Dendritic cell-based vaccines for colorectal cancer: Mechanisms, clinical progress and future directions (Review)

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Abstract. Dendritic cell (DC)-based vaccines have emerged as a promising immunotherapy against a broad range of cancers. DCs are sentinel antigen-presenting cells that play a pivotal role in cancer immunosurveillance by recognizing tumor antigens via innate immune responses and subsequently stimulating adaptive T-cell-mediated anti-tumor responses. Autologous DC-based vaccines prime the individual's immune system to recognize and destroy tumor cells, aiming to achieve durable anti-tumor immunity and impede tumor relapse. The safety and effectiveness of DC-based vaccines for cancer treatment have been demonstrated in several clinical studies, either as monotherapy (such as Sipuleucel-T) or in combination with other therapies such as chemotherapy and immune checkpoint blockade. The present review provides a comprehensive overview of the current progress and clinical applications of DC-based vaccines for colorectal cancer (CRC). Various subsets of DCs and their biological roles in anti-tumor immunity are explored in detail and the mechanistic insights into DC-based vaccine development are discussed. Additionally, the present review highlighted the outcomes of recent clinical trials of autologous DC-based vaccines for advanced CRC, as well as the challenges and strategies for overcoming them in the development and clinical applications for CRC. Taken together, the potential and limitations of DC-based vaccines for CRC are underscored in the present review to provide an improved understanding and guide future directions in developing efficacious DC-based therapeutic vaccines for CRC.

Contents

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide and represents the second principal cause of cancer death. The mortality rates due to CRC have been increasing in recent years and account for 8.5% of total cancer mortality (1). The World Health Organization estimated over 1.9 million newly diagnosed CRC cases globally in 2020 and there were more than 930,000 mortalities due to CRC. The global incidence of CRC is projected to rise to 3.2 million new cases annually by 2040, indicating a 63% increase and the number of fatalities is expected to be 1.6 million per year (1). Within the field of oncology, immunotherapy is fast becoming recognized as a critical approach, though with varying degrees of success for CRC. Immune checkpoint blockade (ICB) therapy,

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for instance, demonstrates limited effectiveness in advanced CRC patients with microsatellite instability-high (MSI-H) tumors (2). This highlights a current limitation in the broader application of this specific immunotherapy for CRC.

The Consensus Molecular Subtypes (CMS) provide a framework for understanding the heterogeneity of CRC and are categorized into four subtypes: CMS1 (MSI Immune), CMS2 (Canonical), CMS3 (Metabolic) and CMS4 (Mesenchymal) (3). Each subtype presents unique molecular aberrations and distinct immune profiles that dictate the choice of antigens that can be exploited for cancer immunotherapy. Integrating these classifications allows for the identification of specific tumor microenvironments, ranging from highly immunogenic to immunosuppressive, that can be more easily targeted through personalized immunotherapy. CMS1 tumors, for example, are hypermutated and characterized by strong immune activation and high infiltration of Th1 and cytotoxic T cells, making them ideal candidates for vaccines targeting high neoantigen burdens. By contrast, CMS4 tumors exhibit prominent angiogenesis and stromal invasion, which often leads to an immunosuppressive environment that hinders T cell infiltration. This mesenchymal phenotype provides a strong rationale for the DC vaccines that target tumor-derived blood vessels to induce anti-angiogenic immunity and overcome stromal barriers. Meanwhile, 'cold' epithelial subtypes such as CMS2 and CMS3, which lack significant immune infiltration, may benefit from DC vaccines designed to initiate *de novo* priming and convert these immunologically inactive environments into 'hot' tumor. The gut microbiome has also emerged as a critical regulator for cancer immunity, fundamentally influencing the effectiveness of anticancer immunosurveillance. Evidence suggests that specific commensal bacteria are essential for the therapeutic effectiveness of immune-checkpoint inhibitors (ICIs) by modulating the functional activity of immune cells, including dendritic cells (DCs) (4,5). Understanding the interactions between the microbiome, immune cells and CRC is important for optimizing cancer immunotherapy, as therapeutic efficacy may depend on a balanced gut microbiota that supports effective systemic immunity.

Looking to the future and the continued emergence of immunotherapy approaches for CRC, therapeutic cancer vaccines are being developed to activate the immune system (6). Therapeutic vaccines are known to activate DCs and promote infiltration of T lymphocytes into the cancer microenvironment to elicit immune responses against tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) that are present in cancer cells. The overexpression of TAAs and TSAs is frequently observed in tumor cells and cancer cells respectively, as compared with normal cells (6). However, the challenge is to precisely identify the TAAs and TSAs present in CRC tumors. If the neoantigens are known, the accurate design of a peptide, DNA- or mRNA-based cancer vaccine is feasible. In the absence of known neoantigens from an individual cancer, using whole tumor cell lysates can elicit broad cytotoxic T-cell responses against multiple unknown antigens and patient-specific neoantigens from CRC (2,6).

DCs are crucial in initiating adaptive immunotherapy for CRC. There are a number of different colon cancer vaccines that function to stimulate antigen-presenting cells (APCs) (2). DC vaccines specifically utilize DCs loaded with tumor antigens under *ex vivo* conditions. This process activates effector immune cells such as $CD4^+$ and $CD8^+$ T cells, leading to a strong and long-term anti-tumor response (2,6). The rationale for utilizing DCs as a vaccine platform, particularly in the context of CRC immunotherapy, is rooted in their pivotal role in initiating adaptive immune responses. DCs are highly effective APCs as they excel at capturing, processing and presenting tumor antigens to immune cells such as $CD4^+$ and $CD8^+$ T cells (6). This capability is central to their function in vaccines. This mechanism is crucial for eliciting a robust immune response against cancer cells, thereby overcoming the challenges that conventional treatments often face in preventing metastasis. Essentially, DCs act as a vital link between recognizing the tumor and mobilizing the body's specific immune defense to combat it.

The primary focus for the present review was to explore the role of DCs as a promising immunotherapeutic tool, building upon the recognized limitations of conventional CRC therapies. The present review summarized the current landscape and mechanisms of DC vaccines specifically tailored for CRC. This encompasses detailing how DCs are utilized, for instance, by being loaded with tumor antigens *ex vivo* and underscoring their crucial function as APCs. It is also important to elucidate how DC vaccines are designed to overcome the significant limitations of conventional CRC therapies. The present review highlighted how these vaccines aim to combat CRC by activating effector immune cells, thereby eliciting a potent and long-term anti-tumor polyclonal response against all tumor antigens. Furthermore, the present review presented the current outcomes and findings from relevant clinical trials specifically involving DC vaccines for CRC, assessing their efficacy, safety and clinical progress. While this narrative review was focused on CRC, it drew on evidence from other cancers, including melanoma and glioblastoma, as supporting context for general DC vaccine principles and generation strategies.

2. Dendritic cells in cancer immunity

DCs are a diverse population of leukocytes that function as the 'sentinels' of the immune system, playing a pivotal role in orchestrating both innate and adaptive immunity (7). Initially discovered by Paul Langerhans back in 1868, these cells are responsible for balancing immunity and tolerance (8).

The general biology of DCs involves their origin from bone marrow progenitors such as common myeloid progenitors or common monocyte-DC precursors (MDPs) (9). From the bone marrow, DC precursors travel through the bloodstream to most non-lymphoid tissues, where they reside in an immature state. In this immature state, DCs continuously sample their environment through mechanisms such as endocytosis, macropinocytosis and phagocytosis (10,11). They have the ability to extend processes across epithelial tight junctions independent of any apparent stimulation including infections and inflammatory triggers. As the most potent type of APCs, DCs are specialized in capturing and processing antigens, converting proteins into peptides that are then presented or cross-presented on both major histocompatibility complex (MHC) class I and II molecules for T cell recognition (11).

