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# Dendritic cell-based therapy

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## Abstract

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Dendritic cells (DCs) stand <sup>37</sup> as a highly promising tool for triggering immune responses in cancer immunotherapy. DCs are recognized as <sup>125</sup> potent antigen-presenting cells (APCs), excel in generating robust immune reactions, and play a <sup>113</sup> vital role in bridging the innate and adaptive arms of the immune system. Consequently, <sup>53</sup> DC-based cancer immunotherapy seeks to leverage these distinctive qualities to enhance the battle against cancer. Over the past decade, extensive research has <sup>59</sup> focused on developing immunotherapeutic strategies against cancer through <sup>32</sup> vaccination. To achieve this goal, a comprehensive understanding of DC immunobiology, the regulation of innate and adaptive immune systems, the tumor microenvironment, and the integration of cutting-edge scientific advances is imperative to unlock their significant anti-tumor immunotherapeutic potential. This chapter concentrates on delving into various aspects of DC immunobiology, including their origin, localization, unique properties, distinct subsets, and their connection to innate and adaptive immunity. Additionally, it explores the contemporary concept of cancer immunoediting and sheds light on insights derived from clinical trials involving DC vaccines. Ultimately, the chapter outlines future perspectives for this burgeoning field.

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## Introduction

Dendritic cells (DCs) originate from pluripotent hematopoietic stem cells (HSCs) in the bone marrow, belonging to a category of antigen-presenting cells (APCs) alongside B-cells and macrophages. First identified in 1973 by Canadian scientists Ralph Steinman and Zanvil Cohn, DCs were initially an undefined cell type in mouse spleens [1]. They were later termed "dendritic cells" due to their characteristic features of multiple extended dendritic or pseudopodia-like cytoplasmic protrusions during maturation [2]. Serving as sentinel cells, DCs are ubiquitously distributed throughout the body, found in the skin, peripheral blood, mucosal surfaces, interstitial tissues, lymphoid and non-lymphoid areas [2].

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Studying DCs is crucial because they are the most potent APCs capable of presenting antigens to T-cells. They play a vital role in host immunity by initiating innate inflammatory responses to pathogens, efficiently priming immature T-cells, activating and generating immunological memory T-cells, and inducing B-cell activation. Additionally, DCs contribute to immune tolerance by maintaining steady-state immune homeostasis, continuously presenting tissue-derived self-antigens to CD4+ and CD8+ T-cells, leading to tolerance against these self-antigens. They are integral to the development of an effective adaptive immune response, serving as a critical link between the innate and adaptive immune systems, making them central to the immune system.

## Role of Dendritic Cells in the Immune System

The role of a dendritic cell (DC) in the immune system involves phagocytosing, processing, and presenting antigens on both MHC Class-I and -II molecules to T-cells (Fig2). Initially generated in the bone marrow through hematopoiesis, immature DCs migrate from the bone marrow to non-lymphoid tissues in the body, where they actively monitor the extracellular environment for foreign antigens [3]. Recognition of foreign antigens by DCs is facilitated through specialized surface receptors known as "pattern-recognition receptors," including TLR, C-type lectins (CLR), and intracellular helicases like retinoic acid-inducible gene-I (RIGI). These receptors detect and bind to distinct components on the microbial surface, recognizing a conserved region called a "pathogen-associated molecular pattern" (PAMP) on the pathogen, such as cell wall components like lipopolysaccharides (LPS), lipopeptides, or nucleic acids like viral or bacterial RNA and DNA. This recognition triggers the receptors, leading to the internalization of the foreign particle. Subsequently, DCs migrate to lymph nodes to present antigens to T-cells. As they mature, DCs lose their antigen-uptake capacity and become antigen-presenting cells (APCs) with enhanced capabilities, including the expression of MHC-I and -II on their surface, up-regulation of costimulatory molecules like CD40, CD80, and CD86, and the production of immunostimulatory cytokines and chemokines.

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Upon interaction with activated naïve CD4+ T-cells, DCs play a crucial role in differentiating these T-cells into antigen-specific helper T-cells (TH-cells) of various types, such as Th1, Th2, Th17 and follicular T-follicular helper cells (Tfh-cells). The specific type of TH-cell generated depends on factors such as the type of captured antigen (bacterial, viral, etc.) and the types of costimulatory molecules and interleukins expressed by the DCs. DCs residing in lymph nodes can trap and process antigens in the lymph, presenting them to naïve CD4+ T-cells, leading to the priming and induction of IL-2 secretion. This process ultimately results in the proliferation and clonal expansion of T-cells, playing a vital role in B-cell development and antibody production [4].

## Dendritic Cell Biology

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The detection of foreign antigens by DCs represents a crucial stage in activating the adaptive immune system. Various DC subsets express diverse sets of pattern recognition receptors (PRRs), allowing for both overlap and exclusivity in recognizing "danger" signals. The maturation and activation of DCs mediated by PRRs can be gauged by alterations in the surface expression of costimulatory molecules, changes in the size and shape of DCs, and the production of different cytokines.

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## Overview of Dendritic Cell Types

Dendritic cells (DCs) play a crucial role as immune sentinels, essential for educating and regulating immune responses. This diverse population exhibits variations in phenotype and function depending on their location within the body and their specific immunological functions. In a non-activated or "steady state", immature DCs continuously survey their local environment, actively seeking foreign antigens (Ag) for presentation to T-cells. These immature DCs typically display elevated levels of CD11c, intermediate levels of MHCII, and

limited expression of surface costimulatory molecules such as CD25, CD40, CD69, CD80, CD83, and CD86. When DCs capture antigens during this steady state, they migrate to lymph nodes to present the captured antigens to T-cells. Although these migratory DCs may not be explicitly "activated," they are often classified as "mature" due to their increased expression of MHCII and costimulatory markers, as well as their migratory 67 pacity. The activation of DCs relies on their recognition of "danger signals" through pattern recognition receptors (PRRs).

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DCs can be generally classified into three distinct subsets based on their maturation/activation status:

1. Immature, nonactivated DCs, "steady-state" DCs found in the spleen, exhibit high levels of CD11c, and low-to-intermediate expression of MHCII and costimulatory markers. In the absence of prior activation 6 these DCs do not generate inflammatory cytokines but can stimulate naïve T cells. Plasmacytoid (p) DCs express lower levels of CD11c and MHCII, are weak stimulators of naïve T cells, and do not produce pro-inflammatory cytokines in the "steady-state".
2. Mature but non-activated, and potentially tolerogenic, migratory DCs exhibit intermediate CD11c expression and higher levels of MHCII and costimulatory markers on their surface and does not produce inflammatory cytokines.
3. Mature, activated DCs have encountered "danger signals" in response to an invading pathogen or damaged self. Depending on the DC 10 subset these DCs produce substantial amounts of inflammatory cytokines very high levels of MHCII and costimulatory molecules on their surface.

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Dendritic cells (DCs) recognize pathogen-associated signals through Pattern Recognition Receptors (PRRs) located within the DC or on its surface. Since the identification of the first mammalian Toll-like Receptor (TLR) that activated the innate system [5], the innate immune system has demonstrated plasticity in responding to invading pathogens. These activating "danger signals" recognized by DCs include the following:

1. Pathogen-Associated Molecular Patterns (PAMPs), which are evolutionarily conserved molecules associated with pathogens, such as bacterial cell wall components or viral/bacterial DNA and RNA segments not typically found within eukaryotic organisms.
2. Damage-Associated Molecular Patterns (DAMPs), such as nuclear or cytosolic proteins released by necrotic cells of self-origin.
3. Inflammatory cytokines released by proximal DCs or other cells.

The response to danger signals triggers alterations in the phenotype and morphology of dendritic cells (DCs). These modifications encompass an augmented distribution of MHC to the cell surface, heightened expression of costimulatory markers, adhesion molecules, and chemokine receptors, the release of cytokines, chemokines, and proteases, and a structural transformation into a stellate morphology characterized by distinctive dendrites—the

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namesake feature of these cells. Microscopy, FACS analysis, or assays measuring soluble protein excretion, such as ELISA or Multiplexed Bead-based Immunoassays, can effectively detect all these changes induced by maturation or activation.

