Dendritic cells in cancer immunology and immunotherapy

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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. An increased understanding of DC subset diversity, functions and of how those 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.

Introduction

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 no longer detected by the immune system[2]. The association of infections with spontaneous tumour regressions and the capacity of the immune system to reject immunogenic tumours in preclinical models[1] supports the role of the immune system in protection against cancers. Moreover, large-scale projects such as The Human Cancer Genome 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. DCs promote immunity or tolerance by sampling and presenting antigens to T cells and through the secretion of immunomodulatory cytokines[9,10]. These DC functions are determined by their integration of environmental signals, which are sensed via surface-expressed and intracellular receptors for cytokines and pathogen- or danger-associated molecular patterns (PAMPs and DAMPs)[11]. Furthermore, specific DC subsets may play distinct roles in antitumour immunity, with key implications for therapy[12,13]. In this Review, we will discuss the functions of

different DC subsets in the tumour microenvironment (TME) and consider how these populations could be manipulated for therapy.

1. DCs in cancer immunology

Diversity within DCs. 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, CD8a/CD103+ cDC1s and CD11b+ cDC2s (**Table 1**)[10,14]. B220+ plasmacytoid DCs (pDCs) develop from both CDPs and lymphoid progenitors, yielding functionally distinct pDC subsets[15]. Additionally, inflammatory conditions can lead to the CC chemokine receptor type 2 (CCR2)-dependent recruitment of monocytes from the blood that differentiate into monocyte-derived DCs (MoDCs) in peripheral tissues[9,11]. Notably, human DC subsets (CD141+ cDC1s, CD1c+ cDC2s and CD123+ pDCs) closely resemble their mouse counterparts in transcriptional and main functional analyses[9,16] (**Table 1**).

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 prime CD8+ T cells[17–19]. 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 T helper 1 (Th1) cell responses[10,11,14,19]. Regarding the heterogeneous cDC2 subset, analysis of IRF4-

-/- mice (which lack cDC2s) suggests that these DCs are potent inducers of CD4+ T cell responses[20,21]. In addition, MoDCs are predominantly generated in response to inflammation and promote context-dependent differentiation of CD4+ T cells towards a Th1, Th2 or Th17 cell phenotype[22]. In the TME, DCs acquire, process and present tumour-associated antigens (TAAs) on MHC molecules (signal 1), provide costimulation (signal 2) and soluble factors (signal 3), to shape T cell responses (**Figure 1**). Below, we discuss how these DC functions within the TME and tumour-draining lymph nodes (TDLNs) can promote immunity or tolerance to tumour cells.

Promotion of antitumour immunity by DCs.

Both tumour-infiltrating DCs and DCs in TDLNs contribute to the antitumour immune response[23,24]. As CD8+ T cells are often the main effectors of antitumour immunity, fostering DC cross-presentation is paramount. cDC1s are associated with superior cross-presentation of antigens, which results in stronger CD8+ T cell immunity, and cDC1s additionally support Th1 polarization of CD4 T cell responses[3,25–28]. BATF3-dependent cDC1s are essential for the rejection of highly immunogenic tumours[25]. This is mediated by their cross-presentation of TAAs and is dependent on the regulator of vesicular trafficking WDFY4[29]. DCs also require the SNARE protein SEC22B for efficient handling and cross-presentation of antigen, leading to protection against immunogenic tumours[30]. By contrast, cDC2s and MoDCs are fundamental for presenting TAAs following treatment with certain cancer chemotherapies, such as anthracyclins[31–33].

Upon sensing of appropriate cues, DCs mature and express costimulatory molecules, such as CD80 and CD86, which control the activation or suppression

of T cells through interaction with CD28 or CTLA4, respectively[34]. Other costimulatory pathways involved in DC priming are a focus of research to tailor T cell-mediated immunity in cancer immunotherapy, including CD40-CD40L, CD137-CD137L, OX40-OX40L, GITR-GITRL and CD70-CD27 (**Figure 1**). CD40 on DCs interacts with CD40L on T cells, leading to DC activation[35]. CD137L (also known as 4-1BBL) is expressed on antigen-presenting cells (APCs) and promotes the activation of CD4+ and CD8+ T cells through CD137[36]. OX40L on DCs and macrophages contributes to T cell survival, thereby favouring antitumour immunity[37]. GITRL on DCs promotes CD8+ T cell immunity and the resistance of T cells to regulatory T (Treg) cell-mediated immunosuppression[38]. Finally, CD70 on DCs supports CD8+ T cell cross-priming and antitumour immunity[39].

The effector activity of T cells depends on DC-derived cytokines, including IL-12 and type I IFNs[40] (**Figure 1**). In mice, IL-12 is mainly generated by cDC1s and contributes to Th1 and CD8+ T cell priming[3,4,41]. In humans, both CD141+ cDC1s and CD1c+ cDC2s can produce IL-12 upon TLR stimulation[26,42], but IL-12 levels within human cancers are associated with increased cDC1 infiltration[4]. Type I IFNs are in clinical use to treat patients with cancer[43] and the sensing of nucleic acids through the cGAS–STING pathway is fundamental for DC activation and type I IFN production in antitumour immunity[44,45]. DCs can also produce chemokines in the TME that attract T cells. For example, tumour-infiltrating cDC1s are the main producers of CXCL9 and CXCL10, which promote the recruitment of CD8+ T cells into the TME[45]. Taken together, 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 promote tolerance through DCs. Presentation of TAAs in the absence of costimulatory signals leads to T cell anergy[8], and high engagement of inhibitory receptors can limit T cell effector activity (**Figure 1**). CTLA4 expressed on T cells binds CD80 and CD86 on DCs with greater affinity than CD28, limiting costimulatory signalling and T cell activation[34]. PDL1 and PDL2 on DCs and other cells in the TME also inhibit proliferation and cytokine production by PD1-expressing activated T cells[46]. VISTA is another inducible member of the PD1 family that is expressed by DCs and constrains T cell antitumour immunity[47]. CD31, a transhomophilic coinhibitory molecule, induces a tolerogenic phenotype in DCs, skewing T cell priming towards Treg cell generation, instead of Th1 cell induction[48].

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) (**Figure 1**). IDO1 is induced in DCs upon their recognition of apoptotic cells or following binding of CTLA4 by CD80 and CD86[49]. Notably, increased IDO1 expression is observed in tumour-associated DCs[50], and DC-expressed IDO1 suppresses the proliferation and effector functions of CD8+ T cells, NK cells and plasma cells and contributes to the differentiation of Treg cells[50].

1.1. 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 (**Figure 2**). 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 survival. However, increased infiltration of cDC1s into 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. 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 E2, and this in turn limits cDC1 density and favours tumour growth[6,51].

The TME also curbs DC development and differentiation. Tumour-infiltrating lymphocytes, particularly NK cells, are the predominant producers of FLT3L in the TME[7], and this cytokine is essential for cDC development and proliferation *in situ* [10,27]. Tumour-derived factors such as VEGF can inhibit FLT3L activity and negatively impact cDC differentiation *in vitro*[52]. Tumour-derived gangliosides and prostanoids also inhibit cDC maturation and survival, as well as

MoDC differentiation[53]. As cDC precursors are found in the TME[54], tumour-derived factors could also affect local pre-DC differentiation.

