 have been approved for therapy for certain rare cancers, and STAT3 inhibitors are being evaluated in clinical trials[65] (TABLE 3). The MAPK p38 signalling also impairs development and maturation of DCs in tumour-bearing mice, and its inhibition restores the T cell-stimulating capacity of DCs[92]. In humans, STAT3 inhibition has a weak effect to prevent DC dysfunction by tumour-derived immunosuppressive factors, but co-inhibition of p38 restores the differentiation capacity of DCs and their immunostimulatory capacity[89].

Activation of the WNT–β-catenin pathway in DCs leads to immunosuppression[93], in part through an mTOR–IL-10-dependent pathway[94]. Consistently, the mTOR inhibitor temsirolimus enhances the efficacy of DC vaccination[95]. The tyrosine kinase inhibitors sorafenib and sunitinib target similar pathways that include signalling downstream of VEGFR, PDGFR, FLT3 and KIT[87]. Sorafenib mitigates the inhibitory effect of renal carcinoma cells on DCs[87]; however, as sorafenib and sunitinib also target FLT3 signalling, which favours the expansion of cDC populations (TABLE 1), their global effects on DCs in the context of antitumour immunity need to be further explored.

Immune checkpoint therapy and DCs. Antibodies that target co-inhibitory receptors (such as the PD1–PDL1 axis) or that trigger the activation of costimulatory receptors (such as CD137) on T cells can amplify basal antitumour immune responses that were initially primed by DCs, with a significant contribution of the cDC1 subset (FIG. 3c). Experimental melanomas with stabilized

Table 3 | **Agents promoting immunogenic functions of DCs in cancer**

Compounds	Characteristics	Effect on DCs and immune consequences	Cancer-treatment approved examples
GM-CSF	Cytokine essential for cDC development	cDC mobilization, attraction and maturation	Talimogene laherparepvec (T-VEC) approved, others in clinical trials
FLT3L	Cytokine essential for cDC development	cDC1 and cDC2 mobilization/expansion	CDX-301 in clinical trials
TLR2/TLR4 agonists	Various synthetic or microbial-derived PRR ligands	Mainly human cDC2 activation: cytokines, $CD8^+$ T cell induction, survival extension	BCG, picibanil and monophosphoryl lipid A approved, others in clinical trials
TLR3 agonists	Synthetic PRR ligands, mainly poly(I:C) derivatives	Direct cancer cell cytotoxicity and cDC (mainly human cDC1) activation: cytokines, T_H1 cell immunity, NK cell and $CD8^+$ T cell induction	Poly-ICLC (Hiltonol), poly(I:C12U) (Ampligen) and BO112 in clinical trials
TLR7/TLR8 agonists	Various ligands for PRRs, TLR7 and/or TLR8, mainly imidazoquinolines	Human pDC and cDC activation: cytokines, T_H1 cell immunity, $CD8^+$ T cell induction, tumoricidal DC activity	Imiquimod approved, others in clinical trials (resiquimod, VTX-2337, protamine RNA)
TLR9 agonists	Synthetic PRR ligands, unmethylated CpG oligodeoxynucleotides	Human pDC and cDC activation: cytokines, T_H1 cell immunity, $CD8^+$ T cell induction	Numerous compounds in clinical trials (including CPG-7909 and CpG-685)
IDO inhibitors	Targeting of IDO	Prevention of IDO-mediated tryptophan depletion, tolerogenic functions and T cell anergy induction (can be mediated by IDO-expressing DCs)	Numerous compounds in clinical trials (including INCB 024360 and indoximod)
STAT3 inhibitors	Small molecules/monoclonal antibodies blocking STAT3 signalling	DC activation, prevention of immune-suppressive DC functions	IL-6/JAK/STAT3 signalling blockers approved (siltuximab, tocilizumab, ruxolitinib), STAT3 inhibitors in clinical trials

Overview of factors used in the clinic to stimulate antitumorigenic and pro-inflammatory functions of dendritic cells (DCs) for DC-mediated anticancer T cell activation. References are provided throughout the main text. BCG, bacillus Calmette–Guérin; cDC, conventional DC; cDC1, conventional type 1 DC; cDC2, conventional type 2 DC; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; IDO, indoleamine 2,3-dioxygenase; JAK, Janus kinase; NK, natural killer; pDC, plasmacytoid DC; poly-ICLC, polyinosinic-polycytidylic acid-poly(L-lysine) carboxymethylcellulose; PRR, pattern recognition receptor; STAT3, signal transducer and activator of transcription 3; T_H1 cell, type 1 $CD4^+$ T helper cell; TLR, Toll-like receptor.

β-catenin signalling are associated with reduced cDC1 tumour infiltration and non-responsiveness to immune checkpoint blockade (ICB) therapy, which was rescued by transfer of in vitro-generated cDC1-like cells preactivated with poly(I:C)[5]. Indeed, vaccination with naturally occurring cDC1s loaded with immunogenic cell death-derived whole tumour antigen can synergize with anti-PD1 treatment[32]. Moreover, tumours grafted onto BATF3-deficient mice, which lack cDC1s, did not respond to anti-PD1, anti-PDL1 or anti-CD137 treatments[30,31], and SEC22B-mediated cross-presentation of TAAs by DCs is necessary for effective PD1 blockade therapy[34]. Infiltration of cDC1s within human tumours is associated with responsiveness to anti-PD1 treatment[7].

Synergy of TLR-mediated activation of DCs and ICB can also be further improved by FLT3L-mediated expansion of DC populations[30,31]. Further evidence that cross-priming is the critical function mediated by cDC1 in this context has come from WDFY4-deficient mice, which fail to reject immunogenic tumours due to a defect in a vesicular transport pathway needed for cross-presentation[33].

Enhancing DC functionality may improve and/or broaden responsiveness to ICB regimens. Both cGAS and STING are necessary for intrinsic antitumour immunity and efficient responses to anti-PDL1, which is at least partially mediated by DCs[96]. Targeting of type I interferons to activate cDC1s also improves anti-PDL1 treatment[97], suggesting that tumour DCs may require activation to support ICB-induced effector T cell activity. Increasing DC production of the chemokines CXCL9 and CXCL10, for example through epigenetic modulation, may also increase responsiveness to ICB[55].

In turn, ICB promotes DC accumulation within the TME. Combining pembrolizumab (anti-PD1) treatment with TLR9 agonists is associated with an elevated tumour-infiltrating DC signature and, preliminarily, clinical benefit[98].

Adoptive T cell transfer and DCs. The transfer of activated tumour-specific T cells to patients with cancer is showing promising clinical efficacy. cDC1s attract T cells to the cancer site, ensuring the efficacy of adoptive T cell transfer in preclinical models (FIG. 3c). Indeed, the adoptive transfer of $CD8^+$ T cells lacks efficacy in patients with melanomas with limited cDC1 infiltration[53]. Reactivation by local DCs may also be critical, as shown in a pancreatic cancer model, where CCR4 transduction of $CD8^+$ T cells increases their capacity to interact with DCs and results in stronger antitumour activity[99]. Notably, cDC1s are necessary for effective reactivation of TAA-specific, circulating memory $CD8^+$ T cells in cancer[54]. Moreover, activation of tumour necrosis factor (TNF)- and inducible nitic oxide synthase (iNOS)-producing cDC2s through the CD40–CD40L axis is necessary for the efficacy of preprimed TAA-specific T cell transfer[100]. These cDC2s function independently of colony-stimulating factor 1 receptor (CSF1R), although blockade of CSF1R further improves cancer control by reducing the number of immunosuppressive tumour-associated macrophages[4,100].

The gut microbiota and DCs. Increasing evidence points towards the relevance of the intestinal microbiota for the outcome of cancer therapies. Faecal microbiota transplantation from healthy individuals to germ-free

Immune checkpoint blockade
(ICB). Blockade of specific interactions between immune cells and cancer cells or other immune cells by targeting inhibitory molecules such as CTLA4, PD1 and PDL1 that dampen immune cell activation. Inhibiting these interactions releases the 'brakes' on the immune system and promotes immune cell activation.

or antibiotic-treated mice enhanced responses to ICB, whereas microbiota from non-responsive patients with cancer failed to do so. *Akkermansia muciniphila* was identified as a necessary commensal for ICB efficacy[101]. Additional microorganisms with beneficial effects on ICB efficacy in patients with metastatic melanoma include *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*[102]. DCs are clear candidates to mediate this link between tumour immunity and the microbiota, which has a relevant impact on other therapies[103]. For instance, vancomycin-mediated modulation of the gut microbiota composition enhances adoptive T cell transfer efficacy in tumour-bearing mice by expanding cDC1 populations and enhancing IL-12 production[104]. The emerging picture is that cooperative populations of gut bacteria produce organic metabolites that tonically control the function of immune cells, including DCs, in the intestinal mucosa and elsewhere.

DC-based cancer immunotherapies

Tolerance to tumours is a major hurdle that must be overcome to fully harness the potential of DCs in cancer immunotherapy. Several strategies to reverse DC-mediated tolerance are currently being pursued (FIG. 4).