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## Antigen Presentation by Dendritic Cells

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Immature dendritic cells recognize pathogen-associated molecular patterns (PAMPs) — structures that are evolutionarily conserved, such as microbial lipids, carbohydrates, nucleic acids, and intermediates of viral replication. This recognition is facilitated through pattern recognition receptors (PRRs). Various PRRs participate in the innate recognition of pathogens, including Toll-like receptors (TLRs), nucleotide-binding oligomerization-domain (NOD-like) receptors, C-type lectin receptors (CLRs), active protein kinase (PKR), and RIG-I-like helicases [6]. After recognition, the capture of antigens can occur through various mechanisms, including macropinocytosis, endocytosis, and receptor-mediated phagocytosis [7–9]. Specifically, in the case of receptor-mediated phagocytosis, it involves the engulfment of large particulate antigens, such as bacterial pathogens. This process requires actin re-modeling to create a cup-shaped structure around the foreign particle, which subsequently closes to form a phagosome. The various processes of antigen capture by DCs are facilitated by numerous receptors that transport the antigen to processing compartments [7–9]. Dendritic cells convert proteins into peptides, presenting them on major histocompatibility complex (MHC) molecules, specifically MHC class I and II [7, 8]. In the case of lipid antigens, their processing differs as they are loaded onto non-classical MHC molecules belonging to the CD1 family [7]. Following antigen uptake and processing, DCs present antigens in the following ways:

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1. Via MHC-II to CD4+ T lymphocytes (exogenous route): This route typically occurs when DCs present exogenous peptides through MHC-II molecules derived from proteins that have undergone endocytosis and degradation by acid-dependent proteases in endosomes [8, 10].
2. Via MHC-I to CD8+ T lymphocytes (endogenous route): This route is employed to present intracellular antigens (endogenous peptides) associated with MHC class I molecules on the cell surface. For instance, during a viral response, DCs can present viral peptides, allowing the immune system to recognize and activate CD8+ T lymphocytes, leading to the elimination of infected cells [8, 10].
3. Via cross-presentation: This involves presenting exogenous antigens on MHC-I molecules, ultimately stimulating CD8+ T lymphocytes or cytotoxic cells. Phagocytosis is a critical process for cross-presentation, and it is noteworthy that this capability is a distinctive feature of DCs, particularly specific subsets such as CD8+ DCs and migratory CD103+ DCs [8, 10, 11].

## Dendritic cell isolation and culture

### Methods for isolating dendritic cells

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Dendritic cells (DC) make up only about 0.2% of human blood mononuclear cells and can be isolated and enriched using several methods. One method is to use density gradient

centrifugation over metrizamide to isolate dendritic cells from human mononuclear cells. Mononuclear cells are isolated from leukapheresis pack or buffy coat preparation by Ficoll-Paque density gradient and the T-cells are depleted using magnetic beads conjugated with anti-TCR $\alpha$ / $\beta$  monoclonal antibodies. The T-cell depleted cell suspension is incubated overnight in RPMI1640 media at 37°C on tissue-culture plates. This allows the monocytes to adhere to the plastic and to the DCs to further differentiate into mature DCs. The non-adherent cells are gently removed and the process is repeated a second time to further enrich DCs. The resulting cell suspension is subjected to gradient centrifugation with sterile 14.5% metrizamide solution. Cells at the interface of the top layer (RPMI1640) and bottom metrizamide layer are carefully removed which contains 20% to 80% dendritic cells and is largely free of lymphocytes [12, 13]. Another method is to isolate adherent monocytes (described above) and incubate with TNF- $\alpha$ , GM-CSF, and IL-4 for 5-7 days. The differentiation process occurs without cell proliferation, making the quantity of monocytes a critical factor for dendritic cell recovery. As monocytes are more abundant than dendritic cells, this approach can lead to higher yields compared to the previous protocol [14]. More highly purified dendritic cell preparations can be obtained from these populations by a process known as magnetic-activated cell sorting (MACS). In this protocol mononuclear cells are incubated with a mix of anti-CD3, -CD14, -CD19, and -CD56 monoclonal antibodies conjugated with magnetic beads. Using a magnetic separation apparatus lymphocytes, monocytes, and NK cells are depleted and the resulting cell suspension is incubated overnight in RPMI1640 media at 37°C on tissue-culture plates. The non-adherent cells are gently removed and incubated with anti-CD83 antibody conjugated to magnetic beads. The resulting CD83 positive population contains highly enriched population of DCs for various downstream applications [12, 13].

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### Maturation of dendritic cells

Maturation of dendritic cells (DCs) is a crucial step that should ideally precede vaccination. However, there is currently no consensus on the most suitable maturation stimulus for generating DCs with robust immunostimulatory activity. Various combinations of maturation stimuli have been explored, including proinflammatory cytokines, CD40 ligand (CD40L or CD154), and Toll-like receptor (TLR) agonists [15-17]. The utilization of TLR agonists leads to elevated levels of IL-12, resulting in a potent activation of DCs and subsequent effective immune responses. The maturation process is pivotal for efficient vaccine production because mature DCs generally exhibit enhanced expression of costimulatory molecules and increased production of cytokines and chemokines. In contrast, immature DCs lack the ability to induce antigen-specific responses and may potentially foster the differentiation of regulatory T cells.

### Optimization of dendritic cell yield

In order to ensure an ample supply of DCs it is crucial for isolating sufficient numbers of human blood mononuclear cells. Small blood volumes are inadequate for dendritic cell isolation, and it is recommended to generate dendritic cells from monocytes, especially when dealing with limited blood samples. Freshly isolated mononuclear cells are ideal, but satisfactory results can be achieved using 24 hour old blood preparations stored on ice. Leukocyte-enriched "leukopaks" obtained within the last 24 hours can provide a significant

number of mononuclear cells, typically ranging from  $2 - 12 \times 10^8$  cells per leukopak. Alternatively, "buffy coats" from donated blood units can be used. Care must be taken with isolation procedures, as it may affect neutrophils within leukopaks, making it difficult to remove neutrophils which eventually will impact DC purity.

A critical step of DC isolation is the removal of non-adherent cells. The tissue culture plate must be washed thoroughly with warm media using a pipette with sufficient force to remove non-adherent cells. Using cold media will dislodge adherent cells from tissue culture vessels. The cell number can be roughly assessed through phase-contrast microscopy. DCs are large and irregular shaped with long membrane processes and can be easily identified (Figure 1). For higher dendritic cell purity, two metrizamide density gradient centrifugation can be performed. Contaminating B lymphocytes and monocytes can be further depleted through adherence to Ig-coated plates. Lineage-associated monoclonal antibodies (MAbs) can also be employed for depletion using magnetic beads or panning. Depleting contaminating cells through F<sub>c</sub> receptor-mediated procedures is effective but may limit dendritic cell heterogeneity, considering dendritic cells express CD32 and CD64 F<sub>c</sub> receptors [18].

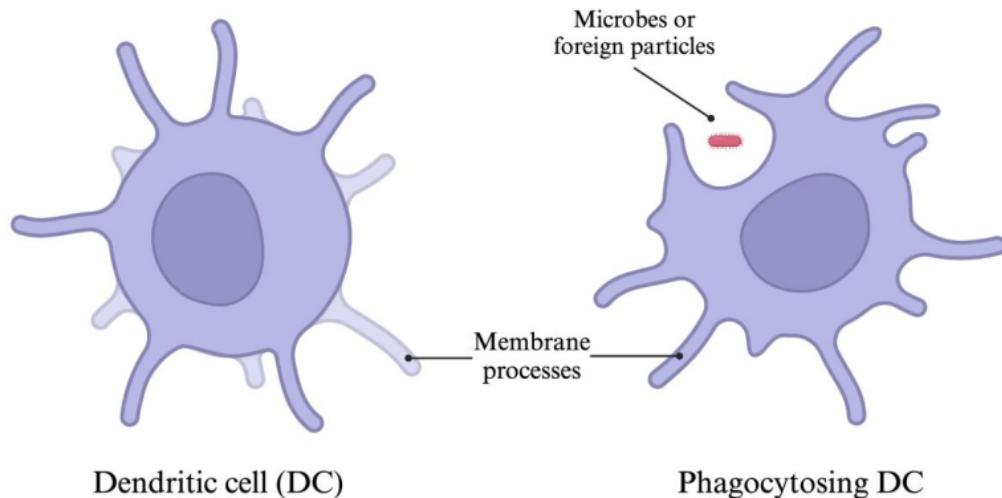


Figure 1: A representative illustration of dendritic cells (DCs) showing long membrane processes.

## Antigen Loading onto Dendritic Cells

### Techniques for Loading Antigens onto Dendritic Cells

1 Loading dendritic cells (DCs) with peptides, proteins, or tumor cells represents the most common method, typically conducted prior to maturation. In this approach, peptides are directly loaded onto MHC-I and MHC-II molecules on the surface of the DCs. Conversely, proteins and tumor cells need to be initially processed and presented by the DCs to activate CD4+ and CD8+ T cells. The primary drawback associated with peptide usage is the requirement to identify the patient's haplotype and the specific peptides that would bind to

these particular **haplotypes**. Contrastingly, utilizing proteins and tumor cells is advantageous as it is not restricted to a particular haplotype. However, in the case of proteins and tumor cells, the MHC-I pathway is not specifically targeted, although studies have observed the activation of CD8+ T cells, indicating the occurrence of cross-presentation [15, 19, 20].