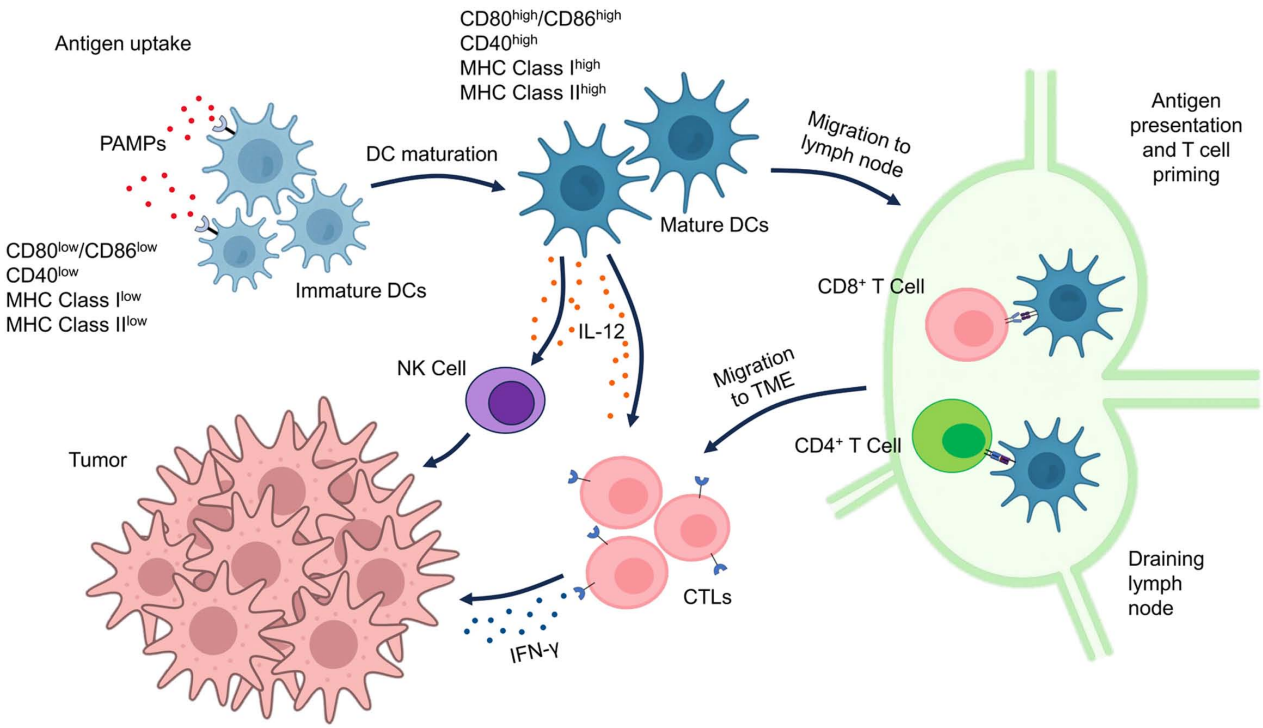

Figure 1. DCs in cancer immunity. Immature DCs in peripheral tissues capture tumor-associated antigens through pattern recognition receptors that recognize PAMPs. This antigen uptake drives their maturation, during which they downregulate phagocytosis but upregulate MHC molecules (MHC Class I and II), co-stimulatory signals (such as CD80, CD86 and CD40) and cytokine production (IL-12). Mature DCs migrate to draining lymph nodes, where they present tumor antigens on MHC I and MHC II to naïve $CD8^+$ and $CD4^+$ T cells, providing the signals necessary for their activation and differentiation. Primed CTLs then migrate into the tumor microenvironment, where they directly kill tumor cells. In parallel, DCs secrete IL-12 to further support CTL and NK cell functions, while activated CTLs release IFN-γ, enhancing tumor immunogenicity and sustaining the anti-tumor immune responses. DCs, dendritic cells; PAMPs, pathogen-associated molecular patterns; MHC, major histocompatibility complex; CTLs, cytotoxic T lymphocytes; NK, natural killer; IFN-γ, interferon-gamma; CD, cluster of differentiation; TME, tumor microenvironment.

Upon detecting intruders via pattern recognition receptors (such as Toll-like receptors) and capturing antigens within the tumor microenvironment (TME), immature DCs undergo phenotypical and functional maturation (12,13). This critical process leads to enhanced expression of MHCs and co-stimulatory molecules (such as CD40, CD80 and CD86), as well as increased secretion of cytokines such as IL-12, thereby equipping them to more effectively prime anti-tumor T cell responses (14-17). These mature DCs then migrate to lymphoid organs, particularly the tumor-draining lymph nodes (TDLNs) where they present processed antigens to naïve T cells to fully activate and differentiate them into effector T cells capable of killing cancer cells as well as supporting the immune response (18,19). The unique ability to directly and indirectly target cancer cells is what makes DCs a powerful platform for generating DC-based cancer vaccines as they effectively prime and activate T cells for a robust anti-tumor immune response (Fig. 1) (20).

The next section explores the biological characteristics of DC subsets such as conventional, plasmacytoid and monocyte-derived DCs, as well as their functional roles that make them an effective and promising approach for cancer vaccine development. It delves into one particular DC subset derived from monocytes as a leading candidate for DC-based cancer vaccines to explore its easy accessibility, antigen-presenting capacity and ability to induce robust T cell responses.

3. Biology and subtypes of DCs

DCs are a heterogeneous population with varying functions and they are categorized into three main populations: Conventional DCs (cDCs), plasmacytoid DCs (pDCs) and monocyte-derived DCs (MoDCs) (9). While the primary classification of DCs remains as plasmacytoid and monocyte-derived, the ever-growing insights into DC biology continue to reveal a more complex and heterogeneous population, with more dynamic states and emerging subtypes particularly within the TME.

cDCs. cDCs arise from common DC precursors (CDPs) and are divided into two primary types: Type 1 conventional DCs (cDC1) and type 2 conventional DCs (cDC2). Type 1 cDCs are defined by their reliance on transcription factors BATF3 and IRF8 for development (21,22). Aside from the common expression of XCR1, CLEC9A, CADM1 (NECL2), BTLA and CD26 found in both species, mouse cDC1s are found to express CD8α in lymphoid organs and C103 in peripheral tissues, while in humans, they are characterized by CD141 (BDCA-3), HLA-DR^{high}, CD11c and the absence of lineage markers (23). There are two major subsets of cDCs present in the TME, cDC1 and cDC2. cDC1s are highly specialized in cross-presenting exogenous antigens and activating $CD8^+$ T cells and are critical for inducing the 'cancer-immune cycle' and anti-tumor immunity, including responses to ICB and adoptive T cell transfer (18-24). They transport tumor antigens

to draining lymph nodes (LNs) and are the main producers of IL-12 and chemokines such as CXCL9/CXCL10 in the TME, which attract $CD8^+$ T cells (25). The functional roles of cDC1s within the TME may however vary across different tumor types.

Type 2 cDCs are typically identified by the absence of cDC1 markers and higher expression of CD11b, CD1c and SIRPα (CD172α), with IRF4 as a key transcription factor (26,27). In humans, they also express HLA-DR^+, $CLEC10A^+$, $CD1c^+$ and $CD172^+$ (27). cDC2s are primarily responsible for presenting endogenous antigens to $CD4^+$ T cells and shaping their polarization (such as Th2, Th17, Treg) (28-30). While the functional demarcation between cDC1 and cDC2 is more pronounced in mice, human cDC2s can also cross-present antigens and produce high levels of IL-12 (31). cDC2s are also important for improved $CD4^+$ T cell priming and tumor rejection, especially when regulatory T cells are absent (19). A previous study reported that cDC2s can activate $CD8^+$ T cells under certain conditions, even in the absence of cDC1s (32). Single-cell RNA sequencing studies have further identified heterogeneity within cDC2s, including cDC2A (T-bet^+) and cDC2B (T-bet^-) subtypes (33,34). Numerous cancers have been found to exhibit cDC2 infiltration within the TME, particularly those that have shown no response to cytotoxic T cells (35).

pDCs. These cells differentiate from myeloid CDPs and also from IL-$7R^+$ lymphoid progenitors, although only myeloid-derived pDCs process and display antigen (36). They are morphologically distinct, resembling plasma cells that secrete antibodies (37). pDCs are characterized by distinct markers found in human and mice, in which humans are defined by the expression of BDCA2 (CD303), BDCA4 (CD304) and IL-3 receptor (CD123) together with the absence of common lineage markers, while in mice, they harbor high expression of MHC II, intermediate CD11c, B220 and Siglec-H (37-39). Transcription factors such as IRF8, RUNX1 and TCF4 are involved in their differentiation (40). pDCs mainly respond to viral RNA and DNA infection through the production of high levels of type I IFN (IFN-α) (41). Their expression of MHC II and co-stimulatory molecules is well-defined; however, their antigen processing capabilities are less clear (42). Their role in cancer is complex; some studies have associated them with immunosuppression (such as promoting regulatory T cells, IL-10 production, programmed cell death ligand 1 (PD-L1) expression), while others reported anti-tumor responses when pDCs were properly activated (43-45).

MoDCs. MoDCs are amongst the most extensively studied DCs and are frequently utilized in clinical studies for cancer therapy, owing to their relative ease of *in vitro* generation and differentiation, as well as the limited availability of cDCs and pre-DCs (46). Due to their foundational and well-established role in DC-based cancer immunotherapy, they provide a viable option for treatment against CRC. These cells differentiate from $Ly6C^+$ monocytes in mice or $CD14^{hi}$ monocytes in humans, typically under inflammatory conditions or exposure to cancer or infection (21,47). Although $Ly6C^{hi}$ monocytes typically exhibit immunosuppressive functions within the TME, their differentiation into inflammatory DCs has been shown to trigger reactive oxygen species (ROS) production and promote an anti-tumor response independent of effector T cells (48,49). In mice, the differentiation of $Ly6C^+$ monocytes into inflammatory MoDCs has been shown to be dependent on activation by interferon regulatory factor 4 (IRF4) and ETV3 (50-52).

There are two subtypes of MoDCs: MoDC1 that expresses PD-L1 and MoDC2 that expresses CD155 (53). Their identification has historically been challenging due to marker overlap with macrophages and $CD11b^+$ DCs, but some studies use markers such as Fc receptors (FcγRI, FcεRI), CD11b, CD1c, SIRPα, HLA-DR, CLEC10A, CD11c and cystatin F (CST7) (53,54). CD14, abundantly expressed in peripheral blood monocytes, is markedly absent from the expression profile of mature MoDCs, although it may be retained at low or variable levels in immature MoDCs (55,56). Upon stimulation, mature MoDCs have been also shown to express high levels of MHC I, MHC II and co-stimulatory molecules such as CD40, CD80 and CD86 (53).

MoDCs are generally not known for transporting antigens to LNs to initiate a *de novo* T cell response. However, they are recruited during inflammation and can differentiate into 'Tip-DCs' (TNF and NOS2-producing inflammatory DCs) (57). They can enhance the survival of adoptively transferred T cells, regulate T cell activity in tumors and, under specific activation conditions, can cross-present antigens to prime $CD8^+$ T cells and reprogram $CD4^+$ T cells to Th1, Th2 and Th17 phenotypes (57-59). The shift toward Th1-polarized immunity and the induction of cytotoxic activity are facilitated by the production of effector molecules and pro-inflammatory cytokines, including TNF-α, IL-12 and nitric oxide (NO) (60-63). Their ability to prime and direct tumoricidal function is what makes them contribute to a successful T cell immunity and anti-tumor response (64-67).