Activation and mobilization of DCs. Cytokines that mobilize DCs, immunostimulatory adjuvants and agents blocking immunosuppressive DC functions can promote the activation of DCs and T cell priming[105] (FIG. 4a,b; TABLE 3). Granulocyte–macrophage colony-stimulating factor (GM-CSF) directly stimulates DC differentiation, activation and migration[10] (TABLES 1,3). Talimogene laherparepvec (Imlygic, T-VEC) is an attenuated oncolytic strain of herpes simplex virus that expresses human GM-CSF; it was approved by the Food and Drug Administration (FDA) after being shown to induce antitumour immune responses and increase survival in patients with advanced melanoma[106]. Moreover, administration of irradiated allogeneic or autologous tumour cells engineered to express GM-CSF (GVAX vaccines) has shown preclinical success and, despite the disappointing outcomes of two phase III clinical studies in prostate cancer, other combinatorial trials using GVAX are ongoing[107].

In this line, encouraging results showing that FLT3L administration enhances tumour immunity, $CD8^+$ T cell activation and cancer control in mouse models[30,108] (TABLES 1,3) are now being followed by clinical trials (NCT01811992, NCT01976585, NCT02129075 and NCT02839265) (FIG. 4b).

Adjuvants that drive immunogenic DC activation are also being actively investigated, particularly derivatives of ligands for TLRs expressed by DCs[78,105,109] (FIG. 4a; TABLES 1,3). Intravesical administration of bacillus Calmette–Guérin (BCG), which is a current standard treatment for superficial bladder cancer, is associated with increased DC viability and activation[110]. The potency of the synthetic TLR3 agonist poly(I:C), which can also engage melanoma differentiation-associated protein 5 (MDA5) and retinoic acid-inducible gene I (RIG-I) receptors, has emerged as a potential cancer immunotherapy[78]. Human $CD141^+$ cDC1s appear to be a main target of this therapy because of their high levels of TLR3 expression[29,30] (TABLES 1,3). In vitro and preclinical studies show the extraordinary efficacy of poly(I:C) to activate DCs and induce pro-inflammatory cytokines, T_H1 cell-type immunity, NK cell activation, cross-presentation and anticancer $CD8^+$ T cell responses culminating in therapeutic cancer suppression[31,111,112]. Of note, in mice, the pronounced antitumour effects of intratumoural nanoplexed poly(I:C) (BO-112) injection rely on BATF3-dependent cDC1s[113]. A pilot trial also demonstrated the general safety and clinical benefit of intratumoural administration of the poly(I:C) derivative polyinosinic-polycytidylic acid-poly(L-lysine) carboxymethylcellulose (poly-ICLC) in one of eight patients with solid cancers; a phase II study is ongoing[114]. In other clinical trials, intratumoural poly(I:C) derivatives in combination with DC-based cancer vaccines also seem to improve clinical outcomes[112]. The TLR7/TLR8 ligand imiquimod has been approved for local treatment of non-melanoma skin cancers, promoting pDC-mediated cytotoxicity[115], and numerous clinical trials with TLR7/TLR8 agonists in cancer are ongoing (NCT02574377 and NCT02692976). TLR7/TLR8 agonists likely target all endogenous DC subsets (TABLES 1,3), activate NF-κB and induce pro-inflammatory cytokine secretion and costimulatory receptor upregulation[109]. Unmethylated CpG oligodeoxynucleotides are a large group of TLR9 agonists that can activate human pDCs and cDCs in vivo (TABLES 1,3), triggering T_H1 cell-type immunity and cancer-specific $CD8^+$ T cell responses[116]. The potential of unmethylated CpG oligodeoxynucleotides in combination with ICB is currently under intense evaluation in the clinic (NCT02521870 and NCT03831295)[78].

Overcoming immunosuppressive activities of cancer-associated DCs is another approach to enhance DC function (TABLE 3). In that regard, inhibition of IDO is being explored in mice and in clinical trials[117]. Also, STAT3 inhibitors, which can foster DC maturation and immunogenic functions[90], are being evaluated in clinical trials[65].

Administration of antigens to boost antitumour immunity. In vivo administration of TAAs that can be presented (or cross-presented) by endogenous DCs has historically been an attractive cancer immunotherapy approach[118]. Such vaccines are mostly composed of TAAs that are delivered as synthetic short or long peptides, recombinant TAA-expressing viruses or whole tumour lysates (FIG. 4c; TABLES 2,4). To further ensure cancer specificity and fuelled by recent technological advances, the use of neoantigens (TAAs derived from mutated proteins) is reviving hopes for TAA-based vaccination[119] (TABLE 2). Efficacy and feasibility of neoantigen vaccines may depend on the mutational rate of individual tumours. Patients with lung cancers or melanomas with a high mutational load experience a higher rate of response to ICB[120,121], and long-term survival in patients with pancreatic cancer correlates with unique antigenic and immunogenic qualities of neoantigens and increased DC and $CD8^+$ T cell infiltrates[122].

Adjuvants
Charles Janeway described adjuvants as the 'immunologist's dirty little secret', as they were substances added to antigens to make vaccines effective, but their mode of action was not known at that time. Adjuvants contain compounds that stimulate the immune system, frequently pathogen-associated molecular patterns acting on pattern recognition receptors.

Neoantigens
Antigens formed by peptides that are absent from the normal human genome. These neoepitopes can be derived from tumour-specific DNA mutations or from viral sequences in the case of virus-associated tumours.