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The use of viral vectors presents an appealing alternative for loading antigenic material onto dendritic cells (DCs), as it enables the insertion of genes encoding tumor antigens or entire proteins, while allowing for the removal of genes encoding virulence or replication factors. In some instances, the vector itself may induce DC maturation, eliminating the need for a separate maturation procedure. Another advantage lies in the ability to incorporate genes encoding cytokines or co-stimulatory molecules, thereby enhancing the immunogenicity of DCs. However, the existence of pre-existing immunity against the vector may diminish the patients' capacity to induce *in vivo* responses, making safety a primary concern. It is noteworthy that lentivirus-based vectors are generally less immunogenic due to the removal of the majority of genes encoding viral proteins. Consequently, their repeated use without provoking immune responses against the vector is feasible. Additionally, lentivirus-based vectors offer specific advantages, including the potential to activate the innate immune system through endosomal or cytoplasmic sensors (i.e., TLRs, RIG-I, PKR, etc.), the ability to transduce quiescent and non-dividing cells, and serving as a potential platform for direct therapeutic vaccination. This is achievable by pseudotyping them with glycoproteins to target specific cell types and directly focusing on DC subtypes *in vivo* [15, 21–24].

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An alternative and appealing approach involves loading dendritic cells (DCs) with messenger RNA (mRNA) encoding tumor-associated antigens (TAAs), a method proven to elicit CD4+ and CD8+ T cell responses. The mRNA, characterized by a short half-life and not integrated into the host genome, can be directly loaded onto DCs without the need for vectors or knowledge of the patient's haplotypes [6, 15]. Furthermore, mRNA transfection allows for the presentation of multiple antigenic epitopes, along with loading options involving maturation stimuli (such as CD40L) or cytokines. Electroporation has been identified as the most efficient method for introducing mRNA into DCs, inducing a temporary increase in cell permeability that facilitates mRNA entry without the requirement for additional reagents [15, 25–27].

### Enhancing Antigen Presentation Efficiency

Article 1 Page 590 Dendritic cells (DCs), acting as **sentinels** in tissues, continuously survey their local environment, capturing antigens for presentation to CD4+ Th cells or CD8+ T cells on MHCII or MHC<sub>I</sub> molecules, respectively [28, 29]. The uptake of exogenous antigen is accomplished through various mechanisms, including phagocytosis [30, 31], receptor-mediated endocytosis [32–34], or micropinocytosis [35, 36], depending on the DC subtype and activation state [32]. For instance, Langerhans cells (LC) and CX3CR1+ macrophages at barrier sites like the intestinal epithelium [37, 38] utilize dendritic projections to sample antigens. Dermal cDC2 can access epicutaneously applied antigen through hair follicles [39]. Following antigen uptake, human LC migrate through the dermis and then to skin-draining lymph nodes (skin-dLN<sub>s</sub>) in a CXCR4-dependent manner,

subsequently transferring antigen-MHCII complexes to dermal cDC through direct contact or indirectly within the dermis [40]. This transfer mechanism potentially enhances the efficiency of antigen transport to the LN, as dermal cDCs migrate faster and disperse more widely within the LN than LCs.

cDC2, with a notable proficiency for antigen uptake in the skin, constitute the majority of antigen-positive cells in skin-dLNs following the administration of particulate antigen [41, 42]. They also play a crucial role in capturing tumor antigens and transporting them to LNs for T cell presentation [43]. Soluble and particulate antigens below 70 kDa can reach LNs without active cellular transport, relying on a conduit network [44, 45]. Nevertheless, cDC2 show a higher capacity for the uptake of soluble antigen per cell and are overrepresented among antigen-positive cells in various settings, indicating an intrinsic capability for exogenous antigen uptake [46, 47]. Their optimal positioning within tissues, especially in proximity to lymph-borne antigens near the subcapsular sinus in LNs, further supports their efficient antigen capture [42, 46].

In contrast, cDC1, situated deep within the LN paracortex [46], excel at capturing cell-associated antigens and dead cells through specific receptors like Clec9A, DEC205, Axl, and TIM3 [48]. This subtype predominantly processes cell-associated antigens via cross-presentation on MHCII, crucial for antiviral and antitumor immunity [48]. Monoclonal antibodies targeting receptors such as CLEC9a and DEC205 have successfully enhanced antigen uptake and cross-presentation in vaccination contexts [49–51]. Additionally, both monocyte-derived DCs (moDC) and cDC2 can engage in cross-presentation, indicating a degree of redundancy in this pathway, particularly notable in humans [52–54].

Dendritic cells (DCs) constitute the primary population of antigen-presenting cells *in vivo*, playing a crucial role in initiating antigen-specific activation and expansion of naive CD4+ T cells through interactions involving peptide-MHCII binding with the TCR and co-stimulatory signaling [49, 55]. Among DC subtypes, cDC2 stands out for its remarkable efficiency in processing exogenous antigens for presentation on MHCII, resulting in superior CD4+ T cell proliferation compared to cDC1. This specialization is believed to be influenced by the expression of Irf4 in cDC2, as IRF4 has been linked to enhanced peptide-MHCII complex formation in these cells [55].

Beyond facilitating CD4+ T cell activation and proliferation, the communication between DCs and CD4+ T cells during antigen presentation plays a crucial role in determining the differentiation fate of T helper (Th) cells. Several factors, including the strength and duration of co-stimulatory signals and interactions between peptide-MHCII and TCR, are implicated in regulating the Th cell differentiation program [56].

## Strategies for Dendritic Cell Activation

### Use of Adjuvants in Dendritic Cell Activation

An effective adjuvant should be designed to specifically target dendritic cells (DCs) to enhance antigen presentation and activate immune responses. DCs, a type 5 white blood cell, are highly proficient in capturing, processing, and presenting antigens to T cells. When appropriately activated, DCs engage with CD4+ T lymphocytes through surface receptors

like major histocompatibility complex class II (MHCII), CD80, and/or CD86, and they release cytokines such as interleukin (IL)-12, initiating T cell activation.

In mice, DCs are identified by the expression of CD11c and can be categorized into broad populations based on the expression of CD8a and CD4, resulting in CD8a+, CD4+, and double-negative (DN) DCs (CD8a-CD4-) subsets [57, 58]. Conversely, in humans, DC subpopulations are not differentiated by CD4 and CD8 expression. DCs also express pattern recognition molecules, specifically Toll-like receptors (TLRs), which provide activation signals. The TLR family comprises 11 members (TLR1–10) that recognize various pathogen-associated molecular patterns [59], inducing the maturation and migration of DCs to lymph nodes to initiate an immune response. While mRNA for TLR2 and TLR4 is present in all murine DC subsets [60], TLR9 protein expression and function have been demonstrated [61, 62]. TLR2 is notable for recognizing a broad range of lipid ligands derived from different microbe types, including bacteria. It forms heterodimers with either TLR1 or TLR6 for recognizing tri- or di-acylated lipids, respectively. However, it is suggested that TLR2 may also function as homodimers for signaling, as seen in the recognition of synthetic lipopeptides.

### Cytokine-Based Activation Methods

A novel approach to antigen loading involves the direct targeting of antigens to dendritic cells (DCs) <sup>2</sup> in vivo, aiming to induce tumor-specific immune responses [63–24]. This strategy presents a promising option for DC immunotherapy, bypassing the costly and labor-intensive ex vivo DC generation process. It allows for <sup>2</sup> the production of vaccines on a larger scale, eliminating the need for customized vaccines for each patient. Importantly, in vivo targeting enables the stimulation of natural DC subsets at multiple sites in vivo.

Early methods for in vivo DC targeting included engineering irradiated tumor cells to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) [66]. GM-CSF was employed to stimulate the recruitment and enhance the function of antigen-presenting cells (APCs). These approaches involved autologous tumor cells engineered to secrete GM-CSF through retroviral- or adenoviral-mediated gene transfer, allogeneic tumor cell lines stably transfected with expression plasmids encoding GM-CSF, or the combination of autologous tumor cells with GM-CSF-secreting cell lines. Clinical trials using these methods demonstrated the recruitment of DCs, granulocytes, macrophages, and T cells into vaccination sites. Patients typically developed delayed-type hypersensitivity reactions involving CD4+ and CD8+ T cells, eosinophils, and macrophages in response to the vaccinations [67–69]. Tumor biopsies from vaccinated patients revealed extensive tumor necrosis and the presence of cytotoxic CD4+ and CD8+ T cells and plasma cells <sup>2</sup> displaying potent cytokine profiles upon restimulation, indicating successful induction of tumor-specific immune responses.

However, prolonged GM-CSF production in the tumor microenvironment has been associated with disease progression in certain experimental models, including a Phase III trial involving immunization with irradiated, GM-CSF-secreting allogeneic prostate carcinoma cells in patients with hormone therapy-refractory metastatic disease [66]. This progression may result from immune tolerance induced by prolonged GM-CSF

administration, leading to the recruitment of myeloid suppressor cells or the differentiation of myeloid precursors into immature tolerogenic DCs [70, 71].