Due to their capacity to be generated *in vitro* from blood mononuclear cells using IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) and subsequently matured with various cytokine cocktails that include prostaglandin E2 (PGE_2) and TNF-α, numerous clinical trials have explored their application in cancer immunotherapy (68). The majority of the clinical trials featuring DC vaccines have centered on the use of autologous MoDCs that were expanded *ex vivo* and introduced back into the patients as adoptive cell therapy. To date, the only DC-based vaccine approved by the U.S Food and Drug Administration (FDA) that utilizes autologous MoDCs is Sipuleucel-T, which has been employed as a cellular immunotherapy for patients with metastatic castration-resistant prostate cancer (mCRPC) (69). The approval was based on a phase III trial which demonstrated an improvement in median overall survival (OS) of 4.1 months (21.7 vs. 25.8 months) without an accompanying delay in disease progression (70). In more than a decade since Sipuleucel-T, there have been numerous clinical trials with MoDC-based vaccines for other cancers, which will be covered in more detail.

Other subsets. Beyond the classical subsets, emerging populations such as mature regulatory DCs (mregDCs) and DC type 3 (DC3s) have gained increasing attention for their distinct immunomodulatory roles within the TME (37). mregDCs, characterized by their expression of regulatory molecules and migratory capacity, have been implicated in both immune

activation and tolerance, while DC3s show functional plasticity and potential in anti-tumor immunity, although their classification is still under refinement. Understanding the diversity of DC populations and heterogeneity may help redefine and shape new approaches in cancer immunotherapy, specifically for CRC. mregDCs represent a newly characterized subset found in the TME that can arise from both cDC1 and cDC2 lineages after maturation and tumor antigen uptake (71,72). They exhibit regulatory activity toward tumor-responding T cells and are defined by coexisting maturation, migration and immunoregulatory profiles [such as PD-L1/PD-L2, indoleamine 2,3-dioxygenase 1 (IDO1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3)] (71,72). DC3s was recently found to be another type of human DC that resembles cDC2 (34,73). Several studies have indicated that DC3s may represent an ontogenetically distinct subset, deriving from MDPs rather than from CDPs, which give rise to cDCs (74). These cells are able to upregulate glucocorticoid-induced tumor necrosis factor receptor ligand (GITRL), thereby potentially sending cues that support T cells after being primed and also promote resident memory T cell survival (75,76).

4. Roles of DCs in anti-tumor response

Antigen uptake, processing and presentation. As the main players of immunity, DCs trigger the immune response by first initiating antigen uptake, processing them and presenting them for T cell activation. The wide range of membrane receptors allows the cells to recognize and subsequently capture and internalize various antigens, using different methods such as phagocytosis, macropinocytosis, micropinocytosis and endocytosis (10,11). Some of these diverse receptors include antibody receptor types I or II (FcγRI or FcγRII), integrins, glycan-binding proteins (lectin), phosphatidylserine receptors, phagocytic receptors and scavenger receptors involved in inflammation (77-80). Phagocytosis is the main process that allows DCs to capture and engulf large particles such as microorganisms, foreign materials and necrotic cells (9). The solubility of antigens also influences antigen uptake and subsequent immune activation, in which particulate antigens are found to elicit a stronger immune response than soluble antigens (81,82).

Activation of naïve T cells (CD4⁺ and CD8⁺). Once antigens are internalized, DCs process them into peptides for presentation through MHC molecules (83). Presenting these antigens along with a costimulatory signal allows DCs to fully activate naïve T cells and shape their differentiation into T effector subsets ($CD8^+$ and $CD4^+$ T cells). The classical antigen presentation pathways include the MHC Class I Pathway (Endogenous Pathway), which typically presents endogenous antigens (such as from viruses or tumor cells) to $CD8^+$ T cells (84). The MHC Class II Pathway (Exogenous Pathway), on the other hand, presents exogenous antigens from the extracellular environment to $CD4^+$ T cells (84). In the MHC II pathway, newly synthesized MHC II molecules are stabilized by binding to the invariant chain, which is later degraded to allow peptide binding facilitated by HLA-DM (85).

A unique and critical capability of DCs is cross-presentation, where they present internalized exogenous antigens on MHC class I molecules to activate $CD8^+$ T cells (84). This is especially important for anti-tumor responses and is particularly significant with cDC1s, as they are highly specialized in cross-presenting exogenous antigens and activating $CD8^+$ T cells (86). This specialization is more pronounced in mice than in humans (87,88). Cross-presentation by cDC1s is enhanced by type I IFN signaling (89). MoDCs can also cross-present antigens to prime $CD8^+$ T cell responses, particularly when triggered under inflammatory or cancerous conditions (90,91). Human cDC2s can also cross-present antigens and produce high levels of IL-12, though this function is largely confined to cDC1s in mice (31,92). Upon activation, tumor-infiltrating $CD11b^+$ cDC2s that express IFN have been shown to present tumor-derived peptide-MHC I complexes via direct interactions, subsequently activating $CD8^+$ T cells and promoting anti-tumor immunity even in the absence of cDC1s (32). There are two principal pathways for cross-presentation: i) The vacuolar pathway, in which antigens are processed and loaded onto MHC I molecules in endo/lysosomes and ii) the endosome-to-cytosol pathway, in which antigens enter the cytosol to be degraded by the proteasome and subsequently have their peptides transported to the endoplasmic reticulum (ER) for MHC I loading (93). WD Repeat- and FYVE Domain-Containing Protein 4 and SNARE family member SEC22B proteins are required for efficient cross-presentation (81). Antigen solubility also affects cross-presentation; soluble antigens are quickly degraded, leading to poor cross-presentation. By contrast, antigens in larger vesicles (~500 nm) are found in early endosomes and protected from complete degradation, resulting in more efficient cross-presentation when compared with smaller particles (<200 nm) or soluble antigens (94).

DCs also employ a mechanism known as cross-dressing, where they capture pre-formed peptide-MHC-I complexes from neighboring cells via trogocytosis, including tumor cells and present them (95,96). This takes place through plasma membrane transfer mediated by direct cell-cell contact or by secretion using membrane vesicles called exosomes (96). This mechanism has been shown to be sufficient for priming antigen-specific $CD8^+$ T cells.

Cytokine signaling and immune orchestration. The process of DC maturation begins with reduced antigen uptake activity and increased antigen presentation activity, which involves an upregulation of MHC molecules and co-stimulatory markers such as CD80, CD83 and CD86 (14-16). This is also accompanied by higher cytokine production (TNF-α and IL-12) and an enhanced migration ability to LNs, largely attributed to higher C-C chemokine receptor 7 (CCR7) expression (17,97). The transition between the two states is clearly defined, in which immature DCs are specialized for antigen capture, whereas mature MoDCs become optimized for T cell activation and immune modulation.

Mature DCs migrate to the draining LNs for T cell activation and differentiation, a process that requires three critical signals (98): i) The first signal (Signal 1) involves the presentation of processed tumor antigens. DCs present antigens on MHC class I molecules to naïve $CD8^+$ T cells through cross-presentation (99,100). This cross-presentation is a dedicated pathway for processing internalized exogenous antigens and loading them onto MHC class I molecules, which

is crucial for generating *de novo* cytotoxic T lymphocytes (CTLs). Simultaneously, DCs present antigens on MHC class II molecules to naïve $CD4^+$ T cells (101). ii) The second signal (Signal 2) provides essential co-stimulation, in which DCs express high levels of costimulatory molecules such as CD80/CD86 and CD40, which interact with their corresponding ligands (such as CD28) on T cells (101,102). This co-stimulation is important for full T cell activation and to prevent clonal anergy (103). iii) A third critical signal (Signal 3) involves the secretion of cytokines, which further shapes the T cell response. MoDCs can secrete inflammatory cytokines (104). Notably, the production of IL-12p70 is crucial, as it favors the development of cytotoxic cellular immunity and supports the differentiation of $CD4^+$ T cells into the Th1 phenotype (61,103). Th1 cells are characterized by the production of IFN-γ (105). This cytokine milieu, along with costimulatory signals, guides naïve T cells to differentiate into specialized helper and effector T cells, including Th1, Th2 and Th17 phenotypes (102,106). Other important co-stimulatory pathways include interactions between CD40-CD40L, CD137-CD137L, OX40-OX40L, GITR-GITRL and CD70-CD27 (107-110). The key cytokines that drive DC function for T cell effector activity lie in IL-12 and type I IFNs (111).

Crosstalk with other immune cells (NK cells, macrophages). The TME can markedly impair DC functionality, often leading to an immunosuppressive or tolerogenic phenotype (86). Inhibitory signals within the TME, such as vascular endothelial growth factor (VEGF), IL-10 and TIM-3, can suppress DC maturation and function (112). Tumors can hinder cDC recruitment and differentiation by reducing chemoattractants (such as CCL4) or affecting growth factors such as VEGF and FLT3L (113). Tumor-derived factors such as PGE_2, IL-6 and gangliosides can inhibit DC maturation and survival (114-116). DCs can also modulate T cell function by altering the availability of metabolic substrates, for instance, by depleting L-tryptophan through IDO1, which promotes regulatory T cells (Tregs) differentiation and suppresses $CD8^+$ T cell and NK cell functions (117,118). Lipid accumulation and ER stress in tumor-associated DCs can impair antigen processing and cross-presentation (119-121). Lactic acid, a metabolic product of tumor cells, can also impair MoDC differentiation and activation (122). Furthermore, some pDCs in the TME are associated with immunosuppression, causing the cells to fail to produce type I IFN and promoting Treg expansion (123,124).