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. 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 HMGB1[33]. HMGB1 recruits nucleic acids into DC endosomes, mediating the innate sensing of nucleic acids from dead tumour cells[55]. This activating axis is prevented in tumour-infiltrating cDCs through high expression of TIM3, which sequesters HMGB1[56]. CD47 expression in tumours inhibits detection of cancer cell-released mitochondrial DNA by signal regulatory protein α (SIRPα) on cDC2s that otherwise induces type I IFNs[57]. 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 STAT3 hyperphosphorylation in DCs and immunosuppression[58]. In addition, macrophages within tumours are a primary source of IL-10 that can abrogate IL-12 production by cDC1s[4]. Chronic exposure of tumour-infiltrating mononuclear phagocytes to IFNγ promotes a transcriptional programme that contributes to immune evasion in a SOCS2-dependent manner[59]. Moreover, metabolites in the

TME can dampen DC function; for example, lactic acid is a metabolic product of tumour cells that impairs MoDC differentiation and activation[60].

Other TME components can also impair cross-presentation of TAAs. For instance, lipid peroxidation byproducts promote endoplasmic reticulum (ER) stress in tumour-associated cDCs, and constitutive activation of the ER stress sensor IRE1α leads to lipid accumulation and reduced T cell activation[61]. Indeed, lipid-laden cDCs show defective processing of exogenous antigen and impaired cross-presentation in cancer[62]. Incorporation of oxidated lipids into cDC lipid bodies inhibits trafficking of peptide–MHC-I complexes to the cell surface[63].

Notably, the ability of pDCs to promote antitumour immunity through production of type I IFN is also inhibited by immunosuppressive factors in the TME[13]. In fact, 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 Treg cell populations in an ICOSL-dependent manner[64]. Tumour-associated pDCs also fail to produce type I IFN in response to TLR9 ligands due to the relocation of TLR9 to late endosomal compartments[65]. However, the antitumour capacity of pDCs can be rescued by stimulation with TLR7 ligands[17,18].

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. In the following section, we consider how different cancer therapies can modulate DC functions to boost antitumour immunity.

2. DCs in the context of cancer therapy

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

2.1. *Chemotherapy and DCs.*

Certain chemotherapeutics used in the clinic — including bortezomib, doxorubicin, epirubicin, idarubicin and mitoxantrone, and oxaliplatin — trigger immunogenic cell death that promotes antitumour immunity[66], and these responses depend on DCs[32] (**Figure 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[67]. Immunogenic death of tumour cells also leads to the release of ATP that promotes DC recruitment (through P2RY2) and activation of the NLRP3 inflammasome (through P2RX7)[68] leading to IL-1β production. ATP also initiates a cell-intrinsic type I IFN 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[69]. 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[33]. Indeed, anthracyclin-induced cell death promotes MoDC recruitment into the TME, and these cells cross-present TAAs to CD8+ T cells[31] (**Figure 3A**). Thus, chemotherapy-induced immunogenic death of cancer cells leads to the release

of stimulatory factors that enhance DC activation and cross-presentation of TAAs, thereby improving antitumour CD8+ T cell responses[24].

However, not all chemotherapies act on DCs by inducing immunogenic cell death and there are additional effects that can influence anti-tumour immunity. Chemotherapy with platinum-based drugs reduces PDL2 expression by DCs and cancer cells, which skews T cell responses towards Th1 cell differentiation and increases TAA-specific T cells[70]. The therapeutic efficacy of paclitaxel, however, is restricted by tumour-associated macrophage production of IL-10, which inhibits IL-12 production by DCs[4]. Thus, different chemotherapeutic agents seem to depend on specific DC subsets and their efficacy may be potentiated accordingly.

2.2. *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 ROS production, release of DAMPs and cytotoxic mediators as well as 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[66] that rely on cDC1 priming of CD8+ T cells[71] (**Figure 3B**). Cytosolic DNA released by cancer cells upon radiation therapy acts as a DAMP and signals through cGAS–STING to induce the production of type I IFN by DCs, contributing to antitumour immunity[72]. However, high non-fractionated radiation doses induce the expression of the

DNase TREX1, which degrades cytosolic DNA and limits its immunostimulatory effect on cDC1s[73]. Additionally, although canonical NF-kB signalling is necessary for the antitumour immune responses induced by radiation therapy, non-canonical NF-kB signalling dampens antitumour immunity by inhibiting STING-mediated induction of type I IFNs[74].

2.3. *Small-molecule inhibitors and DCs.*

Small-molecule inhibitors target key oncogenic signalling pathways — such as the MAPK and PI3K–AKT–mTOR pathways — in tumour cells, but can also affect immune cells. Activation of STAT3 generates a type of inflammation that promotes tumour growth and also inhibits DC-mediated antitumour immune responses[75]. Together with MAPKs, STAT3 signalling leads to the production of IL-10, IL-6 and VEGF, which inhibit IL-12 production by human MoDCs. The STAT3 inhibitor JSI-124 can revert abnormal DC function in cancer[76] and, accordingly, mice with a STAT3 deficiency restricted to CD11c-expressing cells show resistance to tumour growth[77]. Compounds targeting the signaling upstream of STAT3 have been approved for therapy of certain rare cancers and STAT3 inhibitors are evaluated in clinical trials[78] (**Table 3**). Activation of the Wnt–β-catenin pathway in DCs leads to immunosuppression[79], in part through an mTOR–IL10-dependent pathway[80]. Consistently, the mTOR inhibitor temsirolimus enhances the efficacy of DC vaccination[81]. The tyrosine kinase inhibitors sorafenib and sunitinib target similar pathways that include signalling downstream of VEGFR, PDGFR, FLT3 and KIT. Sorafenib mitigates the inhibitory effect of renal carcinoma cells on DCs[82]; however, as sorafenib and sunitinib also

target FLT3, which favours DC population expansion (**Table 1**), their global effects on DCs in the context of antitumour immunity need to be further explored.

2.4. *Immune checkpoint therapy and DCs.*

Antibodies that block inhibitory pathways (such as the PD1–PDL1 axis) or that trigger activation receptors on T cells (such as CD137) can amplify basal antitumour immune responses that were initially primed by DCs, with a significant contribution of the cDC1 subset (**Figure 3C**). Experimental melanomas with stabilized β-catenin signalling associate with reduced cDC1 tumour infiltration and irresponsiveness to immune checkpoint blockade (ICB) therapy, which was rescued by transfer of preactivated cDC1s[5]. Moreover, tumours grafted onto BATF3-deficient mice, which lack cDC1s, did not respond to anti-PD1, anti-PDL1 or anti-CD137 treatments[27,28], and SEC22B-mediated cross-presentation of TAAs by DCs is necessary for effective PD1 blockade therapy[30]. In fact, 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 be further improved by FLT3L-mediated DC expansion[27,28]. Further, both cGAS and STING are necessary for intrinsic antitumour immunity and efficient responses to anti-PDL1, which is at least partially mediated by DCs[83]. Targeting of type I IFNs to activate cDC1s also improves anti-PDL1 treatment[84], suggesting that tumour DCs may require activation to support ICB-induced effector T cell activity.

In turn, ICB promotes DC accumulation within the TME. Combining pembrolizumab (anti-PD1) treatment with TLR9 agonists associates with an elevated tumour-infiltrating DC signature and, preliminarily, clinical benefit[85].

Also, expression of checkpoint counterreceptors may be more critical on DCs than tumour cells as PDL1 expression by TME and TDLN DCs, but not by the tumour, correlates with ICB efficacy in mice and humans[86].