Recent approaches focus on targeting DC-specific molecules, including Fc receptors, CD40, and C-type lectin receptors (CLRs). CLRs are particularly attractive targets because different DC subsets express various CLRs such as DEC205, DC-SIGN, mannose receptor (MR), and Dectin-1. CLRs are involved in recognizing and capturing glycosylated self-antigens and pathogens for antigen presentation, DC trafficking, DC-T-cell interactions, and subsequent immune response activation [71]. Early studies targeting antigens to DCIR2 and DEC205 showed that immune tolerance is elicited in the absence of DC-activating stimuli, whereas co-administration of DC-activating stimuli is necessary to induce immune responses [34]. Subsequent studies targeting antigens to a range of CLRs with a DC-activating stimulus resulted in the effective generation of CD4+ and CD8+ T-cell responses [51, 72]. Furthermore, targeting antigens to CLRs enhanced antibody responses [73]. Although most of these studies are conducted in mice, a number of studies in humans using DC-SIGN [74] and MR [75] have emerged, demonstrating successful induction of naive and memory tumor-specific T-cell responses. However, additional studies are needed to translate this innovative strategy into clinical applications in humans.

#### Genetic Modification for Enhanced Functionality

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Although there is knowledge about signals for dendritic cell (DC) maturation and activation, the optimal sequence and signals to deliver for generating optimal T-cell immunity *in vivo* remain unclear. Current clinical trials of cancer immunotherapy use DCs from various sources, including those isolated directly from unmobilized or Flt3 ligand-mobilized peripheral blood, or generated *in vitro* from CD34+ progenitors, CD14+ monocytes, or adherent peripheral blood mononuclear cells. These DC populations are generally heterologous and often immature, expressing low levels of B7-1, ICAM-1, and LFA-3, despite being efficient in antigen uptake and processing. To address this, some studies have observed increased expression of these molecules after infection with vectors containing TRICOM.

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Immature DCs are loaded with antigens in various forms, such as peptides, proteins, tumor lysates, apoptotic bodies, or tumor cell fusions. Gene transfer through DNA or messenger RNA encoding the antigen has also been investigated for antigen loading and processing. These loaded DCs may undergo further processing by maturation with second signals like tumor necrosis factor- $\alpha$ , CD40 ligand, or monocyte-conditioned media before administration to patients via different routes, such as intravenous, intradermal, subcutaneous, or direct intralymphatic or intranodal injection.

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Mature DCs within secondary lymphoid organs express high levels of costimulatory molecules, facilitating antigen presentation. However, several issues persist regarding gene modification of DCs. These include questions about uniform maturation, persistence of molecule expression, the potential for enhanced function with higher levels of expression, and the broader impact of genetic modification on DC function. Studies suggest that using exogenous expression systems like viral vectors engineered to express crucial molecules can optimize the delivery and processing of antigens.

One study demonstrates the effectiveness of their strategy for modifying DCs and improving their in vitro performance [76]. However, uncertainties remain, including the optimal strategy for gene expression or repression, concerns about the viral vector system's potential impact on DC functions, and questions about the efficiency of migration for matured DCs and whether fully matured DCs should be administered directly or matured in vivo. Despite these uncertainties, Hodge et al.'s experiments show that DC function can be manipulated through genetic modification, paving the way for further exploration of their biologic activity and role in eliciting authentic antitumor responses in patients.

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## Dendritic Cell Vaccines

In recent years, there has been clinical promise in reprogramming the immune system against cancer. Dendritic cells (DCs) emerge as a compelling target for immunotherapy due to their capacity to uptake and present tumor-associated antigens (TAAs) through various mechanisms, thereby initiating robust effector responses against the tumor [77–79]. Beyond direct antigen presentation, other intrinsic properties of DCs play a crucial role in immunotherapy. These include their ability to migrate between lymphoid and non-lymphoid tissues, regulate cytokine and chemokine gradients, and control inflammation and lymphocyte homing, all of which are essential for achieving systemic and enduring anti-tumor effects. Personalized vaccines involving patient-derived DCs manipulated ex vivo have been extensively investigated to harness these features. Typically, these therapies are developed by isolating monocytes or hematopoietic stem and progenitor cells (HSPCs) from peripheral blood. Subsequently, these cells undergo treatment with recombinant cytokines to induce differentiation, are stimulated for maturation, and are loaded with TAAs in various forms. This comprehensive process has been employed in numerous preclinical and clinical studies [80].

### Dendritic Cell Vaccines Design

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Many contemporary DC therapies rely on ex vivo-modified autologous dendritic cells (DCs) that are subsequently re-administered to cancer patients. However, there is a need to develop strategies that enhance the potency of DC therapy. To optimize this approach further, various parameters can be examined, including the specific subtype utilized, as well as the route, dose, and frequency of DC therapy administration. The activation or maturation status of DCs is a critical factor influencing the efficacy of DC-based immunotherapy, impacting their ability to reverse established T cell tolerance to tumor antigens and induce potent antitumor T cells [81–83]. Consequently, extensive efforts have been invested in identifying robust activation stimuli that generate fully mature DCs, either through the addition of inflammatory cytokines [84], Toll-like receptors (TLRs) [85, 86] in vitro cultures, or by inducing maturation of DCs in vivo [87, 88]. Currently, there is a growing focus on approaches that induce maturation 'from within' [89, 90].

In this context, Calderhead et al. [89] compared different protocols for the maturation of DCs electroporated with antigen mRNA, including co-electroporation with mRNA encoding CD40L. Their findings indicated that while there were no significant differences in the expression of co-stimulatory molecules or major histocompatibility complexes (MHC)

among the various maturation protocols, induction of a potent pro-inflammatory cytotoxic T lymphocyte (CTL) response occurred when DCs were matured by adding cytokines followed by CD40L mRNA electroporation. In our work, we demonstrated the maturation and increased T cell stimulatory capacity of DCs when electroporated with mRNA encoding CD40 ligand (CD40L), CD70, and a constitutively active form of TLR4 (caTLR4), collectively termed TriMix [91]. This combination mimics CD40 ligation [92] and TLR4 triggering [93], resulting in the generation of phenotypically and functionally mature DCs. The introduction of CD70 into DCs provides a co-stimulatory signal to CD27<sup>+</sup> naive T cells, inhibiting activated T cell apoptosis and supporting T cell proliferation [94]. Notably, this approach eliminates the need for incubating DCs with soluble maturation signals, preventing potential exhaustion and resulting in DC-based vaccines of superior capacity [95]. Electroporation of DCs with TriMix yielded phenotypically mature, cytokine- and chemokine-secreting DCs capable of potently activating CD4<sup>+</sup> T helper 1 (TH1) cells.

In the subsequent step, tumor antigen mRNA was co-delivered as a replacement for peptide pulsing [96]. The current DC therapy involves ex vivo generation of monocyte-derived DCs loaded with tumor antigen-encoding mRNA and simultaneously potentiated with TriMix mRNA. This streamlined 'one-step' procedure minimizes the manipulations needed for the cellular product's generation, allowing direct injection into patients. Consequently, TriMixDC-cellular therapy leads to *in situ* maturation and secretion of immunostimulatory cytokines and chemokines. This *in situ* maturation approach may enhance T cell immunity, closely resembling physiological responses to pathogen infections [97].

Moreover, employing this approach expands treatment accessibility to more patients by circumventing human leukocyte antigen (HLA) restriction as an inclusion criterion for clinical trials [98]. The use of full-length tumor antigen mRNA enables presentation of all possible epitopes by the patient's HLA repertoire. Our studies have demonstrated both *in vitro* and *in vivo* stimulation of antigen-specific T cells and the induction of antigen-specific T cell responses in melanoma patients through the administration of TriMixDCs co-electroporated with mRNA encoding a fusion of DC-LAMP and one of four melanoma-associated antigens (gp100, tyrosinase, MAGE-A3, and MAGE-C2), referred to as TriMixDC-MEL [96]. Importantly, these activated T cells exhibit a cytolytic phenotype and produce inflammatory cytokines upon specific stimulation, indicating their potential efficacy in combating cancer [96].

### Manufacturing and Quality Control of Dendritic Cell Vaccines

The treatment landscape for various malignant diseases is evolving, with tumor vaccines gaining prominence in therapeutic strategies. The effectiveness of these innovative products is undergoing rigorous evaluation through numerous global clinical trials. In the European Union (EU), cell-based tumor vaccines categorized as somatic cell therapy medicinal products must align with a specified definition. As per this definition, somatic cell therapy involves the use of autologous (derived from the patient), allogeneic (from another human), or xenogeneic (from animals) somatic cells whose biological characteristics have been significantly altered through manipulation for therapeutic, diagnostic, or preventive purposes. Somatic cell therapy medicinal products encompass

cells that are genetically modified, unless the genetic modification is unrelated to therapeutic, diagnostic, or preventive objectives, as is the case with primary tumor cells immortalized through gene transfer.