5. Mechanistic insights into monocyte-derived DC-mediated T cell activation for cancer therapy

MoDCs are more commonly utilized in DC-associated *in vitro* studies. The cells are key players in initiating and influencing anti-tumor responses through their complex interactions with T cells. The mechanism of immune activation by MoDCs is similar to other DC subsets, in which this process begins with antigen uptake, where they engulf and internalize TAAs (12,124). Following this uptake, MoDCs begin to mature and increase the surface expression of key molecules such as MHC class I and II, CD40, CD80 and CD86, alongside the production of cytokines such as IL-12 (15,16,125). Once matured, MoDCs migrate to draining LNs, where their migration to LNs is dependent on CCR7 signaling (126).

Within these LNs, MoDCs engage with naïve T cells as depicted in Fig. 2, providing three signals for T cell activation and differentiation: antigen presentation and cross presentation via MHC-peptide complexes (Signal 1), co-stimulation via molecules from the DC to T cells, such as CD80 and CD86 interacting with CD28 on T cells (Signal 2) and immune-stimulatory cytokines in the microenvironment (Signal 3) (61,124,127). Beyond these three signals, MoDCs can also provide Signal 4, which imprints desirable homing properties onto the activated T cells (128,129). Once activated in the LNs, the progeny of these primed T cells, including CTLs and helper T cells, migrate out of the LNs and traffic to the TME to execute their effector functions (130).

$CD8^+$ CTLs recognize cancer cells through specific tumor antigens presented on MHC class I molecules. The CTLs then proceed to eliminate the cancer cells through direct cytotoxicity, which is primarily mediated by the release of perforin and granzyme B (131). Perforin creates pores in the target cell membrane, allowing granzymes to enter and induce apoptosis (programmed cell death). Additionally, activated $CD4^+$ T cells (Th1 cells), which are polarized by MoDCs, support CTLs by producing cytokines such as IL-2 that are essential for maintaining the responsiveness and proliferation of CTLs and enhancing their anti-tumor activity (20). While MoDCs offer significant promise in immunogenic cell death, their functionality can be influenced by the immunosuppressive TME (132). Despite these challenges, MoDCs remain a viable and adaptable option for cancer immunotherapy, with ongoing efforts to optimize their properties for more potent anti-tumor responses.

T cell exhaustion and dysfunction in the TME. In the TME, T cell exhaustion and dysfunction are dominant characteristics of immune responses against cancer (Fig. 3). Exhausted T cells have a reduced ability to generate pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-2 and to kill cancer cells. They have increased expression of several co-inhibitory receptors, including programmed cell death protein 1 (PD-1), TIM-3, lymphocyte activation gene 3 (LAG-3) and 2B4 (CD244), which contribute to the immunosuppressive environment within the TME (133). MoDCs, while having an established foundation in DC therapy, are considered a double-edged sword as they can both oppose and contribute to the state of TME, particularly under conditions of T cell exhaustion.

While under inflammatory conditions such as exposure to cancer cells, MoDCs become stimulated, enabling them to induce TNF-α and NO and subsequently enhance the survival of adoptively transferred T cells (53,57). These stimulated cells, also called TIP-DCs, are shown to play a crucial role in controlling tumor growth and might be dependent on activation of the CD40-CD40L signaling pathway (57). Building on this, when targeted with agonistic anti-CD40 antibodies, MoDCs are able to differentiate into inducible nitric oxide synthase (iNOS)-producing cells and express high levels of costimulatory molecules that correlate with the presence of effector tumor infiltrating lymphocytes, thereby enhancing PD-1 ICB therapy (53). In some contexts, a combination of MoDCs with adjuvant therapy such as polyinosinic:polycytidilic acid

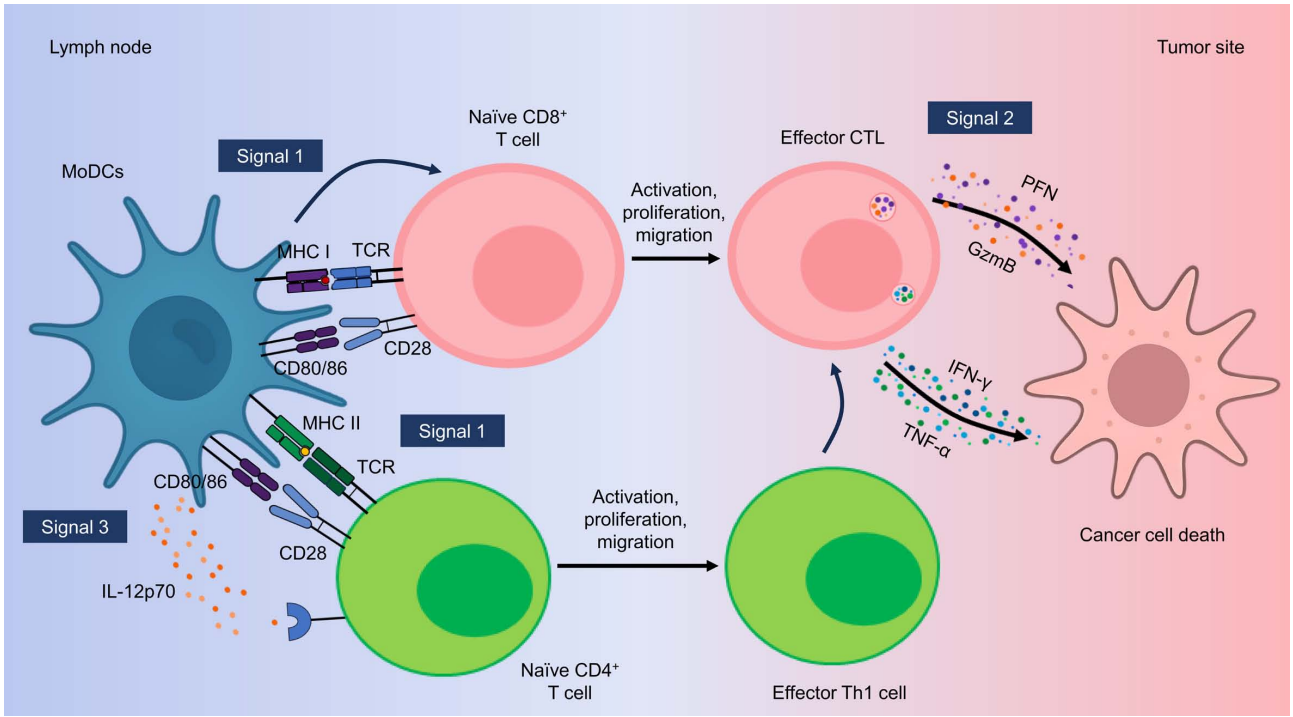

Figure 2. Overview of MoDC-driven activation of T lymphocytes. After antigen uptake and processed by DCs, they are presented or cross-presented as peptides on MHC class I for priming $CD8^+$ T cells and MHC class II for priming $CD4^+$ T cells (Signal 1). Once matured, MoDCs express high levels of costimulatory molecules (such as CD80 and CD86) that activate $CD8^+$ CTLs (Signal 2). Activated $CD8^+$ CTLs directly kill tumor cells via perforin/granzyme secretion. MoDCs also secrete cytokines such as IL-12p70, which shifts the immune response into a Th1 phenotype, further enhancing CTL activity and improve antitumor immunity (Signal 3). MoDCs, monocyte-derived dendritic cells; DCs, dendritic cells; MHC, major histocompatibility complex; CTLs, cytotoxic T lymphocytes; Th1, T helper 1; TCR, T cell receptor; CD, cluster of differentiation; PFN, perforin; GzmB, Granzyme B; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor alpha; IL-12p70, interleukin-12 p70.