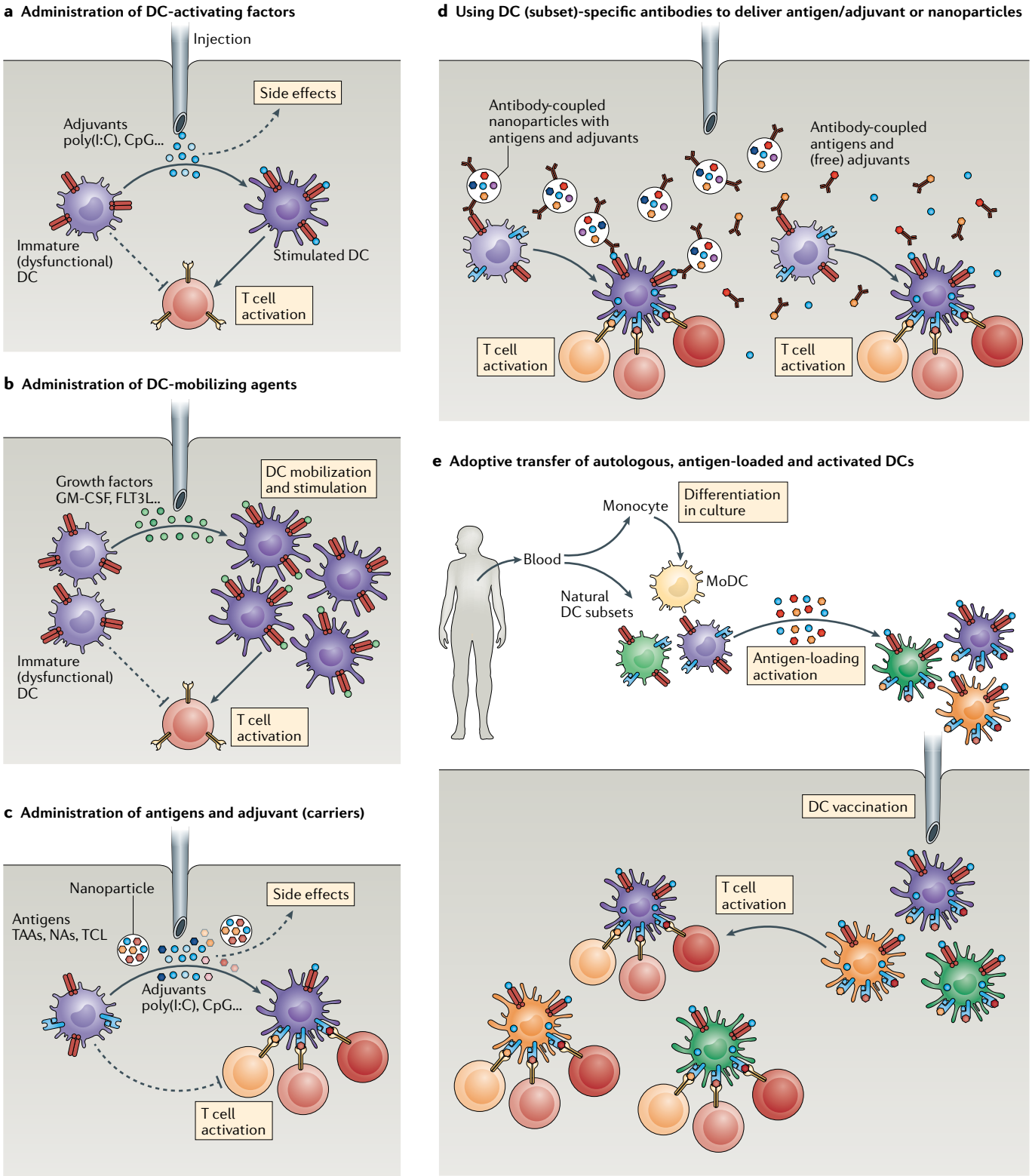

Fig. 4 | **Exploiting DCs for cancer immunotherapy.** Principles underlying functionality of therapeutic approaches (directly) targeting dendritic cells (DCs) are illustrated. **a** | Adjuvants induce stimulation of DCs, circumventing immaturity and potential tolerogenicity. **b** | Growth factors trigger DC population expansion and often activation. **c** | Delivery of free, carrier-associated or viral vector-encoded antigen, together with adjuvants, fosters activation of cancer-specific T cells by DCs. **d** | Direct targeting of (nanoparticle-conjugated) antigen–adjuvant to DCs via DC-specific antibodies can enhance antigen presentation and cancer-specific T cell activation and reduce off-target effects. **e** | Workflow for preparation of DC vaccines and effects of their administration. Natural DC subsets are isolated from blood and monocyte-derived DCs (MoDCs) differentiated in vitro from blood monocytes. After ex vivo activation and antigen loading, autologous DCs are reinfused into the patient to induce antigen-specific T cells with minimal side effects. GM-CSF, granulocyte–macrophage colony-stimulating factor; FLT3L, FMS-like tyrosine kinase 3 ligand; NA, neoantigen; TAA, tumour-associated antigen; TCL, tumour cell lysate antigen.

Table 4 | **Approaches targeting DCs for cancer immunotherapy: advantages and drawbacks**

Strategy	Costs	Applicability	Potential side effects[a]	Feasibility	Other advantages	Other disadvantages	Examples	Results
Free/soluble adjuvant or DC activation factors	Low	Universal	High, compound dependent (local or systemic inflammation)	Easy	–	Low persistence, targeted cells unclear, antigen unspecific	BCG, picibanil, monophosphoryl lipid A (TLR2/TLR4), poly(I:C) (TLR3), imiquimod, resiquimod, VTX-2337 (TLR7/TLR8), CpG-ODN (TLR9)	Imiquimod licensed for skin cancer and BCG licensed for bladder cancer (BCG mechanisms poorly understood). Adjuvants are part of most DC-based immunotherapies under evaluation
DC-mobilizing agents	Low	Universal	Moderate (systemic effects possible)	Easy	–	Eventual immaturity and dysfunction of expanded DCs[b], antigen-unspecific	GM-CSF, FLT3L	Clinically approved talimogene laherparepvec (T-VEC, oncolytic virus + GM-CSF). GM-CSF is added to numerous DC-based immunotherapies. FLT3L is being evaluated in trials
Viral vectors/vaccines expressing TAAs and T cell/DC-activating factors	Moderate[c], depending on virus strain, BSL2 is often required	Limited (TAA expression) or personalized (neoantigens) possible	Moderate/high (reactions to live virus possible)	Easy/moderate[c]	Intrinsic adjuvancy, viral proteins trigger type I interferons and TAAs and T cell/DC-activating factors are produced by infected cells (DCs), can be coupled to antibodies	Often pre-existing immunity neutralizing virus, possible epitope dominance of viral antigens over TAAs, variable transgene expression stability, targeted cells unclear, potential effects on other cells, eventual BSL2 production necessary	mRNA/DNA-expressing virus families: *Poxviridae*, *Adenoviridae*, *Retroviridae*, *Togaviridae*, *Rhabdoviridae*	FDA-licensed YS-ON-001 (inactivated rabies vaccine + poly(I:C)) for liver and pancreatic cancer, clinical trials ongoing for, e.g. TAA-encoding (and costimulatory molecule (TRICOM)-encoding) ALVAC- or PROSTVAC-based viral vectors
Free/soluble antigen (TAAs, TCL, NAs)	Low[c]	Universal (TCL), limited (TAA expression) or personalized (neoantigens)	Moderate/low, adjuvant dependent	Easy[c]	Large antigen diversity possible	Rapid clearance by phagocytes, targeted cells unclear, can cause tolerance without adjuvant	Synthetic peptides, SLPs, dead whole tumour material	Neoantigens show great promise, otherwise generally poor outcomes. Clinical trials ongoing. Antigens are part of most DC-based immunotherapies under evaluation
Adjuvant/antigen carriers (untargeted emulsions, nanoparticles, etc.)	Moderate/low[c]	Universal (TCL), limited (TAA expression) or personalized (neoantigens)	Moderate (local or systemic inflammation)	Easy/moderate[c]	Protection from antigen clearance, slow release, additional adjuvancy	Targeted cells unclear, relies on local DCs, potential effects of carriers on DCs	Peptide/protein conjugates (e.g. nanoparticles), liposomes, virosomes, ISCOMs, water–oil emulsions	Emulsion Montanide ISA 51 (carrying EGF + P64k) licensed for lung cancer. Many clinical trials ongoing
DC-targeted adjuvant/antigen delivery (DC-specific antibody coupled)	Moderate/low[c]	Universal (TCL), limited (TAA expression) or personalized (neoantigens)	Low, antibody specificity dependent	Easy/moderate[c]	Specific DC-targeted, antibody uptake can enhance cross-presentation	Rapid clearance, limited to identified TAAs/neoantigens, TCL challenging, unspecificity of antibody	DC-specific antibodies or receptor-ligands: anti-DEC205, anti-CLEC4A, anti-CD209, anti-CLEC7A, anti-CLEC12A, anti-CD40, anti-MR, oxidized mannan	Early clinical trials ongoing: e.g. anti-DEC205-coupled NY-ESO-1 (+ adjuvants); MR targeting with anti-MR-conjugated hCG-β or oxidized mannan-coupled MUC1

Table 4 (cont.) | **Approaches targeting DCs for cancer immunotherapy: advantages and drawbacks**

Strategy	Costs	Applicability	Potential side effects[a]	Feasibility	Other advantages	Other disadvantages	Examples	Results
DC-targeted adjuvant/antigen carrier delivery (e.g. antibody coupled nanoparticles)	Moderate/low[c]	Universal (TCL), limited (TAA expression) or personalized (neoantigens)	Low, antibody specificity dependent	Moderate/easy[c]	Specific DC-targeted, protected co-delivery of adjuvant/antigen, antibody uptake can enhance cross-presentation, antigen diversity possible	Potential effects of carriers on DCs, unspecificity of antibody	PLGA or ferrous nanoparticles conjugated with anti-CLEC9A, anti-DEC205, anti-CLEC4A	Promising preclinical results in mice and humans (cells)
Adoptive transfer of adjuvant/antigen-loaded DCs	High[c], can be automated	Personalized DC preparation	Low	Difficult, can be automated	Specific DC subsets, controlled adjuvant/antigen co-delivery, unlimited adjuvant/antigen diversity, quality control, antibody-mediated delivery ex vivo possible, personalized product might enhance efficacy	Limited cell number, leukapheresis necessary, potentially poor migration to lymphoid organs	In vitro-generated MoDCs, blood APCs and natural DC subsets activated and antigen loaded ex vivo	Licensed sipuleucel-T (Provenge) for prostate cancer. About 200 clinical trials generally showed induction of anticancer immunity and mild overall responses. Evaluation of neoantigen-loaded DCs, therapy combinations and stage III clinical trials with MoDCs and natural DCs ongoing

Characteristics of different dendritic cell (DC)-based therapeutic strategies are summarized. References are provided throughout the main text. ALVAC, replication-defective canarypox viral vector; APC, antigen-presenting cell; BCG, bacillus Calmette–Guérin; BSL2, biosafety level 2; CpG-ODN, unmethylated CpG oligodeoxynucleotide; EGF, epidermal growth factor; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte–macrophage colony-stimulating factor; hCG-β, human gonadotropin β-chain; ISCOM, immunostimulatory complexes; MoDC, monocyte-derived dendritic cell; MR, mannose receptor; MUC1, mucin 1 cell surface associated; NA, neoantigen; NY-ESO-1, New York oesophageal squamous cell carcinoma 1; P64k, meningococcal protein antigen of 64 kDa; PLGA, poly(lactic-*co*-glycolic acid); PROSTVAC, formulation of recombinant pox viral vectors encoding human prostate-specific antigen (rilimogene galvacirepvec/rilimogene glafolivec); SLP, synthetic long antigen peptide; TAA, tumour-associated antigen; TCL, whole tumour cell lysate; TRICOM, (transgenes for a) triad of immune-enhancing costimulatory molecules (CD80, CD54, CD58); TLR, Toll-like receptor. [a]Potential side effects of treatments are compared among the represented DC-targeted or DC-based approaches only. [b]GM-CSF administration likely has negligible potential to cause DC immaturity. [c]Production cost and feasibility are indicated when known synthetic TAAs or TCL is used; the identification and synthesis of NAs is more expensive, difficult and personalized.