Tumor vaccines contain<sup>66</sup> genetically modified allogeneic or autologous somatic cells fall under the classification of gene-transfer medicinal products. Regulatory oversight for quality, preclinical, and clinical aspects of such products is provided in the "Note for guidance on the quality, preclinical and clinical aspects of gene-transfer medicinal products." The European definition of somatic cell and gene-therapy medicinal products is outlined in Annex 1 to Directive 2001/83/European Commission (EC), as amended by Directive<sup>29</sup> 2003/63/EC. According to this definition, gene-transfer medicinal products involve<sup>3</sup> the transfer of a prophylactic, diagnostic, or therapeutic gene to human/animal cells, either in vivo or ex vivo, followed by its expression in vivo. Consequently, tumor vaccines featuring genetically engineered dendritic cells expressing tumor-associated antigens (TAAs) or tumor cells transduced/transfected with genes encoding cytokines are classified as gene-transfer medicinal products.<sup>3</sup>

As of now, tumor vaccines are not covered by the European Pharmacopoeia (Ph. Eur.). Therefore, specific Ph. Eur. guidance is lacking for both tumor vaccines and therapeutic vaccines. Nonetheless, Expert Group 15 of the European Directorate for the Quality of Medicines is currently working on drafting a general Ph. Eur. monograph for vaccines for human use, which will encompass therapeutic vaccines as well.

Manufacturing and control of medicinal products, including cell-based tumor vaccines, in the EU are subject to compliance with good manufacturing practice (GMP) principles. The principles applicable to the manufacture of investigational medicinal products are summarized in Annex 13 of the GM<sup>3</sup>s. Specific guidance for GMP inspections of gene-therapy products is in preparation by the Committee for Medicinal Products for Human Use at the European Medicines Agency.

Regulatory guidance for cell-based tumor vaccines can be found in documents designed to regulate conventional biotechnologicals, such as recombinant proteins. For instance, the International Conference on Harmonization (ICH) Topic Q 5 D addressed principles related to cell-banking systems, including master and working cell banks. The European Medicines Agency published a "points to consider" document on the manufacture and quality control of somatic cell therapy products, which may be subject to modification in the future to incorporate<sup>19</sup> merging technologies like tissue engineering. Importantly, this document also addresses genetic manipulation of cells and should be read alongside the guidance on the quality, preclinical, and clinical aspects of gene-transfer medicinal products, which deals with specific issues related to gene transfer methodologies involving plasmids or viruses and addresses safety concerns associated with certain gene-transfer approaches.

## Challenges and Limitations

Dendritic cells (DCs) are typically found<sup>21</sup> in an immature state in circulation and peripheral tissues. Upon receiving<sup>50</sup> maturation signals, they undergo changes such as upregulation of chemokine receptors, increased surface expression of MHC molecules, and upregulation of

<sup>20</sup> stimulatory models. These changes facilitate their migration to lymph nodes, enhance antigen presentation, and amplify T-cell responses. The type of maturation signals determines the phenotypes of DCs, influencing their interactions with T cells and the cytokines they secrete.

<sup>34</sup> While DCs play a crucial role in activating the immune system, they can also induce immune tolerance, potentially hindering effective vaccine strategies. Studies have linked immature DCs to the promotion of regulatory T-cell development, contributing to peripheral self-tolerance. In some cases, immature DCs induced nonproliferative responses and IL-10 secretion, characteristic of a Treg population. The use of antigen-pulsed immature DCs has even led to antigen-specific immune suppression, inhibiting pre-existing antigen-specific T-cell function.

To address these challenges, vaccines need to incorporate signals that ensure full maturation and activation of DCs before administration. Attempts to exploit immature DCs to promote anergy have been made in transplantation and autoimmunity settings. However, in cancer vaccines, ensuring full maturation and appropriate activation of DCs is crucial to overcome immune tolerance barriers. Researchers have also demonstrated that inappropriately activated DCs, even with mature features, can induce T-cell tolerance.

Understanding the signals that lead to tolerogenic states is essential for directing the choice of maturation signals and targeting factors present in the tumor microenvironment that mediate tolerance. Moreover, maturation alone may not be sufficient for immune activation, as demonstrated in studies where immature DCs led to the expansion of Treg populations in myeloma patients. Confirming DC maturity and phenotype through cell-surface markers and cytokine secretion before vaccine administration is crucial for cancer vaccines to overcome the challenge of immune tolerance.

## Clinical Trials and Outcomes

<sup>2</sup> Effective cancer vaccines can function as preventive agents against cancers linked to infectious diseases, such as human papillomavirus or hepatitis B virus, and serve as onco-therapeutic agents. The latter approach relies on the recognition of specific tumor-associated antigens (TAAs) by CD3+ T cells within the host body. Vaccination can enhance the existing immune response against TAAs or induce a De Novo response. Dendritic cells (DCs), known for their efficiency as antigen-presenting cells (APCs), play a crucial role in both major histocompatibility complex class I (MHC I) and II (MHC II) presentation to CD8+ and CD4+ T cells, respectively. DCs exhibit migratory capabilities between lymphoid and non-lymphoid tissues, modulating cytokine and chemokine gradients to regulate inflammation and lymphocyte homing.

I will add a table here with the current clinical trials

<sup>2</sup> Numerous clinical trials are underway, testing DCs alone or in combination with other regimens to combat cancer. The failure of first-generation DC vaccine trials in randomized trials stemmed from an incomplete understanding of DCs' role and the immune-suppressive environment created by cancer, leading to tolerance. Overcoming this

<sup>2</sup>  
tolerance challenge involves loading DCs with large amounts of antigens for activation and expansion through immune-stimulatory molecules.

<sup>8</sup>

Sipuleucel-T, the first FDA-approved DC vaccine therapy for metastatic prostate cancer, demonstrated increased average survival but did not show significant tumor size reduction or halted tumor progression. Standard response evaluation criteria in solid tumors (RECIST) criteria, based on cytotoxic effects, may not be suitable for assessing outcomes in cancer immunotherapy. A new version of RECIST has been proposed to evaluate vaccination as a valid alternative for conventional cancer therapies.

<sup>2</sup>

Current DC vaccination approaches involve conventional vaccination, in vivo DC targeting, and DC vaccination. Conventional vaccination uses antigens with an adjuvant, lacking precise targeting. In vivo DC targeting injects anti-DC antibodies with antigens, triggering strong immunity with an appropriate maturation stimulus. DC vaccination entails the adoptive transfer of ex vivo-generated DCs, loaded with TAAs and activated with pro-inflammatory cytokines before re-injection into the host.

Despite promising ex vivo effects in many DC vaccines, clinical efficacy, especially in late-stage cancer, remains modest. Different routes and methodologies in preparing DCs for clinical trials contribute to variations in efficiency. Preclinical studies are underway to develop next-generation DC vaccines, aiming to enhance effectiveness by boosting immunogenicity through different maturation cocktails and improving effector T lymphocyte function.

## Combination Therapies

### Synergy with Other Immunotherapeutic Approaches

<sup>17</sup>

Cytotoxic treatments can exert various positive effects on the immune system, ranging from the simple release of tumor antigens (AGs) due to cancer cell destruction to more complex immunological impacts. The release of tumor AGs enhances the uptake and presentation of a diverse array of antigens, promoting T cell activation. The immunological effects induced by cytotoxic actions involve the upregulation of immunostimulatory molecules (e.g., DAMPs), heightened tumor AG expression, reduction of suppressor cells, and increased proliferation and activation of cytotoxic T lymphocytes (CTLs) [35]. In a clinical example, 26 patients with different types of advanced and treatment-refractory cancers underwent combined therapy involving radiation, immature dendritic cells (DCs), whole limpet hemocyanin (KLH), and T cells. The initial treatment successfully eliminated metastatic and recurrent tumors in 21 out of 26 patients, with half of them exhibiting a complete response and no evidence of disease recurrence. These encouraging outcomes provide impetus for further exploration of research into combining conventional therapies with DC-based anti-tumor immunotherapy [61]. Additionally, low doses of cyclophosphamide and paclitaxel have been demonstrated to stimulate DC maturation. As a result, these agents are employed in combination with DC vaccines to enhance their efficacy [62, 63].

Tumors can create an immunosuppressive environment by expressing negative co-stimulatory molecules and secreting factors that inhibit both innate and adaptive

1

immunity. This immunosuppressive milieu employs various mechanisms to evade immune surveillance, including the loss of tumor antigen (AG) expression, alterations in MHC molecules, lack of co-stimulation, expression of inhibitory ligands, induction of Treg cells, presence of indoleamine 2,3-dioxygenase (IDO) promoting Treg cell generation, and production of immunosuppressive cytokines (such as transforming growth factor  $\beta$  (TGF- $\beta$ ), IL-6, and IL-10). Overcoming this tolerance and suppression within the tumor microenvironment is crucial for enhancing the immunogenicity and effectiveness of DC vaccines *in vivo* [14].