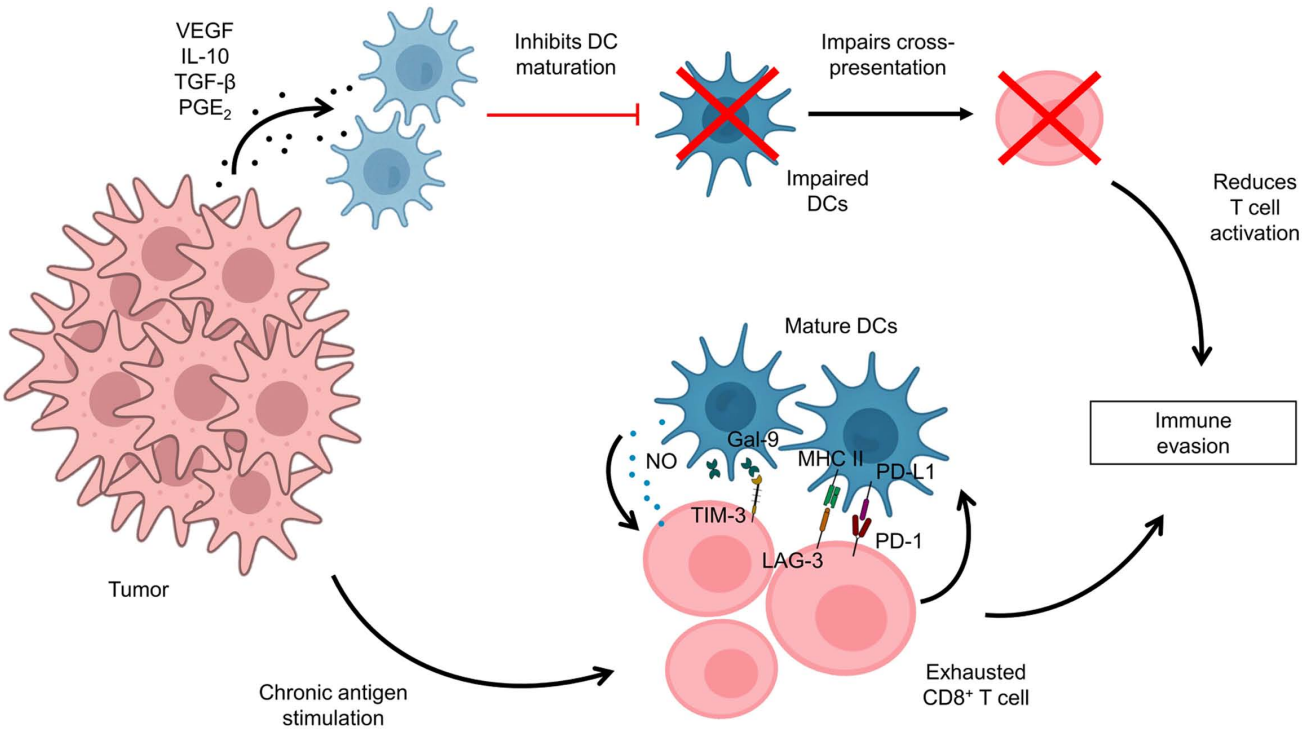

Figure 3. T-cell exhaustion and dysfunction in the TME. Tumor cells secrete VEGF, IL-10, TGF-β and PGE_2, which inhibit DC maturation and cross-presentation. These defects limit the ability of DCs to activate T cells, reducing $CD8^+$ cytotoxic T cell responses and promoting immune evasion within the TME. Chronic antigen stimulation promotes $CD8^+$ T cell exhaustion, which in turn expresses checkpoint receptors such as PD-1, LAG-3 and TIM-3 that act on DCs and also NO secretion from DCs that act on tumor cells and facilitate immune evasion. TME, tumor microenvironment; DCs, dendritic cells; VEGF, vascular endothelial growth factor; IL-10, interleukin-10; TGF-β, transforming growth factor beta; PGE_2, prostaglandin E2; NO, nitric oxide; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3; LAG-3, lymphocyte activation gene 3; Gal-9, Galectin-9; MHC, major histocompatibility complex; PD-L1, programmed cell death ligand 1; PD-1, programmed cell death protein 1; CD, cluster of differentiation.

(Poly(I:C)) was also observed to be significant in eliciting an anti-tumor response compared with other DC subsets (134). These inflammatory MoDCs can foster a microenvironment that not only exerts direct tumoricidal effects but also sustains the expansion of tumor-infiltrating T cells, thereby amplifying anti-tumor immunity.

However, under chronic inflammation or T cell exhaustion, persistent TNF-α and NO might also contribute to immunosuppression and tissue damage, limiting therapeutic benefit. While low/moderate NO could support immune activation, excessive NO might suppress T cell proliferation and contribute to immune exhaustion (135,136). Moreover, monocytes as a source of inflammatory DCs are also known to give rise to myeloid-derived suppressor cells (MDSCs), further hampering anti-tumor activity and promoting tumor growth (137). It is important to acknowledge that while the TME could impair MoDC function and contribute to T cell dysfunction, strategic targeting and activation of MoDCs represent a viable therapeutic avenue to support T cell activity and overcome exhaustion. However, further research is necessary to specifically identify MoDCs and fully comprehend their complex roles within the TME, especially given the challenge of distinguishing them from other DC subsets.

6. Underlying mechanism and advances in DC-based vaccine development

Antigen loading strategies. The strategy used to load antigens onto DCs is pivotal in determining the vaccine efficacy, as it governs both the breadth of tumor antigen recognition and the specificity of the resulting T-cell responses (Fig. 4). Whole tumor cell lysates (TCLs) remained among the most widely employed approaches, since they encompassed the full repertoire of TAAs and TSAs. This poly-antigenic stimulation generated polyclonal T-cell populations capable of recognizing multiple epitopes, thereby reducing the likelihood of immune evasion through antigen-loss variants (138). Clinical studies in glioblastoma and ovarian carcinoma demonstrated that lysate-pulsed DCs could elicit durable CTL responses and long-term immune memory with acceptable safety profiles (139,140). More recently, the phase-III DCVax-L trial validated this proof-of-concept at a clinical level, where the study reported significant prolonged survival in glioblastoma patients treated with autologous tumor lysate-loaded DCs compared with control groups with any treatment/interventions (141). This study firmly established broad tumor-antigen coverage as a clinically relevant strategy, though survival benefits were not similar across multiple different cancers, reflecting the variability of host immune competence and the heterogeneity of TME.

The manner in which lysates are prepared is now understood to critically influence vaccine potency. Roufarshbaf *et al* (142) systematically compared four methods of tumor lysate generation: freeze-thaw, hypochlorous acid (HOCl) oxidation, hyperthermia and ultraviolet irradiation. The results showed that HOCl-treated lysates produced superior DC maturation, higher IFN-γ secretion and reduced IL-10 release. The findings from the study highlighted that oxidative stress markedly induced immunogenic danger signals that could polarize T cells toward Th1 immunity. At the same time, the authors cautioned that excessive oxidation could lead to epitope degradation, potentially impairing T-cell recognition and further stated that such methods demand precise laboratory control to ensure reproducibility. Herzog *et al* (143) also demonstrated that the use of trypsin to detach adherent tumor cells prior to lysate generation resulted in degradation of key surface proteins required for effective antigen presentation, thereby diminishing the stimulatory potential of the final DC product. Collectively, these studies indicate that antigen preparation is not a trivial technical step but a decisive factor that can determine whether lysates generated are immunogenic or non-immunogenic.

Heat shock-based TCL preparation was also observed to provide ample DC stimulation. Heat-shock treatments induced the expression of stress proteins such as HSP70 and HSP90, which served as endogenous danger signals that promoted DC maturation and antigen cross-presentation. Heat-shocked TCLs therefore not only provide a comprehensive repertoire of TAAs but also incorporated intrinsic adjuvant activity. Srivastava *et al* (144) were among the first to identify the immunogenic potential of heat-shock proteins as carriers of tumor peptides, showing that HSP-peptide complexes could elicit protective immunity in mice. Later work by Manjili *et al* (145) demonstrated that TCLs subjected to heat shock enhanced DC activation, leading to stronger $CD8^+$ T-cell priming and tumor rejection compared with lysates prepared under standard conditions. DCs pulsed with heat-shock TCLs generated higher levels of IL-12 and TNF-α, favoring Th1-polarized immunity (146). The strength of this approach is that it coupled antigen breadth with innate activation, thus circumventing the need for external adjuvants. However, the drawbacks included variability in lysate preparations, the potential induction of tolerance if maturation signals were insufficient and difficulty in standardizing clinical-grade heat-shock protocols.

Beyond conventional lysates, fusion-based vaccines could offer an alternative means of broad antigen presentation. Kao *et al* (147) directly compared DCs pulsed with TCLs to DC/tumor fusion hybrids in a murine colon carcinoma model. The authors found that fusion-derived DC vaccines elicited stronger CTL responses and superior tumor rejection, a phenomenon that could be attributed to the continuous endogenous expression of tumor antigens after fusion, which allowed sustained presentation to T cells. While this approach circumvented some of the limitations of TCL-derived DC vaccines, the technical demands of creating fusion cells *ex vivo* and the challenges of regulatory standardization across patient-specific samples remained major hurdles for clinical translation. The immunogenic potential of lysate-pulsed DCs could also be augmented through rational combinations with immune modulators. Bakhshivand *et al* (148) examined the use of breast cancer lysate-pulsed DCs in combination with cytotoxic T-lymphocyte antigen-4 (CTLA-4) blockade. The results demonstrated markedly enhanced T-cell expansion and improved tumor control compared with DC vaccination alone. These findings suggested that checkpoint inhibitors could synergize with DC vaccines by overcoming tumor-induced tolerance and reinvigorating exhausted T-cell populations. Nevertheless, the increased risk of immune-related toxicities and the economic burden of combining two advanced therapies presented practical barriers to generalized clinical