2.5. *Adoptive T cell transfer and DCs.*

Transfer of activated tumour-specific T cells to cancer patients is a growing field with promising clinical efficacy. cDC1s attract T cells to the cancer site ensuring the efficacy of adoptive T cell transfer in preclinical models (**Figure 3C**). Indeed, adoptive transfer of CD8+ T cells lacks efficacy in melanomas with limited cDC1 infiltration[45]. 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[87]. Notably, cDC1s are necessary for effective reactivation of TAA-specific, circulating memory CD8+ T cells in cancer[88]. Moreover, activation of TNF- and iNOS-producing cDC2s through the CD40-CD40L axis is necessary for the efficacy of pre-primed TAA-specific T cell transfer[89]. These cDC2s function independently of CSF1R, although blockade of CSF1R further improves cancer control by reducing the number of immunosuppressive tumour-associated macrophages[4,89].

2.6. *The gut microbiota and DCs?*

Increasing evidence points towards the relevance of the intestinal microbiota for the outcome of cancer therapies. Fecal microbiota transplantation from healthy patients to germ-free or antibiotics-treated mice enhanced responses to ICB, whereas microbiota from non-responsive cancer patients failed. *Akkermansia*

muciniphila was identified as a necessary commensal for ICB efficacy[90]. Additional microorganisms with beneficial effects on ICB efficacy in metastatic melanoma patients are *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*[91]. DCs are clear candidates to mediate this link between tumour immunity and the microbiota, which has a relevant impact on other therapies[92]. For instance, vancomycin-mediated modulation of the gut microbiota composition enhances adoptive T cell transfer efficacy in tumour-bearing mice by expanding cDC1s and IL-12 production[93].

3. DC-based cancer immunotherapies

Tolerance to tumours represents a major hurdle that must be overcome in order to fully harness the potential of DCs in cancer immunotherapy. Several strategies to revert DC-mediated tolerance are currently being pursued (**Table 2 and Figure 4**).

3.1 *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[94] (**Table 3, Figure 4A and 4B**). GM-CSF directly stimulates DC differentiation[10] (**Table 1 and 3**). Talimogene laherparepvec (Imlygic™, T-VEC) is an attenuated strain of HSV that expresses human GM-CSF; it was FDA-approved after being shown to induce antitumour immune responses and improve survival in patients with advanced melanoma[95]. Moreover, encouraging results showing that FLT3L administration enhances tumour immunity, CD8+ T

cell activation and cancer control in mouse models (**Table 1 and 3**)[27,96] are now being followed by clinical trials (NCT01811992, NCT01976585, NCT02129075 and NCT02839265) (**Figure 4B**).

Adjuvants that drive immunogenic DC activation are also being actively investigated, particularly derivatives of ligands for TLRs expressed by DCs[66,94,97] (**Table 1 and 3, Figure 4A**). BCG intravesical administration, a current standard treatment for superficial bladder cancer, associates with increased DC viability and activation[98]. The potency of the synthetic TLR3 agonist poly(I:C), which can also engage MDA5 and RIG-I receptors, has emerged as a potential cancer immunotherapy[66]. Human CD141+ cDC1s appear to be a main target of this therapy because of their high levels of TLR3 expression[26,27] (**Table 1 and 3**). In vitro and preclinical studies show the extraordinary efficacy of poly(I:C) to activate DCs, induce pro-inflammatory cytokines, Th1-type immunity, NK cell activation, cross-presentation and anti-cancer CD8+ T cell responses culminating in therapeutic cancer suppression[28,99,100]. In clinical trials, poly(I:C) derivatives added to cancer (DC) vaccines improve clinical outcomes[100]. The TLR7/TLR8 ligand imiquimod has been approved for local treatment of non-melanoma skin cancers, promoting pDC-mediated cytotoxicity[101] and numerous clinical trials with TLR7/TLR8 agonists in cancer are ongoing (NCT-02574377, NCT02692976). TLR7/TLR8 agonists likely target all natural DC subsets (**Table 1 and 3**), activate NFκB and induce inflammatory cytokine secretion and costimulatory receptor upregulation[97]. Unmethylated CpG oligodeoxynucleotides (CpG-ODN) represent a large group of TLR9 agonists which can activate human pDCs and cDCs in vivo (**Table 1 and 3**) triggering Th1-type immunity and cancer-specific CD8+ T cell responses[102]. Interestingly, antigen and CpG co-localization in DCs correlates

with antitumour immunity[103]. The potential of CpG-ODN in combination with ICB is currently under evaluation in the clinic[66].

Overcoming suppression 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[104]. Also, STAT3 inhibitors, which can foster DC maturation and immunogenic functions[75], are being evaluated in clinical trials[78].

3.2 *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[105]. Such vaccines are mostly composed of TAAs that are delivered as synthetic short or long peptides (SLPs), recombinant TAA-expressing viruses, or whole tumour lysates (**Table 3 and Figure 4C**). To further ensure cancer-specificity and fueled by recent technological advances, the use of neoantigens (TAAs derived from mutated proteins) is reviving hopes for TAA-based vaccination[106]. Efficacy 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 response rate to ICB[107,108] and long-term survival in patients with pancreatic cancer correlates with unique qualities of neoantigens and increased DC and CD8+ T cell infiltrates[109]. Regarding the use of dead whole tumour lysates for vaccination, the type of induced cell death can influence their efficacy to induce immunity[66,110]. Clinically-approved whole tumour lysate preparations include hypochlorous acid oxidation, UVB-irradiation, freeze–thaw cycles and hyperthermia[111].

DC maturation is key for immunogenic antigen presentation[94]. Hence, efforts combining adjuvants with antigens for in vivo provision are on the rise (**Table 2 and Figure 4C**). TAA–adjuvants can be attached and encapsulated to particulate delivery systems such as single and supramolecular peptide conjugates (e.g. nanofibers, gels or nanoparticles), liposomes, virosomes or immunostimulatory complexes (ISCOMs)[112]. The use of self-assembling polymers of degradable biomaterial or nanoparticles in cancer therapy can intrinsically enhance pro-immunogenic DC functions[113]. With regard to DCs, medium size nanoparticles (5-100nm) most efficiently reach the lymph node and negatively charged adjuvants (such as poly(I:C), CpG-ODN) 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 Th1 cell polarization[113].

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 on DC and T cell functions together with technical advances open exciting possibilities for future therapeutic achievements.

3.3 *Targeting DCs in vivo for cancer immunotherapy.*

Targeted delivery of antigens and adjuvants to DCs in vivo can improve antitumour immunity[114] (**Table 2 and Figure 4D**). These therapeutic strategies limit potential side effects and show preclinical efficacy controlling cancer, with 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; using CLEC4A4 (also known as DCIR2) to target cDC2s; use of CLEC7A (also known as Dectin-1) to target cDC2s and MoDCs; use of CD209 (also known as DC-SIGN), the mannose receptor (MR) and macrophage galactose-type lectin (MGL) to target predominantly cDC2s, MoDCs and macrophages; and using CLEC12A to target multiple DC subsets (including cDCs, pDCs and MoDCs)[114]. Of note, antibody-conjugated antigen with adjuvant outperformed the administration of non-conjugated antigen[115–117]. Anti-DEC205 antibodies can target a MAGE-A3 antigen to human MoDCs, stimulating CD4+ T cell responses[118]. Full-length NY-ESO-1 fused to anti-DEC205 antibodies additionally promotes CD8+ T cell activation, contrary to uncoupled NY-ESO-1[119]. A phase I clinical trial shows that cutaneous NY-ESO-1-coupled to anti-DEC205 with resiquimod and/or Hiltonol induces antigen-specific antibodies and T cells with partial clinical responses in cancer patients without toxicity[120]. Primary human MoDCs treated with CD209/DC-SIGN-conjugated antigens (and adjuvants) stimulate specific T cell responses ex vivo[121] 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[122]. In addition, TAAs can also be conjugated to ligands for DC-specific receptors. Administration of MUC1 conjugated to oxidized mannan targeting the MR on DCs induces specific antibody and CD8+ T cell responses in breast cancer patients and improves cancer-free survival[123].