13

Combining DC-based vaccines with the blockade of inhibitory signals holds significant promise for eliciting a more robust immune response against cancer. Disrupting the interaction between the programmed death-1 (PD-1) receptor on activated T cells and its ligand (PD-1) overexpressed on tumors and DCs has shown improved *in vitro* immune responses. Strategies to interfere with this mechanism include the use of anti-PD-1 antibodies or small interfering RNA (siRNA) to silence PD-L1 and PD-L2 ligands on DCs [42, 64].

28

Another valuable target is the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), which inhibits T cell activation. Blocking CTLA-4 with antibodies, such as ipilimumab, has demonstrated therapeutic efficacy and received FDA approval for treating metastatic melanoma in 2011 [42].

Targeting components of signaling pathways involved in the inhibition of DC functions, such as the suppressors of cytokine signaling (SOCS) family and glucocorticoid-induced leucine zipper (GILZ), provides additional avenues. SOCS, known to inhibit JAK/STAT signaling crucial for DC function, can be inhibited using siRNA [65]. Inhibiting GILZ has potential therapeutic benefits by altering DC maturation in response to TLR agonists and CD40L, as demonstrated in studies [66]. Furthermore, GILZ expression in macrophages within Burkitt lymphomas has been linked to immune system failure to reject the tumor [67].

Immunotherapeutic strategies directed at inhibiting immunosuppressive cytokines like IL-10 and TGF- $\beta$  are also under exploration to enhance vaccine effectiveness. IL-10 receptor antibodies have shown promise in enhancing specific immune responses and IL-12 production [68]. Additionally, inhibiting TGF- $\beta$  has been associated with suppressing Treg cell proliferation and increasing the number of TAA-specific T cells [69].

### Prospects for Personalized Combination Therapies

1

Alternative cancer immunotherapies exhibiting promise encompass adoptive cell transfer of tumor-infiltrating lymphocytes (TILs), genetically engineered T cells, and immune checkpoint inhibitor antibodies. Adoptive cellular therapy (ACT) involves *ex vivo* isolation and expansion of antigen-specific T cells for later transfer back to patients. Despite its success in hematologic malignancies and melanoma, ACT's efficacy against most solid tumors is limited, primarily due to T cells' inability to function and persist *in vivo*. Obstacles include the necessity for tumor resection, challenges in isolating or expanding TILs adequately, and the standard lymphodepletion procedure before transfer, causing side effects in roughly 50% of patients.

4

An alternative approach is genetic engineering of T cells to express chimeric antigen receptors (CARs), linking an antigen-binding domain<sup>1</sup> to an intracellular T cell signaling domain. Prominent results have been achieved with CAR T cells targeting CD19 in B cell malignancies, albeit with significant toxicity, including cytokine-release syndrome and neurotoxicity. In contrast, DC-based immunotherapies boast a favorable safety profile, observed over two decades in numerous trials, with minimal toxicity and rare severe immunotoxicity reactions.

DC vaccines have demonstrated tolerability, inducing only localized reactions at injection sites and occasional systemic effects like fever and malaise. In contrast, immune checkpoint blockade can lead to autoimmune complications affecting various organs. CTLA4 blockade, for instance, may disrupt the proper inhibition of autoreactive T cells, resulting in immune-related adverse events affecting the liver, skin, endocrine glands, and bowel.

1

In summary, immunotherapy holds promise for improving tumor outcomes. DC vaccines, monoclonal antibodies, immune checkpoint blockade, and adoptive and genetically engineered<sup>1</sup> cell therapy have shown encouraging results. Future directions involve integrating these approaches with standard chemo- and radiotherapy, determining optimal timing for immunotherapy initiation, and devising strategies to maximize effectiveness while limiting toxicity.

## Conclusion & Future Perspectives

4

Recent successes have sparked interest in enhancing antitumor T cell immunity for cancer therapy. Dendritic cells (DCs) stand out as the most potent antigen-presenting cells capable of activating naïve T cells and eliciting long-term memory responses against cancer. Despite often being dysfunctional or tolerogenic in the tumor microenvironment (TME), a deeper understanding of the regulation of DCs in this context holds therapeutic potential in various clinical settings. Exploring how different DC subsets may elicit distinct functional immune responses in cancer is a notable area of interest. For instance, the cDC1 subset is associated with the induction of cancer-controlling immunity and increased survival in specific cancer types [99–102]. On the other hand, monocyte-derived DCs (MoDCs) play a fundamental role during treatment with immunogenic cell death-inducing chemotherapy agents and radiation therapy [103–105], while cDC2s are crucial for inducing antitumor CD4+ T cell immunity [43, 106]. Although DCs can enhance the effectiveness of established cancer therapies, the development of optimal vaccination strategies requires a deeper understanding of DC biology and functions. Progress in preclinical studies encourages the exploration of DCs in more efficient therapeutic treatments through clinical trials. Strategies to achieve this involve administration in conjunction with (neo)antigens, mobilization of endogenous DCs, and the use of stimulating adjuvants. A more precise and refined targeting of DCs could further enhance the efficacy of these approaches. DC vaccination approaches show promise, especially in delaying or preventing relapse and metastasis following debulking surgical procedures. Overall, there is a need to gain more insights into how specific DC subsets with specialized functions can be optimally exploited to coordinate effective immune responses against cancer.

## References

1. Chen P, Liu X, Sun Y, Zhou P, Wang Y, Zhang Y. Dendritic cell targeted vaccines: Recent progresses and challenges. *Human vaccines & immunotherapeutics*. 2016;12:612–22.
2. Rehman Z, Umar S, Meng C, Ullah Z, Riaz F, Rehman S, et al. Dendritic cell harmonised immunity to poultry pathogens; a review. *World's Poultry Science Journal*. 2017;73:581–90.
3. Steinman RM. Decisions about dendritic cells: Past, present, and future. *Annual review of immunology*. 2012;30:1–22.
4. Wykes M, MacPherson G. Dendritic cell-b-cell interaction: Dendritic cells provide b cells with CD40-independent proliferation signals and CD40-dependent survival signals. *Immunology*. 2000;100:1–3.
5. Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the drosophila toll protein signals activation of adaptive immunity. *Nature*. 1997;388:394–7.
6. Van Brussel I, Berneman ZN, Cools N, et al. Optimizing dendritic cell-based immunotherapy: Tackling the complexity of different arms of the immune system. *Mediators of inflammation*. 2012;2012.
7. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nature Reviews Cancer*. 2012;12:265–77.
8. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature*. 2007;449:419–26.
9. Borghaei H, Smith MR, Campbell KS. Immunotherapy of cancer. *European journal of pharmacology*. 2009;625:41–54.
10. Boudreau JE, Bonehill A, Thielemans K, Wan Y. Engineering dendritic cells to enhance cancer immunotherapy. *Molecular therapy*. 2011;19:841–53.
11. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. *Nature Reviews Immunology*. 2012;12:557–69.
12. Nair S, Archer GE, Tedder TF. Isolation and generation of human dendritic cells. *Current protocols in immunology*. 2012;99:7–32.
13. Tedder TF, Jansen PJ. Isolation and generation of human dendritic cells. *Current protocols in immunology*. 1997;23:7–32.
14. Zhou L-J, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proceedings of the National Academy of Sciences*. 1996;93:2588–92.
15. Sabado RL, Bhardwaj N. Directing dendritic cell immunotherapy towards successful cancer treatment. *Immunotherapy*. 2010;2:37–56.

16. Amedei A, Benagiano M, Bella C della, Niccolai E, D'Elios MM, et al. Novel immunotherapeutic strategies of gastric cancer treatment. BioMed Research International. 2011;2011.
17. Nicolette C, Healey D, Tcherepanova I, Whelton P, Monesmith T, Coombs L, et al. Dendritic cells for active immunotherapy: Optimizing design and manufacture in order to develop commercially and clinically viable products. Vaccine. 2007;25:B47–60.
18. Fanger NA, Wardwell K, Shen L, Tedder TF, Guyre PM. Type i (CD64) and type II (CD32) fc gamma receptor-mediated phagocytosis by human blood dendritic cells. Journal of immunology (Baltimore, Md: 1950). 1996;157:541–8.
19. O'Neill D, Bhardwaj N. Generation of autologous peptide-and protein-pulsed dendritic cells for patient-specific immunotherapy. Adoptive Immunotherapy: Methods and Protocols. 2005;97–112.
20. Schnurr M, Chen Q, Shin A, Chen W, Toy T, Jenderek C, et al. Tumor antigen processing and presentation depend critically on dendritic cell type and the mode of antigen delivery. Blood. 2005;105:2465–72.
21. Schroers R, Sinha I, Segall H, Schmidt-Wolf IG, Rooney CM, Brenner MK, et al. Transduction of human PBMC-derived dendritic cells and macrophages by an HIV-1-based lentiviral vector system. Molecular Therapy. 2000;1:171–9.
22. Dyall J, Latouche J-B, Schnell S, Sadelain M. Lentivirus-transduced human monocyte-derived dendritic cells efficiently stimulate antigen-specific cytotoxic t lymphocytes. Blood, The Journal of the American Society of Hematology. 2001;97:114–21.
23. Lizée G, Gonzales MI, Topalian SL. Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells. Human gene therapy. 2004;15:393–404.
24. Yang L, Yang H, Rideout K, Cho T, Joo K il, Ziegler L, et al. Engineered lentivector targeting of dendritic cells for in vivo immunization. Nature biotechnology. 2008;26:326–34.
25. Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E, et al. Induction of tumor-specific cytotoxic t lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. Annals of surgery. 2002;235:540.
26. Gilboa E, Vieweg J. Cancer immunotherapy with mRNA-transfected dendritic cells. Immunological reviews. 2004;199:251–63.
27. Heiser A, Maurice MA, Yancey DR, Coleman DM, Dahm P, Vieweg J. Human dendritic cells transfected with renal tumor RNA stimulate polyclonal t-cell responses against antigens expressed by primaryand metastatic tumors. Cancer research. 2001;61:3388–93.
28. Doyle C, Strominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. Nature. 1987;330:256–9.