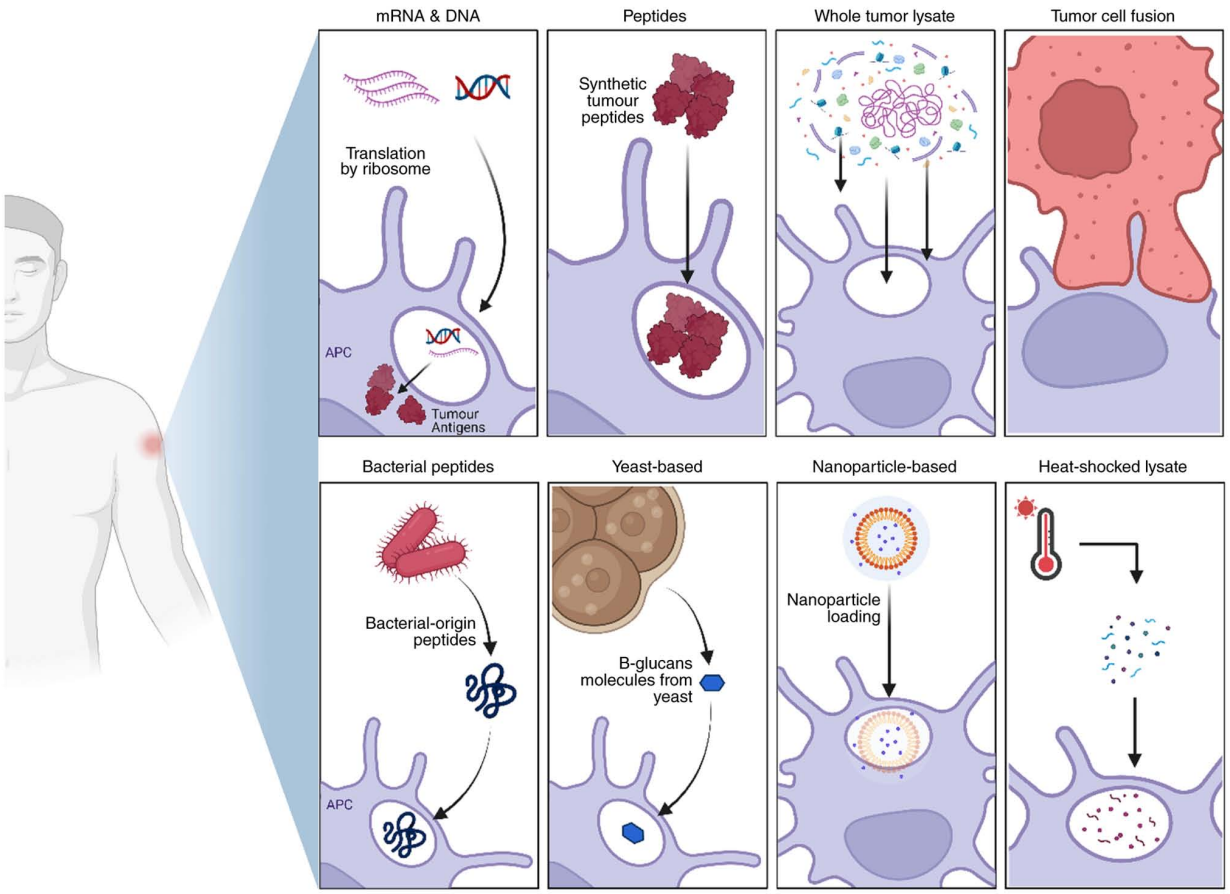

Figure 4. DC-vaccine stimulation and antigen loading strategies. Created by https://app.biorender.com. DC, dendritic cell.

implementation and therapeutic use. Whole tumor cell (WTC) vaccines represented another approach to broaden antigen exposure. As summarized by Pérez-Baños *et al* (149), WTC vaccines not only delivered a comprehensive range of antigens but also provided tumor-derived danger signals that activated innate immune pathways. This dual mechanism was suggested to enhance both antigen-specific responses and pro-inflammatory conditioning of the TME. However, this could also introduce variabilities and complicate the standardization of vaccine preparations. Furthermore, the presence of self-antigens in WTC preparations raised the possibility of tolerance rather than immunity in some patients.

Peptide- and protein-based antigen loading, such as those using carcinoembryonic antigen (CEA) or mucin 1, allowed precise targeting and facilitated monitoring of antigen-specific T-cell responses (150). In the context of colorectal cancer, these antigens are of particular clinical relevance; for instance, CEA is overexpressed in ~90% of CRC cases (151). While targeting these TAAs had successfully elicited T-cell responses in early-phase CRC trials, a major hurdle remains their low immunogenicity in mismatch repair-proficient (pMMR) tumors, which lack the high mutational burden found in MSI-H cases (152,153). Despite these advantages, they are restricted by MHC haplotypes and by the narrow antigenic spectrum they cover, rendering them vulnerable to tumor antigen heterogeneity. To overcome these issues, investigators began exploring neoantigen peptide-pulsed DCs. In pancreatic cancer, Oyama *et al* (154) demonstrated the feasibility of loading DCs with patient-specific neoepitopes identified through sequencing, with early evidence of improved disease control following surgery.

However, recent evidence underscores that the clinical benefit of these vaccines depends more on neoantigen 'quality' than 'quantity' (total mutational burden). As demonstrated by McGranahan *et al* (155), tumors characterized by a high burden of clonal neoantigens, mutations present in every tumor cell, exhibited markedly higher T-cell immunoreactivity and improved survival outcomes compared with tumors with high subclonal heterogeneity. This 'quality' model, which focuses on the best-presented clonal mutation, has proven more predictive of therapeutic success than the 'quantity' model, which is based on the total number of neoantigens (156,157). By prioritizing these high-quality clonal targets, DC vaccines may potentially eliminate the entire tumor population and minimize the risk of subclonal immune evasion (158). This approach allowed highly individualized targeting of mutations, but its dependence on rapid next-generation sequencing, epitope prediction, immuno-proteomic validations and complex manufacturing remained a major limitation.

As an intermediate between lysate breadth and peptide precision, total tumor RNA (ttRNA) was used to load DCs. ttRNA ensured endogenous antigen processing across both MHC class I and II pathways, thereby combining broad coverage with physiologically relevant presentation. Early-phase trials across multiple solid tumors indicated that ttRNA-loaded DCs

were safe and immunogenic (154). Yet, RNA instability and batch-to-batch variability in transcript content continued to present logistical challenges for multicenter applications.

In addition to endogenous tumor antigens, heterologous antigens were also tested as adjuncts. Zhai *et al* (159) reported that DCs pulsed with the mycobacterial antigen Ag85A enhanced immune responses against colorectal carcinoma in murine models. The use of Ag85A not only increased the expression of costimulatory molecules CD80 and CD86 but also promoted IFN-γ secretion and delayed tumor progression. These results suggested that heterologous microbial antigens could serve as powerful adjuvants by further activating innate immunity pathways while augmenting tumor-specific responses. Whether this indirect elicitation could be a long-term, transient, or highly case-by-case basis has yet to be studied in depth. Additionally, concerns also remain regarding the specificity of such approaches and the potential for off-target or autoimmune responses in humans.

Nucleic acid-based strategies, including mRNA and DNA, also provided a new frontier in DC vaccine stimulation and generation. mRNA-transfected DCs could generate endogenous antigen expression and present epitopes via both MHC-I and II pathways, enhancing $CD8^+$ and $CD4^+$ T-cell priming (160,161). Personalized mRNA neoantigen vaccines are known to induce immunogenicity in melanoma and glioblastoma patients, with evidence of durable T-cell activation (162,163). These approaches bypassed central tolerance and focused on patient-specific mutations via prior characterization. However, the substantial costs incurred, time-consuming production periods and the need for individualized manufacturing pipelines could lead to a challenging establishment of personalized mRNA-based vaccines.

Notably, natural microbial products are also exploited for their capacity to activate DCs. One prominent example was the use of *Saccharomyces cerevisiae* (baker's yeast) cell wall components, particularly β-glucans, which engaged pattern recognition receptors such as Dectin-1 and TLR2/4 on DCs. This interaction promoted DC maturation, cytokine secretion and cross-presentation of TAAs. Experimental cancer vaccines using recombinant yeast particles expressing TAAs showed the ability to induce strong $CD8^+$ T-cell responses and tumor regression in preclinical models (164). Moreover, β-glucan particles derived from yeast cell walls were developed as antigen carriers: DCs phagocytosed these hollow glucan shells and presented encapsulated antigens efficiently to T cells, achieving potent immunity with minimal toxicity (165). The advantage of this approach lies in the dual role of β-glucans as both delivery vehicle and adjuvant, providing intrinsic immunostimulatory signals while ensuring efficient antigen loading. However, variability in yeast preparations and the potential for excessive inflammation remained challenges that required careful optimization. Integrating yeast cell wall platforms into DC vaccine design illustrates how naturally occurring microbial ligands could be harnessed to reinforce both immunogenicity and safety.

Recent advances in nanotechnology have also transformed antigen-DC stimulation approaches. Prasad *et al* (166) encapsulated tumor lysates in biodegradable poly(lactic-co-glycolic acid; PLGA) nanoparticles, reporting improved antigen stability, protection from enzymatic degradation and enhanced cross-presentation to CTLs. Scheffel *et al* (167) demonstrated that calcium phosphate nanoparticles not only improved antigen delivery but also promoted DC maturation and stronger $CD8^+$ T-cell responses when compared with soluble antigens. While the nanoparticle systems reduced systemic toxicity and prolonged antigen persistence, questions remained regarding their long-term safety in humans and the reproducibility of their effects across diverse patient populations. Focusing on the importance of DC-therapy manufacturing, authors such as Boudousquié *et al* (168) established a current good manufacturing practice (cGMP)-compliant protocol for generating oxidized tumor lysate-pulsed DCs, demonstrating that HOCl-treated lysates improved immunogenicity and facilitated consistent, reproducible production across multiple clinical centers. Such standardization is essential for large-scale clinical translation. However, the requirement for specialized infrastructure and the high cost of GMP operations remain formidable barriers, particularly in resource-limited healthcare systems.