While the amount of TAAs and adjuvants that can be fused to these targeting molecules could be limited, polymer nanoparticles signify an appealing

approach[113] (**Table 2 and Figure 4D**). Human MoDCs efficiently internalize anti-DEC205 antibody-coated PLGA nanoparticles loaded with MART-1 peptide and display enhanced cross-priming activity, compared with exposure to untargeted nanoparticles[124]. Also, anti-CLEC9A-coated PLGA nanoparticles carrying a GP100 SLP induce more robust CD8+ T cell priming ex vivo by human primary blood CD141+ cDC1s, compared with isotype-coated nanoparticles[125].

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 2**).

4. DC vaccines for cancer

The use of DC vaccines for cancer has been extensively investigated, with over 200 completed clinical trials to date (**Table 2 and Figure 4E**). This approach involves the isolation or in vitro generation and amplification of autologous DCs followed by their ex vivo manipulation and reinfusion into cancer 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 anti-cancer NK cell, CD8+ T cell and CD4+ T cell immune 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[126–129]. The only clinically approved DC-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

rate of patients with prostate cancer patients by about 4 months[130]. Recent scientific advances suggest the efficacy of DC vaccines could be further improved by considering various other factors, which we discuss below.

Influence of DC type. Autologous MoDCs obtained from patient CD14+ blood monocytes or by 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) as well as 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 reports clinical safety and a potential increase in survival[131].

Naturally occurring DC subsets harbour greater antigen-presentation capabilities than in vitro-generated MoDCs due to higher MHC molecule expression and functional specialization and are proposed as the basis of next-generation vaccines[10,127,129] (**Table 1**). Preclinical mouse studies show the efficacy of primary pDCs to induce CD8+ T cell activation in certain settings[17]. However, in a comparative experimental glioma vaccination study, tumour-bearing mice-derived cDCs, rather than pDCs, were more effective in prolonging survival[132]. 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. Interestingly, cDC1s induce CD8+ and CD4+ immunity, while preventive vaccination with cDC2s relies on Th17 cell responses[133].

Advances in natural DC isolation techniques from leukapheresis products have led to the first clinical trials in cancer patients. One clinical trial uses enriched blood cDCs and pDCs from patients with melanoma after FLT3L treatment. This personalized DC preparation, stimulated with CD40L and pulsed with cancer-germline antigen peptides, generates antigen-specific T cell responses[134]. Human blood DC subsets have also been assessed for their suitability for cancer vaccination separately. CD303+ pDCs obtained from melanoma patient leukapheresis products induce specific immunity in some patients when loaded with TAA peptides[18]. Two clinical trials report the safety and feasibility of patient blood-derived CD1c+ cDC2s loaded ex vivo with TAA peptides in prostate cancer and melanoma[135,136], the latter additionally showing vaccine-specific CD8+ T cell responses that correlated with improved progression-free survival in 4 out of 14 patients. These studies led to clinical trials using pDCs and/or cDC2s in various cancer settings (NCT02993315, NCT02692976, NCT02574377, NCT03747744 and NCT03707808). Notably, to our knowledge, the potential of naturally occurring mouse or human cDC1s for therapeutic cancer vaccination was not assessed so far, despite their correlation with favourable prognosis[3,5,6,23] and the data supporting their importance for CD8+ T cell cross-priming and induction of antitumour immune responses (see previous sections).

As potential limitations, natural DCs from cancer patients may be dysfunctional (see previous sections)[129,137] and only represent a small blood cell population (<1%)[26]. New cell culture techniques generating cells largely equivalent to natural DC subsets may overcome issues of DC availability[138,139]. Notably, cytokine secretion by pDC (IFNα), cDC1 and cDC2 (TNFα and IL-12) subsets from breast cancer patients and healthy donors was equal upon R848 stimulation[137],

highlighting the need for proper DC activation to overcome DC dysfunction before re-infusion.

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 3**); however, the nature of the antigen and its internalization influences the induction and upholding of immune reposes by DCs (**Table 2**). Compared with untargeted delivery, coupling of TAA to DC-specific antibodies promotes cross-presentation by human MoDCs and cDC1s, leading to TAA-specific CD8+ T cell responses[124,125,140]. Adoptive transfer of patient-specific neoantigen-loaded MoDCs to melanoma patients amplifies the diversity of neoantigen-specific T cells[141], a strategy currently being tested in several clinical trial phases (e.g. NCT03300843, NCT03674073, NCT01885702). Human MoDCs electro-fused with breast cancer cells (as antigen source) promote stronger CD8+ T cell responses than MoDCs cultured with live cancer cells[142]. 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[143].

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 disappointment of first vaccination attempts with steady-state immature DCs[127]. Indeed, early clinical studies proved the importance of MoDC maturation for their migration and induction of effector T cells leading 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 towards each DC subset since their efficacy depends on the pathogen-recognition receptor profile (**Table 1**).

Route and dosage 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 represent tested DC vaccine administration routes[144,145]. While the clinically-approved Sipuleucel-T/Provenge® vaccine is safely delivered intravenously[130], the most effective fashion of DC delivery is debated and may depend on the 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[146]. Pre-conditioning of the DC vaccination site and injection of higher numbers of DCs was suggested to improve vaccine efficacy[127,145] , although some studies report opposite results[147]. However, these differences might rely on the preconditioning stimulus and DC subset. For DC vaccination, the minimal required DC number remains to be defined, while the largely limiting factor is commonly sufficient generation/isolation of DCs[148].

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 tumour type and burden, immunological fitness of the patient as well as 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[126–129]. Overcoming this immunosuppression is crucial for improving DC vaccination.

Notably, the action of DCs is associated or even underlies efficacy of currently used cancer therapies such as ICB, chemotherapy and radiotherapy (discussed in previous sections). Thus, the combination of DC vaccination with those therapies has been proposed[126,149]. Especially, DC vaccination in combination with ICB appears ideal as transferred DCs might foster initial antigen-specific effector T cell activation[127].

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 adjuvants and antigens (**Table 3**) as well as quality control before inoculation. Some drawbacks include the complexity of optimizing the precise conditions and higher costs due to the need of personalized cell-therapy products (**Table 2 and Figure 4E**). The power and potential of DC vaccination for cancer immunotherapy lies in its clinical safety and its potential synergy with established treatments.

5. Perspective

Recent success has fueled the interest in improving antitumour T cell immunity in 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 on how DCs are regulated in this context may allow for 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 that regard, the cDC1

subset is linked to induction of cancer-controlling immunity and improved survival in certain cancer types[3,5–7,12,25,27–29,45]. However, MoDCs are fundamental during treatment with immunogenic cell death-inducing chemotherapy agents and radiotherapy[31–33] and cDC2s can also be key in particular cancer types[133]. 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 fosters the use of DCs to find more efficient therapeutic treatments in clinical trials. Approaches to attain so 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 efficacy of those strategies. DC vaccination approaches may be particularly effective to delay or prevent both relapse and metastasis after debulking surgeries. Overall, we need to learn more concerning how we can optimally manipulate and exploit specific DC subsets with specialized functions to orchestrate efficacious immune responses against cancer.