Regarding the use of whole tumour lysates for vaccination, the type of induced cell death can influence their efficacy to induce immunity[78,123]. Clinically approved whole tumour lysate preparations for DC vaccines (see later) include hypochlorous acid oxidation, UVB irradiation, freeze–thaw cycles and hyperthermia[124]. Viral vectors are recombinant, replication-deficient or attenuated and mostly RNA or double-stranded DNA viruses that encode TAAs and aim to modify DCs in situ through infection, despite not being DC specific. They show remarkable efficacy in preclinical models and are currently being tested in numerous clinical trials[125].

DC maturation is key for immunogenic antigen presentation[105], as evidenced by the efficacy of viral vectors engineered to promote DC immunity or the coadjuvant effect of GM-CSF[106,107,125]. Hence, efforts combining adjuvants with antigens for in vivo provision are on the rise (FIG. 4c; TABLES 2–4). TAA and/or adjuvants can be attached to and encapsulated in particulate delivery systems such as single and supramolecular peptide conjugates (for example, nanofibres, gels or nanoparticles), liposomes, virosomes or immunostimulatory complexes[126]. The use of self-assembling polymers of degradable biomaterial or nanoparticles in cancer therapy can intrinsically enhance immunogenic DC functions[127]. With regard to DCs, medium-sized nanoparticles (5–100 nm) most efficiently reach the lymph node, and negatively charged adjuvants (such as poly(I:C) and unmethylated CpG oligodeoxynucleotides) are easily internalized in cationic nanoparticles. Notably, negatively charged nanoparticles such as the FDA-approved poly(lactic-*co*-glycolic acid) (PLGA) promote DC maturation, cross-presentation and T_H1 cell polarization[127]. Moreover, viral TAA-encoding vector vaccines are also often designed to co-express costimulatory molecules (for example, the TRICOM vector comprises CD80, CD54 and CD58) or DC activating factors (for example, poly(I:C) or GM-CSF), and recently the FDA granted orphan drug designation to the poly(I:C)-expressing rabies virus-based vaccine YS-ON-001 for treatment of hepatocellular carcinoma and pancreatic cancer[125].

Overall, much has to be learnt about optimal antigens, adjuvants and formulation of TAA-based cancer vaccines for which DCs are a key target to induce specific T cell-mediated cancer immunity. Improved knowledge of DC and T cell functions together with technical advances open exciting possibilities for future therapeutic achievements.

Targeting DCs in vivo for cancer immunotherapy. Targeted delivery of antigens and adjuvants to DCs in vivo can improve antitumour immunity[128] (FIG. 4d; TABLE 4). These therapeutic strategies limit potential side effects and show preclinical efficacy controlling cancer, with the first clinical trials ongoing. C-type lectin receptors (CLRs) show a diverse expression pattern on DCs (TABLE 1) and have been used as preferential target receptors. Examples include the use of DEC205, CLEC9A and langerin to target cDC1s; the use of CLEC4A4 (also known as DCIR2) to target cDC2s; the use of CLEC7A (also known as dectin 1) to target cDC2s and MoDCs; the use of CD209 (also known as DC-SIGN), mannose receptor (MR) and macrophage galactose-type lectin (MGL) to target predominantly cDC2s, MoDCs and macrophages; and the use of CLEC12A to target multiple DC subsets (including cDCs, pDCs and MoDCs)[128]. Of note, antibody-conjugated antigen with adjuvant outperformed non-conjugated antigen[129–131]. Anti-DEC205 antibodies can target a MAGEA3 antigen to human MoDCs, stimulating $CD4^+$ T cell responses[132]. Full-length New York oesophageal squamous cell carcinoma 1 (NY-ESO-1) antigen fused to anti-DEC205 antibodies additionally promotes $CD8^+$ T cell activation, in contrast to uncoupled NY-ESO-1 (REF.[133]). A phase I clinical trial showed that treatment of patients with cancer with cutaneously administered NY-ESO-1 coupled to anti-DEC205 with resiquimod and/or poly-ICLC induced antigen-specific antibodies and T cells and led to partial clinical responses without toxicity[134]. Primary human MoDCs treated with CD209/DC-SIGN-conjugated antigens (and adjuvants) stimulate specific T cell responses ex vivo[135] as well as in humanized mice, limiting cancer growth. Naturally occurring blood-derived pDCs, cDC1s and cDC2s are efficiently targeted ex vivo by (viral) protein antigens conjugated to anti-CLEC12A antibody to induce cross-presentation and $CD8^+$ T cell activation[136]. In addition, TAAs can also be conjugated to non-CLR receptors expressed by DCs or their ligands, such as CD40. For example, viral antigen coupled to anti-CD40 and anti-MR antibodies efficiently stimulates the cross-presentation potential of cDC2s and MoDCs ex vivo[137]. Administration of MUC1 antigen conjugated to oxidized mannan targeting the MR on DCs induces specific antibody and $CD8^+$ T cell responses in patients with breast cancer and increases cancer-free survival[138]. Despite being less DC specific, delivery of anti-CD40 antibody-coupled antigens might at once activate DCs through CD40 ligation and/or enhance cross-presentation due to reduced endosomal degradation of the antigens. Indeed, anti-CD40-mediated targeting of MART1 peptide to MoDCs in vitro outperforms CLR-targeting antibodies in induction of $CD8^+$ T cell responses, but is less potent in activating $CD4^+$ T cells[139]. Moreover, anti-CD40-fused human papillomavirus antigen activates T cells when added to peripheral blood mononuclear cells of patients with cancer and induces $CD8^+$ T cell immunity, resulting in cancer growth control in human CD40 knock-in mice[140]. Also, coupling of anti-CD40 antibodies to TAA-encoding adenovirus-based vectors is currently being pursued to more specifically target DCs in skin[141].

While the amount of TAAs and adjuvants that can be fused to these targeting molecules could be limited, use of polymer nanoparticles is an appealing approach[127] (FIG. 4d; TABLE 4). Human MoDCs efficiently internalize anti-DEC205 antibody-coated PLGA nanoparticles loaded with MART1 peptide and display enhanced cross-priming activity compared with exposure to untargeted nanoparticles[142]. Also, anti-CLEC9A-coated PLGA nanoparticles carrying a GP100 synthetic long peptide induce more robust $CD8^+$ T cell priming ex vivo by human primary blood $CD141^+$ cDC1s, compared with isotype-coated nanoparticles[143].

In summary, delivery of adjuvants and antigens to DCs in vivo by targeting DC-restricted receptors promises to enhance efficacy and reduce side effects of adjuvants (TABLE 4).

DC vaccines for cancer

The use of DC vaccines for cancer has been extensively investigated, with more than 200 completed clinical trials to date (FIG. 4e; TABLE 4). This approach involves the isolation or in vitro generation and amplification of autologous DCs followed by their ex vivo manipulation and reinfusion into patients. These studies were predominantly undertaken in patients with melanoma, prostate cancer, glioblastoma or renal cell carcinoma due to the immunogenic nature of these cancers and, importantly, demonstrated the clinical safety and potency of DC vaccination to induce anticancer NK cell, $CD8^+$ T cell and $CD4^+$ T cell responses. Furthermore, considering that most enrolled patients had advanced cancer after failure of other treatments, the average overall response rate of 8–15% is noteworthy[144–147]. The only clinically approved APC-based vaccine to date is sipuleucel-T (Provenge), which consists of autologous blood APCs loaded with a recombinant fusion protein antigen composed of prostatic acid phosphatase and GM-CSF. It was shown to extend the median overall survival of patients with prostate cancer by about 4 months[148]. Recent scientific advances suggest the efficacy of DC vaccines could be further improved by considering various other factors, which we discuss next.

Influence of DC type. Autologous MoDCs obtained from patient-derived $CD14^+$ blood monocytes or from the differentiation of $CD34^+$ progenitors are effective against different cancer types. Phase III clinical trials using MoDC-based cancer vaccination are ongoing in uveal melanoma (NCT01983748, autologous tumour RNA antigen), castration-resistant prostate cancer (NCT02111577, irradiated prostate cancer cell line antigen) and metastatic colorectal cancer (NCT02503150, autologous tumour lysate), and preliminary results of a large trial (NCT00045968) adding autologous tumour lysate-loaded MoDC vaccination (DCVax-L) to standard treatment of glioblastoma indicate clinical safety and a potential increase in survival[149].

Naturally occurring DC subsets harbour greater antigen-presentation capabilities than do in vitro-generated MoDCs due to higher MHC molecule expression and functional specialization and are proposed as the basis of next-generation vaccines[10,145,147] (TABLES 1,4).

Preclinical mouse studies show the efficacy of primary pDCs to induce $CD8^+$ T cell activation in certain settings[19]. However, in a comparative experimental glioma vaccination study, mouse cDCs, rather than pDCs, were more effective in prolonging survival in tumour-bearing mice[150]. Another comparative study in mice reported the efficacy of prophylactic transfer of tumour-derived cDC1s and cDC2s to reduce growth of a subsequently grafted tumour. cDC1s induce $CD8^+$ T cell-mediated immunity, while preventive vaccination with cDC2s relies on T_H17 cell responses[151].