Maturation and activation of DCs. Immature DCs capture antigens efficiently but are poor T-cell stimulators, while mature DCs express co-stimulatory molecules and secrete cytokines essential for T-cell activation. TLR ligands such as poly(I:C), lipopolysaccharide (LPS) and CpG oligonucleotides could drive DC maturation and IL-12 production, favoring Th1 polarization (169,170). These studies highlighted the promise of pathogen-associated molecular pattern-based cues in generating immunogenic DCs, though they also revealed the risk of overstimulation, leading to DC exhaustion or variable cytokine responses between patients. The kinetics of maturation were increasingly recognized as critical. Giermasz *et al* (171) and Poženel *et al* (172) demonstrated that short-term stimulation of DCs with IFN-γ and TLR ligands could generate 'type-1 polarized DCs' that retained IL-12p70 secretion at the time of T-cell encounter, thereby supporting durable Th1 and CTL responses. These findings emphasized that not only the presence but also the time window of maturation signals could be important in determining the functional capacity of the final DC product. While type-1 polarized DCs offered superior immunogenicity, their shorter culture duration might have reduced antigen uptake and the precise implementation of tightly timed protocols posed challenges for clinical-scale manufacturing.

Cytokine-based differentiation protocols using GM-CSF and IL-4, followed by TNF-α or PGE_2-based maturation cocktails, were conventional standards for generating monocyte-derived DCs (173,174). These methods yielded reproducible results and remained foundational for clinical-grade DC vaccines. However, limitations included reduced IL-12 production with PGE_2-based maturation and extended culture times that reduced GMP-level scalability. Addressing these issues, Nava *et al* (175) optimized a GMP-compliant process for producing DC vaccines for glioblastoma, demonstrating that careful selection of cytokine cocktails and standardized monocyte isolation techniques could increase yield and reproducibility. Although such refinements made clinical translation feasible, they also increased operational complexity and final production costs. Alternative differentiation strategies were also explored. IFN-DCs, generated using IFN-α, displayed superior cross-presentation ability, higher costimulatory

molecule expression and stronger innate crosstalk compared with IL-4 DCs (176,177). These features might have made them particularly suitable for poorly immunogenic tumors. Nonetheless, their distinct *ex vivo* culture requirements complicated large-scale production and might have limited their use outside of specialized facilities.

Novel immune modulators had further expanded the DC maturation toolkit. CD40 ligand stimulation augmented cross-presentation, while STING agonists induced type I IFN production with enhanced $CD8^+$ T-cell priming (178,179). These strategies provided potent stimulation approaches but held risks of systemic inflammation and cytokine-related toxicities due to possible over- or hyperimmune stimulation. Clinical translation was demonstrated in clinical trials such as TriMixDC-MEL, which used tumor antigen mRNA alongside CD70, CD40L and caTLR4 mRNAs to generate a highly immunogenic DC phenotype. Trials of TriMixDC-MEL against melanoma confirmed safety and indicated potential improvements in disease-free survival (180,181). These findings highlighted the feasibility of inducing stimulatory and maturation signaling directly into DC vaccine constructs, though the added complexity of mRNA-based engineering posed practical barriers that might lead to permanent, transcriptomic-level side effects in patients. Nickles *et al* (182) extended this concept with CD137L-DC-EBV-VAX for nasopharyngeal carcinoma, engineering DCs to express CD137L and thereby enhance $CD8^+$ memory T-cell generation against Epstein-Barr virus. This study demonstrated safety and promising immunological activity, indicating that targeted receptor-ligand engineering could fine-tune DC maturation to favor long-lived immunity. However, such disease-specific tailoring might not generalize across tumor types.

In parallel, novel adjuvants were being tested as maturation stimuli. Hackstein *et al* (183) showed that TLR7/8 agonists such as resiquimod (R848) markedly enhanced DC activation and T-cell priming. Alam *et al* (184) further reported that combining R848 with the alarmin HMGN1 synergistically amplified Th1 response pathways. These findings suggested that combinatorial TLR stimulation could maximize immunogenicity, but they also suggested the need for careful titration, as overstimulation could suppress IL-12 and led to DC dysfunction. Biomaterial-based systems were also emerging as tools to enhance maturation. Prasad *et al* (166) and Scheffel *et al* (167) showed that nanoparticle formulations acted both as antigen carriers and as stimulants of DC maturation, while Kim *et al* (185) demonstrated that *in situ* cross-linking hydrogels sustained local DC activation without systemic toxicity. These systems offered an integrated approach to antigen delivery and immune activation, though their long-term biosafety and large-scale reproducibility remained unproven. Furthermore, oxidative antigen preparations were also reported to influence DC maturation. Roufarshbaf *et al* (142) showed that HOCl-treated lysates promoted expression of maturation markers CD83 and CD86 and induced a pro-Th1 cytokine profile. Boudousquié *et al* (168) translated this observation into a GMP-compliant process, enabling reproducible production of oxidized lysate-pulsed DCs for multicenter trials. Together, these findings highlighted how biochemical processing of tumor material could serve both as an antigen source and maturation signal. Collectively, the studies indicated that the quality of DC maturation, apart from the event of maturation, also determined clinical outcomes (168,175,180-182).

Optimization of these maturation protocols must specifically account for the unique barriers of the CRC microenvironment. A significant challenge is CMS4, which is characterized by high levels of transforming growth factor-beta (TGF-β) and VEGF, as reported previously. These factors could actively suppress DC maturation and promote an 'immune-excluded' phenotype (186). Furthermore, a number of CRC tumors exhibit a paucity of infiltrating DCs, particularly in microsatellite-stable (MSS) lesions, which limits the initial priming of anti-tumor response (187). Therefore, DC vaccines for CRC must be engineered not only to present antigens but also to actively reverse the local tolerance. Strategies that preserved IL-12 competence, incorporated rational costimulatory engineered signals, leveraged biomaterials, or harnessed oxidative danger signals appeared as promising strategies for future DC vaccine developments.

Delivery routes, vaccine administration and clinical translations. The route of administration profoundly influences the distribution and immunological activity of DC vaccines that in turn have an effect on the OS of treated patients. Intradermal and subcutaneous injections are the most commonly used, capitalizing on the abundance of skin-resident APCs and efficient access to draining LNs. Clinical imaging studies confirmed that intradermal delivery promoted greater DC migration to lymphoid tissues compared with intravenous administration, where cells were often sequestered in non-lymphoid organs such as the liver and lungs (150,188). While intradermal delivery enhanced lymph node access, variability between patients persisted and repeated injections might be required to maintain immunity. Subcutaneous administration, though slower in kinetics, was simpler and more easily tolerated (189). Intravenous delivery was tested in metastatic disease settings to exploit systemic distribution (161), but lymph node targeting remained inefficient.

Lesterhuis *et al* (190) directly compared intradermal and intranodal injections in melanoma and observed route-dependent differences in immunological outcomes, with intranodal delivery providing direct lymph node access and enhanced T-cell responses. However, the technical precision required for intranodal injections and variability in nodal accessibility limited their routine use. Building on this, Radomski *et al* (191) reported that intralymphatic infusion using implantable ports was both feasible and safe, enabling prolonged antigen delivery into draining LNs. This approach ensured continuous immune stimulation but introduced procedural risks and logistical challenges, including the risk of patients facing sterility and possible infections via these ports. The phase III trial by Bol *et al* (192) provided strong evidence for nodal delivery, showing that intranodal vaccination with autologous conventional and plasmacytoid DC subsets in stage III melanoma resulted in tumor-specific immune responses and encouraging clinical outcomes. This trial validated the principle that direct nodal delivery could enhance efficacy, though its general application to other tumor types warranted further studies.

Imaging technologies have also refined delivery approaches. Bulte and Daldrup-Link (193), Bulte and Shakeri-Zadeh (194) and Zhang *et al* (195) developed MRI and PET/SPECT-based

cell-tracking methods to visualize DC migration *in vivo*, allowing investigators to identify whether vaccines reached draining LNs and to troubleshoot failures in targeted cell trafficking. These techniques served as excellent *in vivo* quality-control tools for clinical trials but added complexity and cost. In parallel, Grippin *et al* (196) and Fink *et al* (197) demonstrated that magnetic nanoparticles could guide DC migration toward LNs, further improving homing efficiency. These advances pointed toward delivery systems that were actively controlled and monitored in real time, rather than based on blind assumptions. This technical optimization is vital for addressing the clinical disparities observed in CRC immunotherapy. Currently, although MSI-H CRC patients show encouraging responses, the vast majority of patients have MSS tumors, in which the objective response rates to DC vaccines remain suboptimal, often ranging from 5-10% (186). By using targeted delivery systems, such as scaffolds or nanoparticles, to concentrate antigens and adjuvants within TDLNs, researchers aim to bridge this gap and induce meaningful responses in the 'cold' MSS CRC population (187).

Biomaterials were also exploited as novel delivery vehicles. Sánchez-Paulete *et al* (198) and Park *et al* (199) showed that scaffolds and nanoparticles prolonged antigen delivery, sustained DC activation and improved persistence *in vivo*. Ali *et al* (200) demonstrated that *in situ* biomaterial implants could program DCs within the host, bypassing the need for *ex vivo* culture while still inducing tumor regression. These approaches simplified logistics but at the cost of less precise control over DC phenotype and possibly less cGMP-compliant procedures at a clinical level. Chen *et al* (201) further advanced delivery innovations with microneedle patches that enabled painless, targeted administration to skin-resident DC subsets. While this technology improved patient compliance, its limited antigen payload created a technical barrier. This delivery mode could be especially helpful for the treatment of melanoma.