Author contributions

FJC and SKW contributed equally to this work and share first authorship. SKW and FJC prepared tables and figures, conceptualized and wrote the manuscript. AMM and MFK conceptualized and wrote part of the manuscript. IM helped conceptualization and edited the manuscript. DS conceptualized and wrote the manuscript. All authors contributed to manuscript editing, read and approved the final version.

Competing interests

IM reports receiving commercial research grants from BMS and ROCHE and serves as a consultant/advisory board member for BMS, Merck-Serono, Roche-Genentech, Genmab, Incyte, Bioncotech, Tusk, Molecular partners F-STAR, Alligator and AstraZeneca. The authors have no additional financial interests.

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DC subset	Morphology	Presence in vivo	Development, growth & transcription factors	Main surface markers		Main pathogen recognition receptors		Main functional specialization	
				Mouse	Human	Mouse	Human	Mouse	Human
Plasmacytoid DCs (pDCs)	Plasma-cell like	Resident in lymphoid tissues; found in blood, lung (mouse) and tonsil (human)	HSC, CDP / depend on FLT3L / E2-2, IRF7	CD11c-low, MHC-II-low, B220+, CD317+, SiglecH+, 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 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.
Conventional type 1 DCs (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 LNs.	HSC, CDP, pre-cDC / depend on FLT3L, GM- CSF / BATF3, IRF8, BCL6, ID2, ZBTB46, NFIL3, NOTCH signaling	CD11c+, MHCII+, CD8α+, (resident) CD103+, (migratory) CD24+, XCR1+, Clec9A+, DEC205+	CD11c+/low, HLA-DR+, CD141+, XCR1+, CLEC9A+, DEC205+	TLR2-4, TLR11-13, STING, Clec12A	TLR1, TLR3, TLR6, TLR8, TLR10, STING, CLEC12A	Cellular immunity against tumours and intracellular pathogens, CD8+ T cell and Th1 type immunity. Specialized on cross-presentation. High secretion of IL-12, type I and III interferons. Implicated in self-tolerance in the steady-state (via cross-presentation).	Cellular immunity against tumours and intracellular pathogens, CD8+ T cell and Th1 type immunity. Specialized on cross-presentation. Produce type I and III interferon and IL-12 at lower levels. Correlate with beneficial prognosis in cancer. Role in tolerogenic settings poorly described.
Conventional type 2 DCs (cDC2s)			HSC, CDP, pre-cDC / depend on FLT3L, GM-CSF / IRF4, ID2, RBPJ, NOTCH2, KLF4, ZBTB46	CD11c+, MHCII+, CD11b+/hi, CD172a+	CD11c+, HLA-DR+, CD1c+, CD11b+, CD172a+	TLR1-2, TLR4-9, TLR13, RLR, NLR, STING, Clec4A, Clec6A, Clec7A, (Clec12A)	TLR1-9, RLR, NLR, STING, CLEC4A, CLEC6A, CLEC7A, CLEC10A, CLEC12A	Context-dependent, large repertoire of PRRs and pro- and anti-inflammatory cytokines. Humoral and cellular immunity against extracellular pathogens, T follicular helper cell, Th2 and Th17 type immunity. Implicated in Th17 homeostasis in gut and lung.	Context-dependent, large repertoire of PRRs and pro- and anti-inflammatory cytokines, including IL-12. Mainly induce Th17, but also Th1, Th2, Treg and CD8+ T cell (cross-presentation) activation, depending on the context and precise cDC2 subpolulation. Maintain Treg/Th17 homeostasis in gut (and lung).
Monocyte-derived DCs (moDCs)	Context-dependent	Differentiate from monocytes in peripheral tissues upon inflammation. Resident in skin, lung and intestine.	Monocytes / mainly depend on CSF-1R, in vitro GM-CSF + IL-4 / MAFB, KLF4, express ZBTB46	CD11c+, MHCII+, CD11b+, Ly6C+, CD64+, CD206+, CD209+, CD14+, CCR2+	CD11c+, HLA-DR+, CD1c+, CD11b+, CD14+, CD64+, CD206+, CD209+, CD172a+, CCR2+	Not well defined	Not well defined	Mainly generated during inflammation conditioning their functions: Direct anti-microbial effector functions and induction of CD8+ T cell, Th1, Th2 and Th17-type immunity. Implicated in Treg generation and immune-suppression 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, Treg, Th1, Th2 and Th17-type immunity. Implicated in regulatory functions in steady state skin.

Table 1: Human and mouse DC subsets

Overview on characteristics of the predominant DC subsets found in humans and mice: plasmacytoid DCs (pDCs), conventional/classical type I (cDC1s) and type 2 (cDC2s) DCs as well as monocyte-derived DCs (MoDCs).

BATF3, Basic Leucine Zipper ATF-Like Transcription Factor 3; BCL6, B-cell lymphoma 6 protein; CDP, common DC progenitor; CSF-1R, Colony-stimulating factor 1 receptor; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HSC, hematopoietic stem cell; ID2, inhibitor of DNA binding 2; IRF, Interferon-regulatory factor; KLF4, Kruppel-like factor 4; MAFB, MAF BZIP Transcription Factor B; MHC, major histocompatibility complex; NFIL3, Nuclear Factor, Interleukin 3 Regulated; NLR, NOD-like receptor; PRR, pathogen recognition receptor; RBPJ, Recombining binding protein suppressor of hairless; RLR, RIG-I-like receptor; Th, CD4+ T helper cell; TLR, Toll-like receptor; Treg, regulatory CD4+ T cell; ZBTB46, Zinc Finger And BTB Domain Containing 46.

Therapeutic strategy	Costs	Applicability	Potential side effects	Feasibility	Other advantages	Other disadvantages	Examples	Reported successes
Free / soluble adjuvant/DC activation factor(s)	Low	Universal	High, (local or systemic inflammation)	Easy	/	Low persistence, targeted cells unclear, antigen-unspecific	BCG, picibanil, monophosphoryl lipid A (TLR2/4), poly(I:C) (TLR3), imiquimod, resiquimod, VTX-2337 (TLR7/8), CpG-ODN (TLR9)	Imiquimod licensed for skin cancer and BCG for bladder cancer (BCG mechanisms poorly understood). Adjuvants are part of most DC-based immunotherapies under evaluation.
DC mobilizing agent(s)	Low	Universal	Moderate (systemic effects possible)	Easy	/	Eventual immaturity and dysfunction of expanded DCs, antigen-unspecific	GM-CSF, FLT3L	Clinically approved Talimogene laherparepvec (oncolytic virus + GM-CSF). GM-CSF is added to numerous DC-based immunotherapies. FLT3L is evaluated in trials.
Free / solube antigen (TAAs, TCL, NAs)	Low*	Universal (TCL), Limited (TAA expression) or Personalized (NAs)	Moderate / Low, adjuvant-dependent	Easy*	Large antigen diversity possible	Rapid clearance by phagocytic cells, targeted cells unclear, can cause tolerance w/o adjuvant	Synthetic peptides, SLPs, mRNA/DNA, expressing viruses, 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*		Moderate (local or systemic inflammation)	Easy / Moderate*	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*		Low, antibody specificity-dependent	Easy / Moderate*	Specific DC-targeted, antibody uptake can enhance cross-presentation	Rapid clearance, limited to identified TAAs/NAs, TCL challenging, unspecificity of antibody	DC-specific antibodies or receptor-ligands: anti-DEC205, anti-Clec4A, anti-CD209, anti-Clec7A, anti-Clec12A, anti-MR, oxidized mannan	Early clinical trials ongoing: e.g. anti-DEC205-coupled NY-ESO-1-(+ adjuvants); MR targeting with anti-MR--conjugated hCG-b or oxidized mannan-coupled MUC1.
DC-targeted adjuvant/antigen carrier delivery (e.g. antibody-coupled nanoparticles)	Moderate / Low*		Low, antibody specificity-dependent	Moderate / Easy*	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 pre-clinical results in mice and humans.
Adoptive transfer of adjuvant/antigen-loaded DCs	High*, 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 possible, personalized product might enhance efficacy	Limited cell number, leukapheresis necessary	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 anti-cancer immunity and mild overall responses. Evaluation of neoantigen-loaded DCs, therapy combinations and stage III clinical trials with moDCs and natural DCs ongoing.