Advances in natural DC isolation techniques from leukapheresis products led to the first clinical trials in patients with cancer. One clinical trial used enriched blood cDCs and pDCs from patients with melanoma after FLT3L treatment. This personalized DC preparation was stimulated with CD40L and pulsed with cancer germ-line antigen peptides and was found to generate antigen-specific T cell responses[152]. Human blood DC subsets have also been assessed for their suitability for cancer vaccination separately. $CD303^+$ pDCs obtained from leukapheresis products of patients with melanoma induce specific immunity in some patients when loaded with TAA peptides[20]. Two clinical trials reported the safety and feasibility of patient blood-derived $CD1c^+$ cDC2s loaded ex vivo with TAA peptides in prostate cancer and melanoma[153,154]; the latter trial additionally showed vaccine-specific $CD8^+$ T cell responses that correlated with increased progression-free survival in 4 of 14 patients. These studies led to clinical trials using pDCs and/or cDC2s in various cancer settings (NCT02993315, NCT02692976, NCT02574377, NCT03747744 and NCT03707808). Therapeutic transfer of tumour antigen-loaded splenic cDC1s induced notable vaccine-specific $CD8^+$ and $CD4^+$ T cell activation, which relied on their intrinsic cross-presentation potential and led to improved cancer control in mice[32]. However, to our knowledge, the potential of naturally occurring human cDC1s for therapeutic cancer vaccination has not been assessed so far, likely due to their low frequency in peripheral blood, despite their correlation with favourable prognosis[3,5,6,16].

As potential limitations, ex vivo DCs derived from patients with cancer may be dysfunctional (see previous sections)[147,155] and may only represent a small blood cell population (less than 1%)[29]. New cell culture techniques that can generate cells largely equivalent to naturally occurring DC subsets may overcome issues of DC availability[156,157]. Notably, pDCs, cDC1s and cDC2s isolated from patients with breast cancer and healthy controls showed similar cytokine secretion when stimulated with R848 (REF.[155]). Therefore, proper DC activation before reinfusion into patients can overcome potential DC dysfunctions.

Antigen loading of DCs. The ideal antigen for ex vivo DC loading depends on the precise clinical setting (for example, TAA expression and the availability of tumour tissue; TABLE 2); however, the nature of the antigen and its internalization influence the induction and upholding of immune responses by DCs (TABLE 4). Compared with untargeted delivery, coupling of TAAs to DC-specific antibodies promotes cross-presentation by human MoDCs and cDC1s, leading to TAA-specific $CD8^+$ T cell responses[142,143,158]. Adoptive transfer of patient-specific neoantigen-loaded MoDCs to patients with melanoma amplifies the diversity of neoantigen-specific T cells[159], a strategy currently being tested in several clinical trials (for example, NCT03300843, NCT03674073 and NCT01885702). Human MoDCs electrofused with breast cancer cells (as an antigen source) promote stronger $CD8^+$ T cell responses than MoDCs cultured with live cancer cells[160]. In a phase I clinical trial, three antigen-delivery regimes for MoDCs were compared with cocultured DCs and irradiated (dead) melanoma cells, achieving slightly higher immune responses than freeze–thaw melanoma cell lysate or DC–melanoma cell fusion[161].

DC maturation and activation. In the steady state, an important function of DCs is to maintain central and peripheral tolerance, which likely contributed to the disappointing outcomes of the first vaccination attempts with non-activated immature DCs[145]. Indeed, early clinical studies proved the importance of maturation of MoDCs for their migration and induction of effector T cells and led to the creation of MoDC maturation cocktails with diverse activating cues, such as cytokines, PAMPs and DAMPs (TABLE 3). Of note, the nature of these adjuvants and activating agents has to be tailored to each DC subset since their efficacy depends on the PRR profile (TABLE 1).

Route and dose of DC vaccination. Migration of transferred DCs to TDLNs for T cell priming is important for DC vaccination efficacy. This feature is not only influenced by DC maturation and activation but also depends on the injection site. Subcutaneous, intratumoural, intravenous, intradermal, intranodal and, recently, intralymphatic DC vaccine administration routes have been tested[162,163]. While the clinically approved sipuleucel-T vaccine is safely delivered intravenously[148], the most effective fashion of DC delivery is debated and may depend on the DC and cancer type. Intriguingly, the administration route and tissue location of DCs seem to imprint migration cues in responding T lymphocytes to recirculate to cancer tissue[164]. Preconditioning of the DC vaccination site and injection of higher numbers of DCs was suggested to increase vaccine efficacy[145,163], although some studies reported opposite results[165]. However, these differences might rely on the preconditioning stimulus and DC subset. For DC vaccination, the minimal required number of DCs remains to be defined, while the largely limiting factor is commonly sufficient generation/isolation of DCs[166].

Combination treatments. A daunting challenge of DC vaccination and immunotherapy in general is the immunosuppressive microenvironment created by the tumour. Such immunosuppression is influenced by the tumour type and burden, the immunologic fitness of the patient and the immunologic, metabolic and hypoxic features of the TME and is manifested by antigen loss or masking and production of

immunosuppressive mediators/cytokines, among other factors[144–147]. Overcoming this immunosuppression is crucial for improving DC vaccination.

Notably, the action of DCs is associated or even underlies the efficacy of currently used cancer therapies such as ICB, chemotherapy and radiation therapy (discussed in previous sections). Thus, the combination of DC vaccination with those therapies has been proposed[144,167]. Especially, DC vaccination in combination with ICB appears ideal as transferred DCs may foster initial antigen-specific effector T cell activation[145], eventually curtailed by co-inhibitory activity that is tackled by ICB.

Future potential of DC vaccines. In summary, antigen loading and maturation of DCs in a controlled environment ex vivo offers several advantages, such as avoiding tolerogenic signals, a wide selection of usable adjuvants and antigen types (TABLES 2,3) and quality control before inoculation. Some drawbacks include the complexity of optimizing the precise conditions and higher costs due to the need for personalized cell-therapy products (FIG. 4e; TABLE 4). The power and potential of DC vaccination for cancer immunotherapy lies in its clinical safety and its potential synergy with established treatments.

Perspective

Recent successes have fuelled interest in improving antitumour T cell immunity for cancer therapy. DCs are the most potent APCs able to activate naive T cells and can induce immune memory responses in cancer. While DCs are often found to be dysfunctional or tolerogenic in the TME, improved knowledge of how DCs are regulated in this context may allow therapeutic exploitation in several clinical settings. A topic of interest is how different DC subsets may lead to unique functional immune responses in the context of cancer. In this regard, the cDC1 subset is linked to induction of cancer-controlling immunity and increased survival in certain cancer types[3,5–7,12,28,30–33,53]. However, MoDCs are fundamental during treatment with immunogenic cell death-inducing chemotherapy agents and radiation therapy[35–37], and cDC2s are key for induction of antitumour $CD4^+$ T cell immunity[17,151]. DCs can promote the efficacy of established cancer therapies, but the development of optimal vaccination strategies still requires a better understanding of DC biology and functions. Achievements in preclinical studies foster the use of DCs to find more efficient therapeutic treatments in clinical trials. Approaches to achieve this include administration in conjunction with (neo)antigens, mobilization of endogenous DCs and the use of stimulating adjuvants. More refined and precise DC targeting might enhance the efficacy of those strategies. DC vaccination approaches may be particularly effective to delay or prevent both relapse and metastasis after debulking surgical procedures. Overall, we need to learn more about how we can optimally exploit specific DC subsets with specialized functions to orchestrate efficacious immune responses against cancer.