29. Engleman EG, Benike C, Grumet FC, Evans R. Activation of human t lymphocyte subsets: Helper and suppressor/cytotoxic t cells recognize and respond to distinct histocompatibility antigens. *Journal of immunology* (Baltimore, Md: 1950). 1981;127:2124–9.
30. Reis e Sousa C, Austyn JM. Phagocytosis of antigens by langerhans cells. *Dendritic Cells in Fundamental and Clinical Immunology*. 1993;199–204.
31. Hoffmann E, Kotsias F, Visentin G, Bruhns P, Savina A, Amigorena S. Autonomous phagosomal degradation and antigen presentation in dendritic cells. *Proceedings of the National Academy of Sciences*. 2012;109:14556–61.
32. Garrett WS, Chen L-M, Kroschewski R, Ebersold M, Turley S, Trombetta S, et al. Developmental control of endocytosis in dendritic cells by Cdc42. *Cell*. 2000;102:325–34.
33. Platt CD, Ma JK, Chalouni C, Ebersold M, Bou-Reslan H, Carano RA, et al. Mature dendritic cells use endocytic receptors to capture and present antigens. *Proceedings of the National Academy of Sciences*. 2010;107:4287–92.
34. Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S-I, Soares H, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves t cell vaccination. *The Journal of experimental medicine*. 2004;199:815–24.
35. Sallusto F, Celli M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: Downregulation by cytokines and bacterial products. *The Journal of experimental medicine*. 1995;182:389–400.
36. Norbury CC, Chambers BJ, Prescott AR, Ljunggren H-G, Watts C. Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. *European journal of immunology*. 1997;27:280–8.
37. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005;307:254–8.
38. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature immunology*. 2001;2:361–7.
39. Tordesillas L, Lozano-Ojalvo D, Dunkin D, Mondoulet L, Agudo J, Merad M, et al. PDL2+ CD11b+ dermal dendritic cells capture topical antigen through hair follicles to prime LAP+ tregs. *Nature communications*. 2018;9:5238.
40. Yao C, Kaplan DH. Langerhans cells transfer targeted antigen to dermal DC and acquire MHC-II in vivo. *The Journal of investigative dermatology*. 2018;138:1665.

41. Deckers J, Sichien D, Plantinga M, Van Moorleghem J, Vanheerswynghels M, Hoste E, et al. Epicutaneous sensitization to house dust mite allergen requires interferon regulatory factor 4-dependent dermal dendritic cells. *Journal of Allergy and Clinical Immunology*. 2017;140:1364–77.
42. Gerner MY, Torabi-Parizi P, Germain RN. Strategically localized dendritic cells promote rapid t cell responses to lymph-borne particulate antigens. *Immunity*. 2015;42:172–85.
43. Binnewies M, Mujal AM, Pollack JL, Combes AJ, Hardison EA, Barry KC, et al. Unleashing type-2 dendritic cells to drive protective antitumor CD4+ t cell immunity. *Cell*. 2019;177:556–71.
44. Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP, et al. The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the t cell area of the lymph node. *Immunity*. 2005;22:19–29.
45. Gretz JE, Norbury CC, Anderson AO, Proudfoot AE, Shaw S. Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *The Journal of experimental medicine*. 2000;192:1425–40.
46. Gerner MY, Casey KA, Kastenmuller W, Germain RN. Dendritic cell and antigen dispersal landscapes regulate t cell immunity. *Journal of Experimental Medicine*. 2017;214:3105–22.
47. Gilfillan CB, Kuhn S, Baey C, Hyde EJ, Yang J, Ruedl C, et al. Clec9A+ dendritic cells are not essential for antitumor CD8+ t cell responses induced by poly i: C immunotherapy. *The Journal of Immunology*. 2018;200:2978–86.
48. Gutiérrez-Martínez E, Planès R, Anselmi G, Reynolds M, Menezes S, Adiko AC, et al. Cross-presentation of cell-associated antigens by MHC class i in dendritic cell subsets. *Frontiers in immunology*. 2015;6:363.
49. Dudziak D, Kamphorst AO, Heidkamp GF, Buchholz VR, Trumppheller C, Yamazaki S, et al. Differential antigen processing by dendritic cell subsets in vivo. *Science*. 2007;315:107–11.
50. Lehmann CH, Baranska A, Heidkamp GF, Heger L, Neubert K, Lühr JJ, et al. DC subset-specific induction of t cell responses upon antigen uptake via fcγ receptors in vivo. *Journal of Experimental Medicine*. 2017;214:1509–28.
51. Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JC, et al. The dendritic cell subtype-restricted c-type lectin Clec9A is a target for vaccine enhancement. *Blood, The Journal of the American Society of Hematology*. 2008;112:3264–73.
52. Haan JM den, Bevan MJ. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8+ and CD8- dendritic cells in vivo. *The Journal of experimental medicine*. 2002;196:817–27.

53. Ballesteros-Tato A, León B, Lund FE, Randall TD. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8+ t cell responses to influenza. *Nature immunology*. 2010;11:216–24.
54. Baker K, Qiao S-W, Kuo TT, Aveson VG, Platzer B, Andersen J-T, et al. Neonatal fc receptor for IgG (FcRn) regulates cross-presentation of IgG immune complexes by CD8-CD11b+ dendritic cells. *Proceedings of the National Academy of Sciences*. 2011;108:9927–32.
55. Vander Lugt B, Khan AA, Hackney JA, Agrawal S, Lesch J, Zhou M, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. *Nature immunology*. 2014;15:161–7.
56. Van Panhuys N. TCR signal strength alters t-DC activation and interaction times and directs the outcome of differentiation. *Frontiers in immunology*. 2016;7:6.
57. McLellan AD, Kapp M, Eggert A, Linden C, Bommhardt U, Bröcker E-B, et al. Anatomic location and t-cell stimulatory functions of mouse dendritic cell subsets defined by CD4 and CD8 expression. *Blood, The Journal of the American Society of Hematology*. 2002;99:2084–93.
58. Vremec D, Pooley J, Hochrein H, Wu L, Shortman K. CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *The Journal of Immunology*. 2000;164:2978–86.
59. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annual review of immunology*. 2003;21:335–76.
60. Edwards AD, Diebold SS, Slack EM, Tomizawa H, Hemmi H, Kaisho T, et al. Toll-like receptor expression in murine DC subsets: Lack of TLR7 expression by CD8 $\alpha$ + DC correlates with unresponsiveness to imidazoquinolines. *European journal of immunology*. 2003;33:827–33.
61. Boonstra A, Asselin-Paturel C, Gilliet M, Crain C, Trinchieri G, Liu Y-J, et al. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing t helper type 1 and 2 cell development: Dependency on antigen dose and differential toll-like receptor ligation. *The Journal of experimental medicine*. 2003;197:101–9.
62. Boonstra A, Rajsbaum R, Holman M, Marques R, Asselin-Paturel C, Pereira JP, et al. Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88-and TRIF-dependent TLR signals, and TLR-independent signals. *The Journal of Immunology*. 2006;177:7551–8.
63. Shortman K, Lahoud MH, Caminschi I. Improving vaccines by targeting antigens to dendritic cells. *Experimental & molecular medicine*. 2009;41:61–6.
64. Tacken PJ, Torensma R, Figdor CG. Targeting antigens to dendritic cells in vivo. *Immunobiology*. 2006;211:599–608.