Table 2. Approaches targeting DCs for cancer immunotherapy: advantages and drawbacks

Characteristics of different dendritic cell (DC)-based therapeutic strategies are summarized. References are provided throughout the main text.

APC, antigen-presenting cell; BCG, Bacille Calmette-Guérin; CpG-ODN, Unmethylated CpG oligodeoxynucleotides; EGF, Epidermal growth factor; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, Granulocyte-macrophage colony-stimulating factor; hCG-b, Human gonadotropin-b chain; ISCOM, immunostimulatory complexes; moDC, monocyte-derived DC; MR, mannose receptor; MUC1, Mucin 1 cell surface associated; NA, neoantigen; NY-ESO-1, New York esophageal squamous cell carcinoma 1; P64k, meningococcal protein antigen of 64 kDa; PLGA, poly(latic-co-glycolic acid); SLP, synthetic long antigen peptides; TAA, tumour-associated antigen; TCL, whole tumour cell lysate; TLR, Toll-like receptor; XP, cross-presentation.

Agents promoting immunogenic functions of dendritic cells 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	Imlygic™ approved, others in clinical trials
FLT3L	Cytokine essential for cDC development	cDC1 & cDC2 mobilization/expansion	CDX-301 in clinical trials
TLR2/4 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 & cDC (mainly human cDC1) activation: cytokines, Th1 immunity, NK and CD8+ T cell induction	Hiltonol™ (poly I:C LC), Ampligen™ (poly I:C 12U) & BO-112 in clinical trials
TLR7/8 agonists	Various ligands for PRRs TLR7 and/or TLR8, mainly imidazoquinolines	Human pDC & cDC activation: cytokines, Th1 immunity, CD8+ T cell induction, tumouricidal DC activity	Imiquimod approved, others in clinical trials (resiquimod, VTX-2337, protamine RNA)
TLR9 agonists	Synthetic PRR ligands, unmethylated CpG oligodeoxynucleotides	Human pDC & cDC activation: cytokines, Th1 immunity, CD8+ T cell induction	Numerous compounds in clinical trials (including CPG-7909 and CpG-685)
IDO inhibitors	Targeting of indoleamine 2,3-dioxygenase enzyme	Prevention of DC-derived IDO-mediated tryptophan--depletion, tolerogenic functions and T cell anergy induction	Numerous compounds in clinical trials (including INCB 024360 and Indoximod)
STAT3 inhibitors	Small molecules/ monoclonal antibodies blocking STAT3 signaling	DC activation, prevention of immune-suppressive DC functions	IL-6/JAK/STAT3 signalling blockers approved (Siltuximab, Tocilizumab, Ruxolitinib), STAT3 inhibitors in clinical trials

Types of tumour associated antigens for DC-mediated anti-cancer T cell activation

TAA type	Examples for proteins/ source for TAAs	Cancer specificity	Advantages	Disadvantages
Differentiation antigens	Melan-A/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-, EBV-derived proteins	High	Very specific, often cancer-causative (oncoviruses), allow conjugation	Limited prevalence of virus-associated tumours
Cancer--germline / cancer-testis antigens	NY-ESO-1, MAGE (e.g. MAGE-A3), 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. MAGE-A3), limited prevalence
Mutated neoantigens	Mutated proteins specific to (individual) cancers	Highest	Very specific, high efficacy being often unique to cancer / patient, might allow conjugation	Expensive, labor- & 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 neoantigens 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

Table 3. Adjuvants and antigens frequently used for in vivo/in vitro DC activation in cancer

Overview of factors to enhance anti-tumourigenic and pro-inflammatory functions of dendritic cells (DCs) and tumour-associated antigens (TAAs) for DC-loading exploited in the clinic. 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; FLT3L, FMS-like tyrosine kinase 3 ligand; GAGE, G antigen; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GP100, glycoprotein 100; HPV, human papillomavirus; IDO, indoleamine 2,3-dioxygenase; MAGE, melanoma-associated antigen; MART1, melanoma antigen recognized by T cells 1; MUC1, Mucin 1 cell surface associated; NY-ESO-1, New York esophageal squamous cell carcinoma 1; PAP, prostatic acid phosphatase; PRR, pattern recognition receptor; STAT3, signal transducer and activator of transcription 3; Th1, CD4+ T helper cell type 1; TLR, Toll-like receptor; WT1, Wilms' tumour 1.

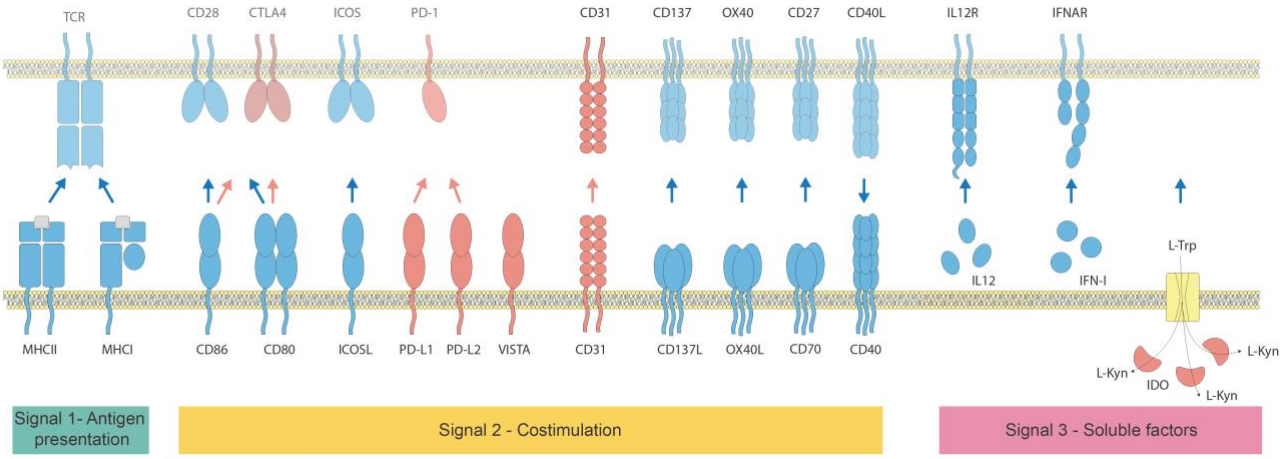

Figure 1. Mechanisms through which DCs induce immunity or tolerance in T cells.