Published online 29 August 2019

1. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
2. Mittal, D., Gubin, M. M., Schreiber, R. D. & Smyth, M. J. New insights into cancer immunoediting and its three component phases-elimination, equilibrium and escape. *Curr. Opin. Immunol.* **27**, 16–25 (2014).
3. 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).
4. 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).
5. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
6. Böttcher, J. P. et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* **172**, 1022–1028.e14 (2018).
7. Barry, K. C. et al. A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments. *Nat. Med.* **24**, 1–14 (2018).
8. Steinman, R. M. Decisions about dendritic cells: past, present, and future. *Annu. Rev. Immunol.* **30**, 1–22 (2011).
9. Collin, M. & Bigley, V. Human dendritic cell subsets: an update. *Immunology* **154**, 3–20 (2018).
10. Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev Immunol* **31**, 563–604 (2013).
11. Schlitzer, A., McGovern, N. & Ginhoux, F. Dendritic cells and monocyte-derived cells: two complementary and integrated functional systems. *Semin. Cell Dev. Biol.* **41**, 9–22 (2015).
12. Böttcher, J. P. & Reis e Sousa, C. The role of type 1 conventional dendritic cells in cancer immunity. *Trends Cancer* **4**, 784–792 (2018).
13. Demoulin, S., Herfs, M., Delvenne, P. & Hubert, P. Tumor microenvironment converts plasmacytoid dendritic cells into immunosuppressive/tolerogenic cells: insight into the molecular mechanisms. *J. Leukoc. Biol.* **93**, 343–352 (2013).
14. Mildner, A. & Jung, S. Development and function of dendritic cell subsets. *Immunity* **40**, 642–656 (2014).
15. Rodrigues, P. F. et al. Distinct progenitor lineages contribute to the heterogeneity of plasmacytoid dendritic cells. *Nat. Immunol.* **19**, 711–722 (2018).
16. 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).
17. Binnewies, M. et al. Unleashing type-2 dendritic cells to drive protective antitumor CD4+ T cell immunity. *Cell* **177**, 556–571.e16 (2019).
18. Villani, A. C. et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* **356**, eaah4573 (2017).
19. Salio, M., Palmowski, M. J., Atzberger, A., Hermans, I. F. & Cerundolo, V. CpG-matured murine plasmacytoid dendritic cells are capable of in vivo priming of functional CD8 T cell responses to endogenous but not exogenous antigens. *J. Exp. Med.* **199**, 567–579 (2004).
20. Tel, J. et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. *Cancer Res.* **73**, 1063–1075 (2013).
21. Chiang, M.-C. et al. Differential uptake and cross-presentation of soluble and necrotic cell antigen by human DC subsets. *Eur. J. Immunol.* **46**, 329–339 (2016).
22. Sittig, S. P. et al. A comparative study of the T cell stimulatory and polarizing capacity of human primary blood dendritic cell subsets. *Mediators Inflamm.* **2016**, 3605643 (2016).
23. 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).
24. Schlitzer, A. et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* **38**, 970–983 (2013).
25. Williams, J. W. et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat. Commun.* **4**, 2990 (2013).
26. Yin, X. et al. Human blood $CD1c^+$ dendritic cells encompass $CD5^{high}$ and $CD5^{low}$ subsets that differ significantly in phenotype, gene expression, and functions. *J. Immunol.* **198**, 1553–1564 (2017).
27. Segura, E. et al. Human inflammatory dendritic cells induce Th17 cell differentiation. *Immunity* **38**, 336–348 (2013).
28. Hildner, K. et al. Batf3 deficiency reveals a critical role for CD8 + dendritic cells in cytotoxic T cell immunity. *Science* **322**, 1097–1100 (2008).
29. Jongbloed, S. L. et al. Human $CD141^+$ (BDCA-3)$^+$ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J. Exp. Med.* **207**, 1247–1260 (2010).
30. 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).
31. Sanchez-Paulete, A. R. et al. Cancer immunotherapy with immunomodulatory anti-CD137 and anti–PD-1 monoclonal antibodies requires BATF3-dependent dendritic cells. *Cancer Discov.* **6**, 71–79 (2016).
32. Wculek, S. K. et al. Effective cancer immunotherapy by natural mouse conventional type-1 dendritic cells bearing dead tumor antigen. *J. Immunother. Cancer* **7**, 100 (2019).
33. Theisen, D. J. et al. WDFY4 is required for cross-presentation in response to viral and tumor antigens. *Science* **362**, 694–699 (2018).
34. Alloatti, A. et al. Critical role for Sec22b-dependent antigen cross-presentation in antitumor immunity. *J. Exp. Med.* **214**, 2231–2241 (2017).
35. Ma, Y. et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* **38**, 729–741 (2013).
36. Casares, N. et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J. Exp. Med.* **202**, 1691–1701 (2005).
37. Apetoh, L. et al. Toll-like receptor 4–dependent contribution of the immune system to anticancer