65. Jinushi M, Hodi FS, Dranoff G. Enhancing the clinical activity of granulocyte-macrophage colony-stimulating factor-secreting tumor cell vaccines. *Immunological reviews*. 2008;222:287–98.
66. Jinushi M, Tahara H. Cytokine gene-mediated immunotherapy: Current status and future perspectives. *Cancer science*. 2009;100:1389–96.
67. Luiten RM, Kueter EW, Mooi W, Gallee MP, Rankin EM, Gerritsen WR, et al. Immunogenicity, including vitiligo, and feasibility of vaccination with autologous GM-CSF-transduced tumor cells in metastatic melanoma patients. *Journal of clinical oncology*. 2005;23:8978–91.
68. Small EJ, Sacks N, Nemunaitis J, Urba WJ, Dula E, Centeno AS, et al. Granulocyte macrophage colony-stimulating factor-secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. *Clinical Cancer Research*. 2007;13:3883–91.
69. Higano CS, Corman JM, Smith DC, Centeno AS, Steidle CP, Gittleman M, et al. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. *Cancer*. 2008;113:975–84.
70. Filipazzi P, Valenti R, Huber V, Pilla L, Canese P, Iero M, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *Journal of clinical oncology*. 2007;25:2546–53.
71. Sica A, Bronte V, et al. Altered macrophage differentiation and immune dysfunction in tumor development. *The Journal of clinical investigation*. 2007;117:1155–66.
72. Carter RW, Thompson C, Reid DM, Wong SY, Tough DF. Preferential induction of CD4+ t cell responses through in vivo targeting of antigen to dendritic cell-associated c-type lectin-1. *The Journal of Immunology*. 2006;177:2276–84.
73. Boscardin SB, Hafalla JC, Masilamani RF, Kamphorst AO, Zebroski HA, Rai U, et al. Antigen targeting to dendritic cells elicits long-lived t cell help for antibody responses. *The Journal of experimental medicine*. 2006;203:599–606.
74. Tacken PJ, Vries IJM de, Gijzen K, Joosten B, Wu D, Rother RP, 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*. 2005;106:1278–85.
75. Ramakrishna V, Treml JF, Vitale L, Connolly JE, O'Neill T, Smith PA, et al. Mannose receptor targeting of tumor antigen pmel17 to human dendritic cells directs anti-melanoma t cell responses via multiple HLA molecules. *The Journal of Immunology*. 2004;172:2845–52.
76. Hodge JW, Rad AN, Grosenbach DW, Sabzevari H, Yafal AG, Gritz L, et al. Enhanced activation of t cells by dendritic cells engineered to hyperexpress a triad of costimulatory molecules. *Journal of the National Cancer Institute*. 2000;92:1228–39.

77. Collin M, Bigley V. Human dendritic cell subsets: An update. *Immunology*. 2018;154:3–20.
78. Eisenbarth S. Dendritic cell subsets in t cell programming: Location dictates function. *Nature Reviews Immunology*. 2019;19:89–103.
79. Canel J-C, Crozat K, Dalod M, Mattiuz R. Are conventional type 1 dendritic cells critical for protective antitumor immunity and how? *Frontiers in immunology*. 2019;10:9.
80. Mastelic-Gavillet B, Balint K, Boudousquie C, Gannon PO, Kandalaft LE. Personalized dendritic cell vaccines—recent breakthroughs and encouraging clinical results. *Frontiers in immunology*. 2019;10:766.
81. Steinman RM, Nussenzweig MC. Avoiding horror autotoxicus: The importance of dendritic cells in peripheral t cell tolerance. *Proceedings of the National Academy of Sciences*. 2002;99:351–8.
82. Vries IJM de, Lesterhuis WJ, Scharenborg NM, Engelen LP, Ruiter DJ, Gerritsen M-JP, et al. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. *Clinical cancer research*. 2003;9:5091–100.
83. Jonuleit H, Giesecke-Tuettenberg A, Tüting T, Thurner-Schuler B, Stuge TB, Paragnik L, et al. A comparison of two types of dendritic cell as adjuvants for the induction of melanoma-specific t-cell responses in humans following intranodal injection. *International journal of cancer*. 2001;93:243–51.
84. Jonuleit H, Kühn U, Müller G, Steinbrink K, Paragnik L, Schmitt E, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *European journal of immunology*. 1997;27:3135–42.
85. Mailliard RB, Wankowicz-Kalinska A, Cai Q, Wesa A, Hilkens CM, Kapsenberg ML, et al.  $\alpha$ -type-1 polarized dendritic cells: A novel immunization tool with optimized CTL-inducing activity. *Cancer research*. 2004;64:5934–7.
86. Lutz MB, Schuler G. Immature, semi-mature and fully mature dendritic cells: Which signals induce tolerance or immunity? *Trends in immunology*. 2002;23:445–9.
87. Vonderheide R, Flaherty K, Khalil M, Stumacher M, Bajor D, Hutnick N. Sull ivan p, mahany JJ, gallagher m, kramer a, green SJ, o'dwyer PJ, running KL, huhn RD, antonia SJ: Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J Clin Oncol*. 2007;25:876–83.
88. Turner JG, Rakhamilevich AL, Burdelya L, Neal Z, Imboden M, Sondel PM, et al. Anti-CD40 antibody induces antitumor and antimetastatic effects: The role of NK cells. *The Journal of Immunology*. 2001;166:89–94.
89. Calderhead DM, DeBenedette MA, Ketteringham H, Gamble AH, Horvatinovich JM, Tcherepanova IY, et al. Cytokine maturation followed by CD40L mRNA electroporation

- results in a clinically relevant dendritic cell product capable of inducing a potent proinflammatory CTL response. *Journal of Immunotherapy*. 2008;31:731–41.
90. DeBenedette MA, Calderhead DM, Tcherepanova IY, Nicolette CA, Healey DG. Potency of mature CD40L RNA electroporated dendritic cells correlates with IL-12 secretion by tracking multifunctional CD8+/CD28+ cytotoxic t-cell responses in vitro. *Journal of Immunotherapy*. 2011;34:45–57.
91. Bonehill A, Tuyaerts S, Van Nuffel AM, Heirman C, Bos TJ, Fostier K, et al. Enhancing the t-cell stimulatory capacity of human dendritic cells by co-electroporation with CD40L, CD70 and constitutively active TLR4 encoding mRNA. *Molecular Therapy*. 2008;16:1170–80.
92. Kikuchi T, Moore MA, Crystal RG. Dendritic cells modified to express CD40 ligand elicit therapeutic immunity against preexisting murine tumors. *Blood, The Journal of the American Society of Hematology*. 2000;96:91–9.
93. Cisco RM, Abdel-Wahab Z, Dannull J, Nair S, Tyler DS, Gilboa E, et al. Induction of human dendritic cell maturation using transfection with RNA encoding a dominant positive toll-like receptor 4. *The Journal of Immunology*. 2004;172:7162–8.
94. Borst J, Hendriks J, Xiao Y. CD27 and CD70 in t cell and b cell activation. *Current opinion in immunology*. 2005;17:275–81.
95. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: Impact on priming of TH1, TH2 and nonpolarized t cells. *Nature immunology*. 2000;1:311–6.
96. Bonehill A, Van Nuffel AM, Corthals J, Tuyaerts S, Heirman C, François V, et al. Single-step antigen loading and activation of dendritic cells by mRNA electroporation for the purpose of therapeutic vaccination in melanoma patients. *Clinical Cancer Research*. 2009;15:3366–75.
97. Nair S, McLaughlin C, Weizer A, Su Z, Boczkowski D, Dannull J, et al. Injection of immature dendritic cells into adjuvant-treated skin obviates the need for ex vivo maturation. *The Journal of Immunology*. 2003;171:6275–82.
98. Wilgenhof S, Van Nuffel AM, Corthals J, Heirman C, Tuyaerts S, Benteyn D, et al. Therapeutic vaccination with an autologous mRNA electroporated dendritic cell vaccine in patients with advanced melanoma. *Journal of immunotherapy*. 2011;34:448–56.
99. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, et al. Expansion and activation of CD103+ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity*. 2016;44:924–38.
100. Sánchez-Paulete AR, Cueto FJ, Martínez-López M, Labiano S, Morales-Kastresana A, Rodríguez-Ruiz ME, et al. Cancer immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal antibodies requires BATF3-dependent dendritic cells. *Cancer discovery*. 2016;6:71–9.

101. Wculek SK, Amores-Iniesta J, Conde-Garrosa R, Khouili SC, Melero I, Sancho D. Effective cancer immunotherapy by natural mouse conventional type-1 dendritic cells bearing dead tumor antigen. *Journal for immunotherapy of cancer*. 2019;7:1–16.
102. Theisen DJ, Davidson IV JT, Briseño CG, Gargaro M, Lauron EJ, Wang Q, et al. WDFY4 is required for cross-presentation in response to viral and tumor antigens. *Science*. 2018;362:694–9.
103. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, et al. Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity*. 2013;38:729–41.
104. Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, et al. Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *The Journal of experimental medicine*. 2005;202:1691–701.
105. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature medicine*. 2007;13:1050–9.
106. Laoui D, Keirsse J, Morias Y, Van Overmeire E, Geeraerts X, Elkrim Y, et al. The tumour microenvironment harbours ontogenically distinct dendritic cell populations with opposing effects on tumour immunity. *Nature communications*. 2016;7:13720.

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