To control T cell activity, DCs can present TAAs on MHC-I and MHC-II molecules. However, that is not sufficient to prime effective antitumour immunity, which requires a positive signaling (blue arrows and receptors) through costimulatory molecules (belonging to the B7 and TNF protein families) and soluble factors, such as IL-12 and type I IFN. Conversely, inhibitory mechanisms (red arrows and receptors) limit T cell activation.

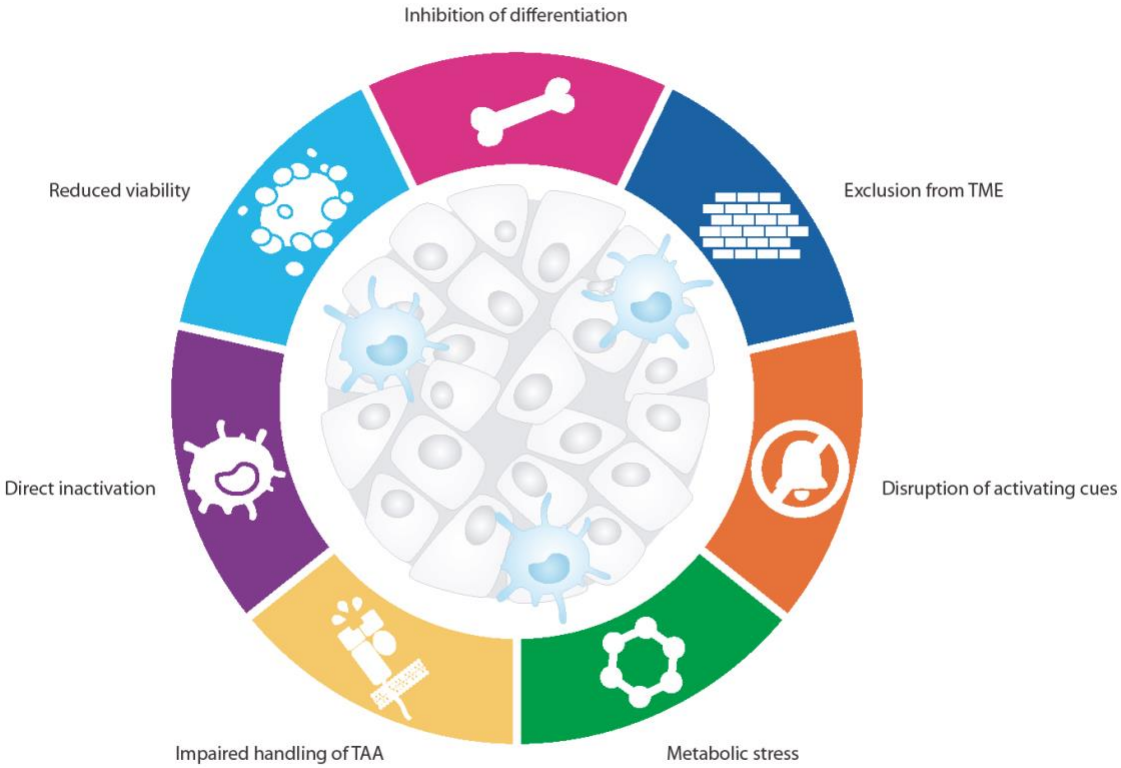

Figure 2. Regulation of DC function by tumours.

Cancer suppresses DC-mediated antitumour immunity by impairing the indicated main aspects of DC biology. 1. Decreased availability of FLT3L in the TME can reduce the terminal differentiation of pre-DCs, as well as tumour-derived prostanoids and gangliosides can affect both in situ or BM generation of DCs. 2. Tumours can block the infiltration of dendritic cells by reducing the expression of DC-attracting chemokines like CCL4, or by preventing other attractors such as NK cells from doing so. 3. Tumours avoid detection by DCs by limiting the release of activating molecular cues, such as TREX1 that degrades ATP and prevents MoDC recruitment into the TME or TIM3 that avoids HMGB1-mediated detection of dying cancer cells. 4. 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. 5. TAA handling and (cross-) presentation are impaired by tumours by promoting the accumulation of half-degraded lipids that interfere with cargo trafficking within DCs. 6. Tumours can regulate the appropriate maturation of DCs by direct or indirect (via CSF1-recruited tumour-associated macrophages) production of soluble compounds such as IL-10, TGFβ, IL-6 or VEGF, which end up hijacking standard signaling pathways, as it occurs with the hyperphosphorylation of STAT3. 7. Tumours can compromise DC viability by targeting factors such as the hypoxia response, ER stress, or the Bcl-2 protein family.