chemotherapy and radiotherapy. *Nat. Med.* **13**, 1050–1059 (2007).
38. Sánchez-Paulete, A. R. A. R. R. et al. Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy. *Ann. Oncol.* **28**, xii44–xii55 (2017).
39. Novak, L., Igoucheva, O., Cho, S. & Alexeev, V. Characterization of the CCL21-mediated melanoma-specific immune responses and in situ melanoma eradication. *Mol. Cancer Ther.* **6**, 1755–1764 (2007).
40. Shields, J. D., Kourtis, I. C., Tomei, A. A., Roberts, J. M. & Swartz, M. A. Induction of lymphoidlike stroma and immune escape by tumors that express the chemokine CCL21. *Science* **328**, 749–752 (2010).
41. Villablanca, E. J. et al. Tumor-mediated liver X receptor-α activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. *Nat. Med.* **16**, 98–105 (2010).
42. Rowshanravan, B., Halliday, N. & Sansom, D. M. CTLA-4: a moving target in immunotherapy. *Blood* **131**, 58–67 (2018).
43. Saoulli, K. et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. *J. Exp. Med.* **187**, 1849–1862 (1998).
44. Dannull, J. et al. Enhancing the immunostimulatory function of dendritic cells by transfection with mRNA encoding OX40 ligand. *Blood* **105**, 3206–3213 (2005).
45. Cohen, A. D. et al. Agonist anti-GITR antibody enhances vaccine-induced CD8+ T-cell responses and tumor immunity. *Cancer Res.* **66**, 4904–4912 (2006).
46. Buchan, S. L. et al. PD-1 blockade and CD27 stimulation activate distinct transcriptional programs that synergize for CD8 + T-cell–driven antitumor immunity. *Clin. Cancer Res.* **24**, 2383–2394 (2018).
47. Curtsinger, J. M. & Mescher, M. F. Inflammatory cytokines as a third signal for T cell activation. *Curr. Opin. Immunol.* **22**, 333–340 (2010).
48. Leõn, B., Lõpez-Bravo, M. & Ardavín, C. Monocyte-derived dendritic cells formed at the infection site control the induction of protective T helper 1 responses against Leishmania. *Immunity* **26**, 519–531 (2007).
49. Martínez-López, M., Iborra, S., Conde-Garrosa, R. & Sancho, D. Batf3-dependent CD103 + dendritic cells are major producers of IL-12 that drive local Th1 immunity against Leishmania major infection in mice. *Eur. J. Immunol.* **45**, 119–129 (2015).
50. Nizzoli, G. et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* **122**, 932–942 (2013).
51. Parker, B. S., Rautela, J. & Hertzog, P. J. Antitumour actions of interferons: implications for cancer therapy. *Nat. Rev. Cancer* **16**, 131–144 (2016).
52. Woo, S.-R. et al. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. *Immunity* **41**, 830–842 (2014).
53. 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).
54. Enamorado, M. et al. Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8+ T cells. *Nat. Commun.* **8**, 16073 (2017).
55. 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).
56. Chemnitz, J. M., Parry, R. V., Nichols, K. E., June, C. H. & Riley, J. L. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *J. Immunol.* **173**, 945–954 (2004).
57. Flies, D. B. et al. Coinhibitory receptor PD-1H preferentially suppresses CD4+ T cell–mediated immunity. *J. Clin. Invest.* **124**, 1966–1975 (2014).
58. Clement, M. et al. CD31 is a key coinhibitory receptor in the development of immunogenic dendritic cells. *Proc. Natl Acad. Sci. USA* **111**, E1101–E1110 (2014).
59. Fallarino, F. et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol.* **4**, 1206–1212 (2003).
60. Munn, D. H. & Mellor, A. L. IDO in the tumor microenvironment: inflammation, counter-regulation, and tolerance. *Trends Immunol.* **37**, 193–207 (2016).
61. Zelenay, S. et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* **162**, 1257–1270 (2015).
62. Ohm, J. E. et al. Effect of vascular endothelial growth factor and FLT3 ligand on dendritic cell generation in vivo. *J. Immunol.* **163**, 3260–3268 (1999).
63. Tang, M. et al. Toll-like receptor 2 activation promotes tumor dendritic cell dysfunction by regulating IL-6 and IL-10 receptor signaling. *Cell Rep.* **13**, 2851–2864 (2015).
64. Zong, J., Keskinov, A. A., Shurin, G. V. & Shurin, M. R. Tumor-derived factors modulating dendritic cell function. *Cancer Immunol. Immunother.* **65**, 821–833 (2016).
65. Johnson, D. E., O'Keefe, R. A. & Grandis, J. R. Targeting the IL-6/JAK/STAT3 signalling axis in cancer. *Nat. Rev. Clin. Oncol.* **15**, 234–248 (2018).
66. Diao, J., Zhao, J., Winter, E. & Cattral, M. S. Recruitment and differentiation of conventional dendritic cell precursors in tumors. *J. Immunol.* **184**, 1261–1267 (2010).
67. Yanai, H. et al. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* **462**, 99–103 (2009).
68. 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).
69. Xu, M. M. et al. Dendritic cells but not macrophages sense tumor mitochondrial DNA for cross-priming through signal regulatory protein α signaling. *Immunity* **47**, 363–373.e5 (2017).
70. Nirschl, C. J. et al. IFNγ-dependent tissue-immune homeostasis is co-opted in the tumor microenvironment. *Cell* **170**, 127–141 (2017).
71. Cubillos-Ruiz, J. R. et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell* **161**, 1527–1538 (2015).
72. Cao, W. et al. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. *J. Immunol.* **192**, 2920–2931 (2014).
73. Veglia, F. et al. Lipid bodies containing oxidatively truncated lipids block antigen cross-presentation by dendritic cells in cancer. *Nat. Commun.* **8**, 2122 (2017).
74. Gottfried, E. et al. Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood* **107**, 2013–2021 (2006).
75. 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 Immunol. Res.* **1**, 402–415 (2013).
76. Combes, A. et al. BAD-LAMP controls TLR9 trafficking and signalling in human plasmacytoid dendritic cells. *Nat. Commun.* **8**, 913 (2017).
77. Humbert, M., Guery, L., Brighouse, D., Lemeille, S. & Hugues, S. Intratumoral CpG-B promotes anti-tumoral neutrophil, cDC, and T cell cooperation without reprograming tolerogenic pDC. *Cancer Res.* **78**, 3280–3292 (2018).
78. Galluzzi, L., Buqué, A., Kepp, O., Zitvogel, L. & Kroemer, G. Immunogenic cell death in cancer and infectious disease. *Nat. Rev. Immunol.* **17**, 97–111 (2017).
79. Obeid, M. et al. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat. Med.* **13**, 54–61 (2007).
80. Ghiringhelli, F. et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1B-dependent adaptive immunity against tumors. *Nat. Med.* **15**, 1170–1178 (2009).
81. Vacchelli, E. et al. Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. *Science.* **350**, 972–978 (2015).
82. Lesterhuis, W. J. et al. Platinum-based drugs disrupt STAT6-mediated suppression of immune responses against cancer in humans and mice. *J. Clin. Invest.* **121**, 3100–3108 (2011).
83. Rodriguez-Ruiz, M. E. et al. Abscopal effects of radiotherapy are enhanced by combined immuno-stimulatory mAbs and are dependent on CD8 T cells and crosspriming. *Cancer Res.* **76**, 5994–6005 (2016).
84. Deng, L. et al. STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. *Immunity* **41**, 843–852 (2014).
85. Vanpouille-Box, C. et al. DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat. Commun.* **8**, 15618 (2017).
86. Hou, Y. et al. Non-canonical NF-κB antagonizes STING sensor-mediated DNA sensing in radiotherapy. *Immunity* **49**, 490–503 (2018).
87. Ott, P. A. & Adams, S. Small-molecule protein kinase inhibitors and their effects on the immune system: Implications for cancer treatment. *Immunotherapy* **3**, 213–227 (2011).
88. Nefedova, Y. et al. Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. *J. Immunol.* **175**, 4338–4346 (2005).
89. Oosterhoff, D. et al. Tumor-mediated inhibition of human dendritic cell differentiation and function is consistently counteracted by combined p38 MAPK and STAT3 inhibition. *Oncoimmunology* **1**, 649–658 (2012).
90. Nefedova, Y. et al. Hyperactivation of STAT3 is involved in abnormal differentiation of dendritic cells in cancer. *J. Immunol.* **172**, 464–474 (2004).
91. Li, H. S. et al. Bypassing STAT3-mediated inhibition of the transcriptional regulator ID2 improves the antitumor efficacy of dendritic cells. *Sci. Signal.* **9**, ra94 (2016).
92. Zhao, F. et al. Activation of p38 mitogen-activated protein kinase drives dendritic cells to become tolerogenic in *ret* transgenic mice spontaneously developing melanoma. *Clin. Cancer Res.* **15**, 4382–4390 (2009).
93. Liang, X. et al. β-Catenin mediates tumor-induced immunosuppression by inhibiting cross-priming of CD8+ T cells. *J. Leukoc. Biol.* **95**, 179–190 (2014).
94. Fu, C. et al. β-Catenin in dendritic cells exerts opposite functions in cross-priming and maintenance of CD8+ T cells through regulation of IL-10. *Proc. Natl Acad. Sci. USA* **112**, 2823–2828 (2015).
95. Wang, Y., Wang, X. Y., Subjeck, J. R., Shrikant, P. A. & Kim, H. L. Temsirolimus, an mTOR inhibitor, enhances anti-tumour effects of heat shock protein cancer vaccines. *Br. J. Cancer* **104**, 643–652 (2011).
96. Wang, H. et al. cGAS is essential for the antitumor effect of immune checkpoint blockade. *Proc. Natl Acad. Sci. USA* **114**, 1637–1642 (2017).
97. Cauwels, A. et al. Delivering type I interferon to dendritic cells empowers tumor eradication and immune combination treatments. *Cancer Res.* **78**, 463–474 (2018).
98. Ribas, A. et al. SD-101 in combination with pembrolizumab in advanced melanoma: results of a phase Ib, multicenter study. *Cancer Discov.* **8**, 1250–1257 (2018).
99. Rapp, M. et al. C-C chemokine receptor type-4 transduction of T cells enhances interaction with dendritic cells, tumor infiltration and therapeutic efficacy of adoptive T cell transfer. *Oncoimmunology* **5**, e1105428 (2016).
100. Marigo, I. et al. T cell cancer therapy requires CD40-CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells. *Cancer Cell* **30**, 377–390 (2016).
101. Routy, B. et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2018).
102. Matson, V. et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science.* **359**, 104–108 (2018).
103. Zitvogel, L. et al. Cancer and the gut microbiota: an unexpected link. *Sci. Transl Med.* **7**, 271ps1 (2015).
104. Uribe-Herranz, M. et al. Gut microbiota modulates adoptive cell therapy via CD8α dendritic cells and IL-12. *JCI Insight* **3**, 94952 (2018).
105. Saxena, M. & Bhardwaj, N. Turbocharging vaccines: emerging adjuvants for dendritic cell based therapeutic cancer vaccines. *Curr. Opin. Immunol.* **47**, 35–43 (2017).
106. 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).
107. Yan, W.-L., Shen, K.-Y., Tien, C.-Y., Chen, Y.-A. & Liu, S.-J. Recent progress in GM-CSF-based cancer immunotherapy. *Immunotherapy* **9**, 347–360 (2017).
108. Saito, T. et al. Combined mobilization and stimulation of tumor-infiltrating dendritic cells and natural killer cells with Flt3 ligand and IL-18 in vivo induces systemic antitumor immunity. *Cancer Sci.* **99**, 2028–2036 (2008).
109. Chi, H. et al. Anti-tumor activity of Toll-like receptor 7 agonists. *Front. Pharmacol.* **8**, 304 (2017).
110. Jiang, L. et al. The combination of MBP and BCG-induced dendritic cell maturation through TLR2/TLR4 promotes Th1 activation in vitro and vivo. *Mediators Inflamm.* **2017**, 1953680 (2017).
111. 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. *Immun.* **44**, 924–938 (2016).
112. Martins, K. A. O., Bavari, S. & Salazar, A. M. Vaccine adjuvant uses of poly-IC and derivatives. *Expert. Rev. Vaccines* **14**, 447–459 (2015).
113. Aznar, M. A. et al. Immunotherapeutic effects of intratumoral nanoplexed poly I:C. *J. Immunother. Cancer* **7**, 116 (2019).

114. Kyi, C. et al. Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. *Clin. Cancer Res.* **24**, 4937–4948 (2018).
115. Drobits, B. et al. Imiquimod clears tumors in mice independent of adaptive immunity by converting pDCs into tumor-killing effector cells. *J. Clin. Invest.* **122**, 575–585 (2012).
116. Molenkamp, B. G. et al. Local administration of PF-3512676 CpG-B instigates tumor-specific CD8+ T-cell reactivity in melanoma patients. *Clin. Cancer Res.* **14**, 4532–4542 (2008).
117. Moon, Y. W., Hajjar, J., Hwu, P. & Naing, A. Targeting the indoleamine 2,3-dioxygenase pathway in cancer. *J. Immunother. Cancer* **3**, 1–10 (2015).
118. Finn, O. J. Human tumor antigens yesterday, today, and tomorrow. *Cancer Immunol. Res.* **5**, 347–354 (2017).
119. Sahin, U. & Türeci, Ö. Personalized vaccines for cancer immunotherapy. *Science* **359**, 1355–1360 (2018).
120. Snyder, A. et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
121. Rizvi, N. A. et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128 (2015).
122. Balachandran, V. P. et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. *Nature* **551**, 512–516 (2017).
123. Garg, A. D. et al. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell-driven rejection of high-grade glioma. *Sci. Transl Med.* **8**, 328ra27 (2016).
124. Chiang, C., Coukos, G. & Kandalaft, L. Whole tumor antigen vaccines: where are we? *Vaccines* **3**, 344–372 (2015).
125. 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).
126. Moyer, T. J., Zmolek, A. C. & Irvine, D. J. Beyond antigens and adjuvants: formulating future vaccines. *J. Clin. Invest.* **126**, 799–808 (2016).
127. Chesson, C. B. & Zloza, A. Nanoparticles: augmenting tumor antigen presentation for vaccine and immunotherapy treatments of cancer. *Nanomedicine* **12**, 2693–2706 (2017).
128. Kreutz, M., Tacken, P. J. & Figdor, C. G. Targeting dendritic cells-why bother? *Blood* **121**, 2836–2844 (2013).
129. Bonifaz, L. C. et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J. Exp. Med.* **199**, 815–824 (2004).
130. Idoyaga, J. et al. Comparable T helper 1 (Th1) and CD8 T-cell immunity by targeting HIV gag p24 to CD8 dendritic cells within antibodies to Langerin, DEC205, and Clec9A. *Proc. Natl Acad. Sci. USA.* **108**, 2384–2389 (2011).
131. 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).
132. Birkholz, K. et al. Targeting of DEC-205 on human dendritic cells results in efficient MHC class II-restricted antigen presentation. *Blood* **116**, 2277–2285 (2010).
133. Tsuji, T. et al. Antibody-targeted NY-ESO-1 to mannose receptor or DEC-205 in vitro elicits dual human CD8+ and CD4+ T cell responses with broad antigen specificity. *J. Immunol.* **186**, 1218–1227 (2011).
134. Dhodapkar, M. V. et al. Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. *Sci. Transl Med.* **6**, 1–10 (2014).
135. Tacken, P. J. et al. Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti-DC-SIGN antibody. *Blood* **106**, 1278–1285 (2005).
136. Hutten, T. J. A. et al. CLEC12A-mediated antigen uptake and cross-presentation by human dendritic cell subsets efficiently boost tumor-reactive t cell responses. *J. Immunol.* **197**, 2715–2725 (2016).
137. Chatterjee, B. et al. Internalization and endosomal degradation of receptor-bound antigens regulate the efficiency of cross presentation by human dendritic cells. *Blood* **120**, 2011–2020 (2012).
138. Apostolopoulos, V. et al. Dendritic cell immunotherapy: clinical outcomes. *Clin. Transl Immunol.* **3**, e21 (2014).
139. Yin, W. et al. Functional specialty of CD40 and dendritic cell surface lectins for exogenous antigen presentation to CD8+ and CD4+ T cells. *EBioMedicine* **5**, 46–58 (2016).
140. Yin, W. et al. Therapeutic HPV cancer vaccine targeted to CD40 elicits effective CD8+ T-cell immunity. *Cancer Immunol. Res.* **4**, 823–834 (2016).
141. Hangalapura, B. N. et al. CD40-targeted adenoviral cancer vaccines: the long and winding road to the clinic. *J. Gene Med.* **14**, 416–427 (2012).
142. Saluja, S. S. et al. Targeting human dendritic cells via DEC-205 using PLGA nanoparticles leads to enhanced cross-presentation of a melanoma-associated antigen. *Int. J. Nanomed.* **9**, 5231–5246 (2014).
143. Schreibelt, G. et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells. *Blood* **119**, 2284–2292 (2012).
144. Bol, K. F., Schreibelt, G., Gerritsen, W. R., De Vries, I. J. M. & Figdor, C. G. Dendritic cell-based immunotherapy: state of the art and beyond. *Clin. Cancer Res.* **22**, 1897–1906 (2016).
145. Garg, A. D., Coulie, P. G., Van den Eynde, B. J. & Agostinis, P. Integrating next-generation dendritic cell vaccines into the current cancer immunotherapy landscape. *Trends Immunol.* **38**, 577–593 (2017).
146. Melero, I. et al. Therapeutic vaccines for cancer: an overview of clinical trials. *Nat. Rev. Clin. Oncol.* **11**, 509–524 (2014).
147. Saxena, M. & Bhardwaj, N. Re-emergence of dendritic cell vaccines for cancer treatment. *Trends Cancer* **4**, 119–137 (2018).
148. Cheever, M. A. & Higano, C. S. PROVENGE (sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clin. Cancer Res.* **17**, 3520–3526 (2011).
149. 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**, 1 (2018).
150. Dey, M. et al. Dendritic cell–based vaccines that utilize myeloid rather than plasmacytoid cells offer a superior survival advantage in malignant glioma. *J. Immunol.* **195**, 367–376 (2015).
151. Laoui, D. et al. The tumour microenvironment harbours ontogenically distinct dendritic cell populations with opposing effects on tumour immunity. *Nat. Commun.* **7**, 13720 (2016).
152. Davis, I. D. et al. Blood dendritic cells generated with Flt3 ligand and CD40 ligand prime $CD8^+$ T cells efficiently in cancer patients. *J. Immunother.* **29**, 499–511 (2006).
153. Prue, R. L. et al. A phase I clinical trial of CD1c (BDCA-1)+ dendritic cells pulsed with HLA-A*0201 peptides for immunotherapy of metastatic hormone refractory prostate cancer. *J. Immunother.* **38**, 71–76 (2015).
154. Schreibelt, G. et al. Effective clinical responses in metastatic melanoma patients after vaccination with primary myeloid dendritic cells. *Clin. Cancer Res.* **22**, 2155–2166 (2016).
155. Verronèse, E. et al. Immune cell dysfunctions in breast cancer patients detected through whole blood multi-parametric flow cytometry assay. *Oncoimmunology* **5**, 1–15 (2016).
156. Kirkling, M. E. et al. Notch signaling facilitates in vitro generation of cross-presenting classical dendritic cells. *Cell Rep.* **23**, 3658–3672 (2018).
157. Balan, S. et al. Large-scale human dendritic cell differentiation revealing notch-dependent lineage bifurcation and heterogeneity. *Cell Rep.* **24**, 1902–1915 (2018).
158. Moeller, I., Spagnoli, G. C., Finke, J., Veelken, H. & Houet, L. Uptake routes of tumor-antigen MAGE-A3 by dendritic cells determine priming of naïve T-cell subtypes. *Cancer Immunol. Immunother.* **61**, 2079–2090 (2012).
159. Carreno, B. M. et al. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* **348**, 803–808 (2015).
160. Pinho, M. P. et al. Dendritic-tumor cell hybrids induce tumor-specific immune responses more effectively than the simple mixture of dendritic and tumor cells. *Cytotherapy* **18**, 570–580 (2016).
161. Geskin, L. J. et al. Three antigen-loading methods in dendritic cell vaccines for metastatic melanoma. *Melanoma Res.* **28**, 211–221 (2018).
162. Radomski, M. et al. Prolonged intralymphatic delivery of dendritic cells through implantable lymphatic ports in patients with advanced cancer. *J. Immunother. Cancer* **4**, 1–9 (2016).
163. Seyfizadeh, N., Muthuswamy, R., Mitchell, D. A., Nierkens, S. & Seyfizadeh, N. Migration of dendritic cells to the lymph nodes and its enhancement to drive anti-tumor responses. *Crit. Rev. Oncol. Hematol.* **107**, 100–110 (2016).
164. Sandoval, F. et al. Mucosal imprinting of vaccine-induced CD8+ T cells is crucial to inhibit the growth of mucosal tumors. *Sci. Transl Med.* **5**, 172ra20 (2013).
165. Aarntzen, E. H. J. G. et al. Targeting of ^{111}In-labeled dendritic cell human vaccines improved by reducing number of cells. *Clin. Cancer Res.* **19**, 1525–1533 (2013).
166. Butterfield, L. H. Dendritic cells in cancer immunotherapy clinical trials: are we making progress? *Front. Immunol.* **4**, 3–9 (2013).
167. Van Willigen, W. W. et al. Dendritic cell cancer therapy: vaccinating the right patient at the right time. *Front. Immunol.* **9**, 2265 (2018).

Acknowledgements

The authors thank all members of the D.S. laboratory at Centro Nacional de Investigaciones Cardiovasculares (CNIC) for scientific discussions. S.K.W. is supported by a European Molecular Biology Organization Long-Term Fellowship (grant ALTF 438–2016) and a CNIC–International Postdoctoral Program Fellowship (grant 17230–2016). F.J.C. is the recipient of a PhD 'La Caixa' fellowship. Work in the D.S. laboratory is funded by the CNIC, by the European Research Council (ERC Consolidator Grant 2016 725091), by the European Commission (635122-PROCROP H2020), by the Ministerio de Ciencia, Innovación e Universidades (MCNU), Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (FEDER) (SAF2016-79040-R), by the Comunidad de Madrid (B2017/BMD-3733 Immunothercan-CM), by FIS-Instituto de Salud Carlos III, MCNU and FEDER (RD16/0015/0018-REEM), by Acteria Foundation, by Atresmedia (Constantes y Vitales prize) and by Fundació La Marató de TV3 (201723). The CNIC is supported by the Instituto de Salud Carlos III, the MCNU and the Pro CNIC Foundation, and is a Severo Ochoa Centre of Excellence (SEV-2015-0505).

Author contributions

F.J.C. and S.K.W. contributed equally to this work and share first authorship. S.K.W. and F.J.C. prepared tables and figures and conceptualized and wrote the manuscript. A.M.M. and M.F.K. conceptualized and wrote part of the manuscript. I.M. helped with conceptualization and edited the manuscript. D.S. conceptualized and wrote the manuscript. All authors contributed to manuscript editing, and read and approved the final version.

Competing interests

I.M. reports receiving commercial research grants from Bristol-Myers Squibb and Roche and serves as a consultant/advisory board member for Bristol-Myers Squibb, Merck Serono, Roche-Genentech, Genmab, Incyte, Bioncotech, Tusk, Molecular Partners, F-STAR, Alligator and AstraZeneca. The other authors declare no competing interests.

Publisher's note

Reviewer information

Nature Reviews Immunology thanks V. Bigley, T. de Gruijl and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.