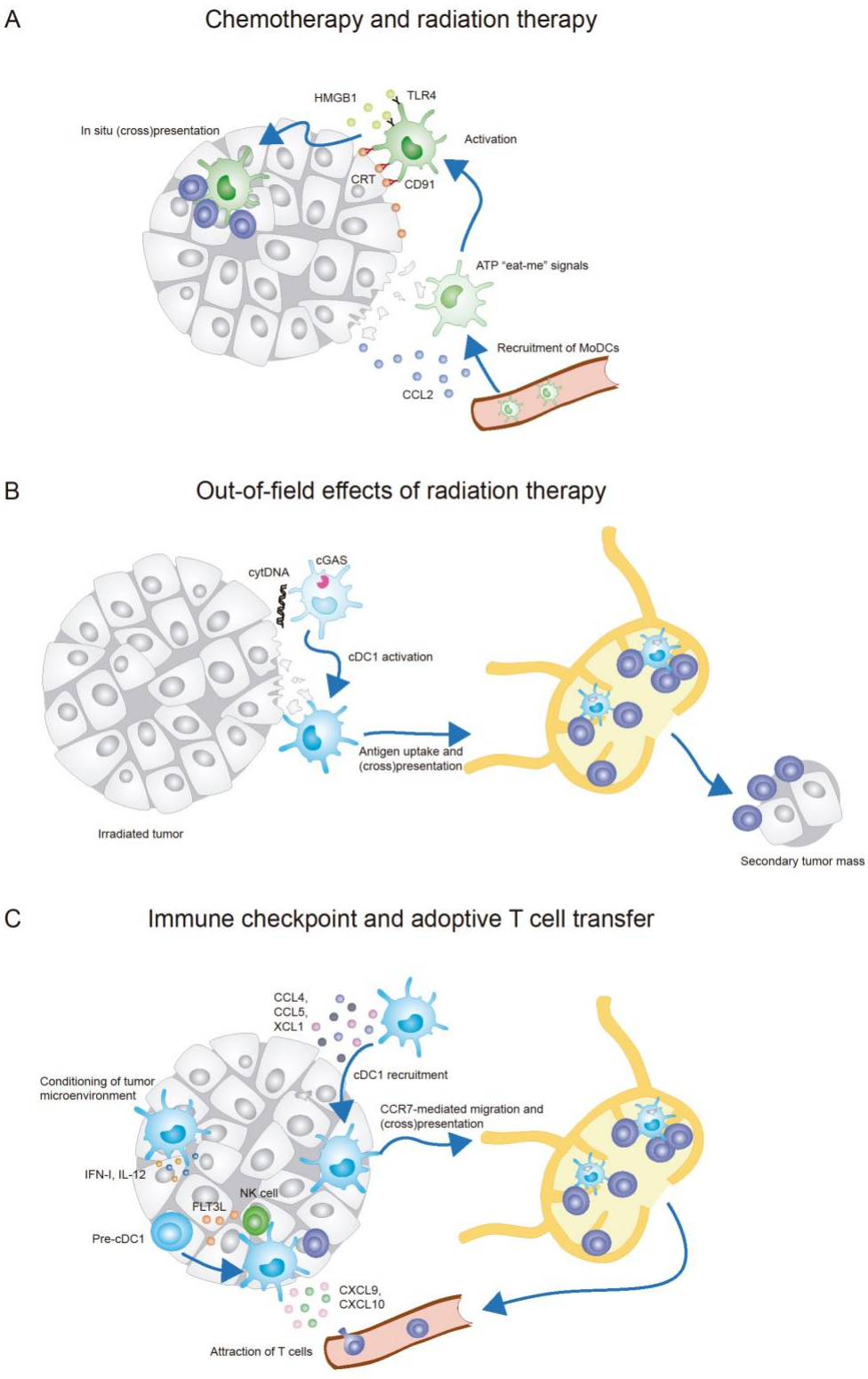

Figure 3. Dendritic cells in the context of cancer therapy

DCs play an essential role in the generation of efficient antitumour immune responses triggered by different therapeutic strategies against cancer. (A) MoDCs mediate antitumour immunity triggered by chemotherapy and local radiation therapy-induced immunogenic cell death. In summary, MoDCs are strongly recruited into the TME of tumours treated with immunogenic cell death-inducers, and prime robust CD8 T cell responses. (B) 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 by the cGAS-STING pathway. (C) cDC1s strongly associate with the efficacy of immune checkpoint therapy and adoptive cell transfer, due to their capacity to prime T cell responses locally and in the TDLNs, to recruit T cells into the TME, and to condition the TME by producing soluble factors.

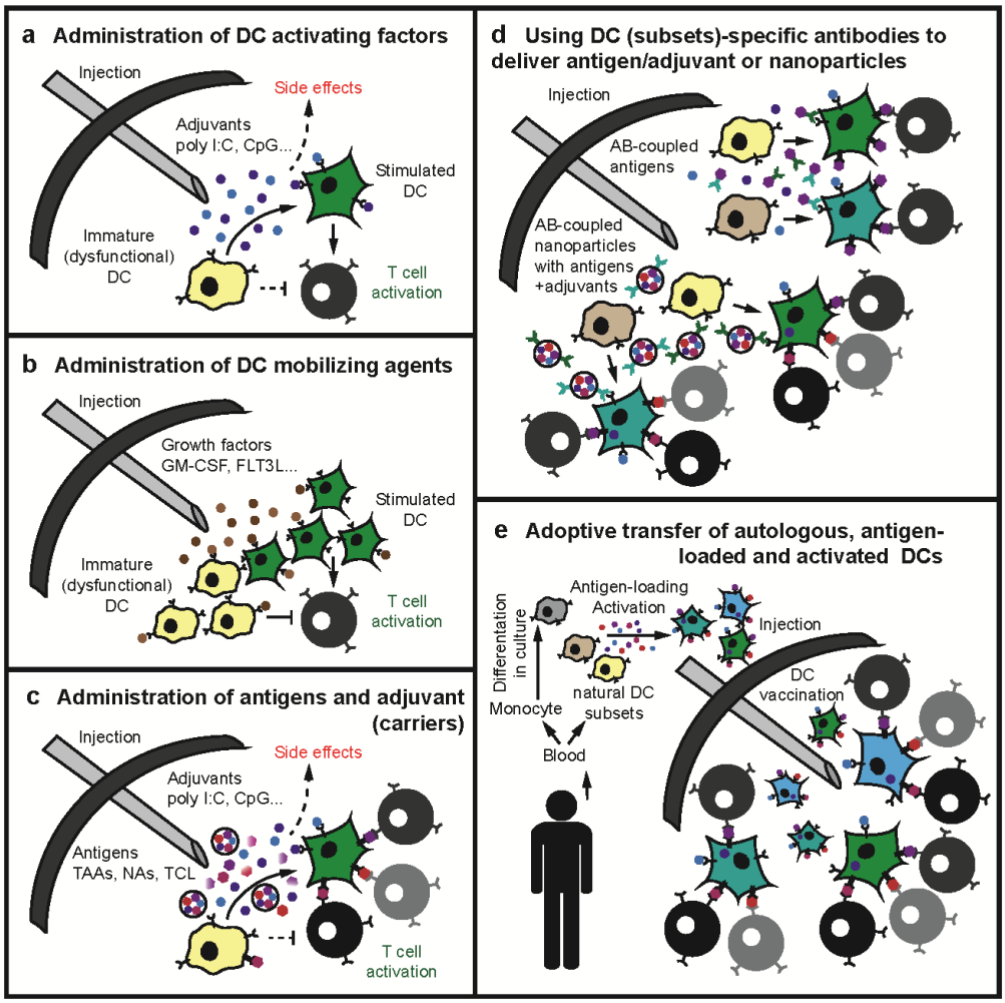

Figure 4. Exploiting dendritic cells for cancer immunotherapy

Principles underlying functionality of therapeutic approaches (directly) targeting dendritic cell (DCs) are illustrated. (A) Adjuvants induce stimulation of DCs, circumventing immaturity and potential tolerogenicity. (B) Growth factors trigger DC expansion and often activation. (C) Delivery of free or carrier-associated 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, cancer-specific T cell activation and reduce off-site effects. (E) Schematic workflow of preparation of DC vaccines and effects of their administration. Natural DC subsets are isolated from blood and 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. NAs, neoantigen; TCL, tumour cell lysate antigen; TAAs, tumour-associated antigens.

Glossary terms:

Pathogen- or danger-associated molecular patterns (PAMPs and DAMPs): variety of molecules derived from pathogens or from endogenous danger signals that are exposed or released from cells and that alert the immune system and activate transduction signals through the interaction with pattern recognition receptors.

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.

Pattern recognition receptors (PRR): germline-encoded host sensors that detect PAMPs, although many of them have also been described to sense DAMPs. This interaction triggers signalling in the host cell.

Adjuvant: 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 moment. Adjuvants contain chemicals that stimulate the immune system, frequently PAMPs acting on PRRs.

Tumour associated antigens (TAAs): autologous cellular antigen 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.

Neoantigen: antigens formed by peptides that are absent from the normal human genome. These neo-epitopes can be derived from tumour-specific DNA mutations or from viral sequences in the case of virus-associated tumours.

Cross-presentation: presentation in MHC class I of external soluble antigens through a process that can be in the endocytic vacuole (vacuolar pathway) leading to loading of peptides in MHC-I in the phagosome or can involve the transfer of peptides to the cytosol, where exogenous antigens are processed by the proteasome and degraded to peptides that are transported to the endoplasmic reticulum for loading on MHC-I. The stimulation of naïve cytotoxic CD8+ T cells following cross-presentation is known as *cross-priming*, and is needed for anti-tumour immunity.

Immunogenic cell death: form of cell death that induces an effective immune response through activation of DCs, in contrast to silent apoptosis, which is not immunogenic.

Immune checkpoint blockade (ICB): blockade of specific interactions between immune cells (e.g PD1) and cancer cells or other immune cells (e.g. PDL1) that dampen immune cell activation. Inhibiting these interactions releases the breaks and promotes immune cell activation.

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