Clinical translation required attention not only to delivery routes but also to manufacturing logistics. Boudousquié *et al* (168) demonstrated that oxidized lysate-pulsed DCs could be reliably produced for multicenter use, while Nava *et al* (175) developed large-scale protocols that balanced yield and quality. These examples underscored that effective delivery could not be divorced from reproducibility and scalability. An optimized DC vaccine framework, combining the aspects of broad or specific antigen loading, IL-competent maturation, lymph node-targeted delivery and integration with rational checkpoint or cytokine adjunct therapies, might lead to an improved avenue of therapeutic potency (202). The clinical feasibility of DC vaccination was definitively demonstrated by the FDA approval of Sipuleucel-T (Provenge) in 2010 for mCRPC. The findings from a Phase III clinical trial of Sipuleucel-T have highlighted one of the key advantages of DC vaccines, the capacity to extend survival through immune modulation even when conventional chemotherapy/radiotherapy responses were absent (70). However, Sipuleucel-T also highlighted challenges that permeated the field. The therapy required highly individualized manufacturing, multiple leukapheresis sessions and complex logistics, contributing to high costs and limiting widespread adoption. Furthermore, the vaccine's clinical benefit could be improved via combinations with checkpoint inhibitors or other immunomodulators. Sipuleucel-T thus occupies a milestone place in the history of cancer vaccinations via DC vaccines, establishing regulatory precedent and validating feasibility, while also revealing the economic and logistical barriers that should be overcome for broader clinical implementation.

7. Clinical applications of DC-based vaccines in cancer immunotherapy

DC-based therapeutic cancer vaccines have been intensively investigated with the aim of inducing adaptive, cell-mediated immune responses in cancer patients, thereby converting the immunologically 'cold tumors' into 'hot tumors' that are more susceptible to cancer therapy (203-205). In general, DC-based cancer vaccines are generated by pulsing a patient's DCs with TSAs and TAAs derived from the patient's tumor tissue, in the form of recombinant proteins, mRNA, peptides, total RNA, or tumor cell lysate (TCL) (206,207). The first FDA-approved autologous DC-based vaccine, Sipuleucel-T (APC-8015), has been marketed since 2010, at a cost of US$ 93,000-100,000 for a complete course of treatment (70,208,209). Sipuleucel-T was developed by Dendreon Pharmaceuticals LLC in the US as a therapeutic cancer vaccine against mCRPC. It stimulates the patient's immune cells to specifically target and destroy prostate cancer cells without harming healthy cells. Sipuleucel-T cancer vaccine consists of autologous monocyte-derived DCs that are *ex vivo*-activated with a recombinant fusion protein containing prostatic acid phosphatase fused to GM-CSF (70). The antigen-loaded DCs are incorporated into Dendreon's proprietary Antigen Delivery Cassette and re-infused into the patient via three intravenous (IV) infusions at 2-week intervals for one month. Based on the latest multicenter, retrospective study spanning 14 years (2010-2024) in the US that evaluated the efficacy of Sipuleucel-T treatment in men with mCRPC, the cohort treated with Sipuleucel-T (n=5,281) had a median OS of 44 months, while the non-treated cohort (n=5,984) had a median OS of only 24 months (210). In the cohort treated with Sipuleucel-T, 28% of the patients were alive at 5 years, with a median OS of 97 months.

Furthermore, recent and ongoing clinical trials using autologous DCs pulsed with autologous TCL that were prepared from patients' tumor have shown promising clinical outcomes in treating metastatic melanoma, glioblastoma (GBM) and CRC, as well as in prolonging the median OS, progression-free survival (PFS) and/or disease-free survival (DFS) of cancer patients (141,211-217). Moreover, these TCL-pulsed DC vaccines have been consistently shown to be safe and well tolerated across clinical studies. In particular, a Phase III multicenter clinical trial of DCVax-L consisting of autologous DCs pulsed with TCL for the treatment of GBM (NCT00045968), demonstrated that the addition of DCVax-L to standard-of-care (SOC) temozolomide (TMZ) was associated with a clinically meaningful and statistically significant extension of OS in patients with both newly diagnosed and recurrent GBM, when compared with the control group who received SOC alone (141). The DCVax-L-treated cohort of 232 patients with newly diagnosed GBM had a median OS of 19.3 months, with a 2-fold increase in the survival rate at 5 years (13%) compared with the control group (5.7%). The

DCVax-L treatment was also found to extend the median OS of 64 patients with recurrent GBM by 5.4 months when compared with the control group, which had a median OS of 7.8 months from relapse. The survival rate of these patients was also increased by 2-fold, reaching 20.7% at 2 years and 11.1% at 2.5 years.

In addition, a randomized Phase II trial (NCT00948480) of an autologous TCL-pulsed DC vaccine was found to markedly prolong the survival of 42 patients with metastatic melanoma by 22.9 months, with a median OS of 43.4 months and reduced mortality risk by 70% (211). In addition, the safety and effectiveness of an autologous TCL-pulsed DC vaccine (DCvax) in patients with unresectable malignant melanoma (stage III or IV) have been demonstrated in a completed Phase II clinical trial, ABSIDE (EudraCT 2012-001410-41), in which a median OS of 20.7 months in seven treated-patients was observed (216). This clinical study also showed that monotherapy with DCvax alone was associated with an improved OS and a higher immune-related disease control rate of 57% compared with the combined immunotherapies of DCvax with radiotherapy, IFN-α or both. The efficacy and safety of this DC-based vaccine approach were further evaluated in patients with GBM in an ongoing monocentric Phase II clinical trial, Combi G-vax (EudraCT 2020-003755-15). This trial was conducted in combination with the standard therapy consisting of concurrent radiotherapy and temozolomide chemotherapy, known as the Stupp regimen, followed by a maintenance phase using TMZ alone. Vaccination with autologous TCL-pulsed DCs in nine patients with GBM was well tolerated and safe, resulting in a median OS of 23.1 months and a median PFS of 11.3 months (215).

Early Phase I and Phase II clinical trials of an allogeneic melanoma TCL vaccine (Melacine), which was prepared from two melanoma cell lines and co-administered with the adjuvant Detox, demonstrated limited anti-tumor responses in patients with stage IV melanoma (218,219). Subsequently, in a randomized Phase III trial conducted by the Southwest Oncology Group (SWOG; SWOG-9035), Melacine did not improve DFS and OS in the overall cohort of 300 patients with resected intermediate-thickness, node-negative melanoma (T3N0M0), in which a 5-year DFS rate of 65% was observed in the vaccinated patients (220). The survival benefit of Melacine was restricted to patients with HLA class I antigens A2 and/or HLA-C3, limiting its broader application. A total of 178 Melacine-treated patients with HLA-A2 and/or HLA-Cw3 serotype had a markedly improved relapse-free survival (RFS) and OS, with 5-year RFS of 78% and 5-year OS of 90% (221).

In addition, a Phase I clinical trial (NCT06556004) of direct vaccination with allogeneic heat-shocked melanoma cell lysate prepared from three human melanoma cell lines (TRIMELVax) in combination with mollusk-derived hemocyanin as an adjuvant, showed moderate preliminary clinical outcomes, with a median OS of 11.6 months and PFS of 4.5 months in 17 nivolumab-refractory patients with unresectable stage III or IV melanoma (222). Although TRIMELVax vaccination presented an acceptable safety profile, further studies are needed to determine the effectiveness of this vaccine that did not use *ex vivo*-generated DCs. These early clinical trials of allogeneic TCL vaccines provided foundational evidence supporting the immunologic and clinical potential of lysate-based active immunotherapy. However, their limited clinical outcomes highlighted the need to use DCs that are *ex vivo*-activated with TCLs to achieve greater clinical benefits in cancer treatment. Moreover, *ex vivo* loading of DCs with TAAs has been shown to outperform irradiated autologous tumor cell-based vaccine, as evidenced in a Phase II trial (NCT00948480) (211).

8. Ongoing and completed clinical studies of DC-based vaccines in metastatic colorectal cancer

In clinical trials, DC-based vaccines have been applied as both stand-alone and complementary therapeutic therapies for various types of cancers with solid tumors, with the primary aim of stimulating a tumor-specific cell-mediated immune response against cancer cells. As a complementary approach, DC vaccines are often combined with other established treatments such as chemotherapy, radiotherapy or newer immunotherapies, such as ICB to enhance overall cancer treatment efficacy. Notably, pMMR or MSS metastatic CRC accounts for ~95% of CRC cases (223). pMMR/MSS CRC typically results in a 'cold' TME characterized by limited infiltration of T cells into the tumors, making it resistant to standard ICB immunotherapy alone (223-225). By contrast, metastatic CRC with mismatch repair-deficiency (dMMR) or MSI-H has been shown to be susceptible to ICB immunotherapy using pembrolizumab, a humanized monoclonal IgG1 antibody targeting PD-1 in a Phase III clinical trial, Keynote-177 (NCT02563002), resulting in a significant median PFS of 16.5 months and overall response rate of 43.8% in 153 patients (226). Apart from pembrolizumab, nivolumab in combination with ipilimumab has recently been approved by the FDA following significant clinical outcomes in a Phase III CheckMate 8HW trial (NCT04008030) for the treatment of patients with dMMR/MSI-H metastatic CRC (227,228). Nevertheless, as most CRC cases belong to pMMR/MSS and are not susceptible to ICB immunotherapy (223), DC vaccines remain a promising alternative immunotherapy for pMMR/MSS metastatic CRC. Hence, the efficacy of DC-based vaccines against metastatic CRC has been evaluated in combination with ICB treatment in recent clinical trials (214).

Tumor cell lysate-pulsed DC vaccines for treatment of advanced CRC. An earlier Phase II randomized clinical trial (NCT01413295) using autologous TCL-pulsed DCs to treat 28 patients with progressive metastatic CRC showed no clinical benefit for OS and PFS, although this DC vaccine induced a tumor-specific T-cell response in 84% of patients (21/25) (229). This vaccine treatment only extended the median OS by 1.5 months and median PFS by 0.4 months compared with untreated-patients, who had a median OS of 4.7 months and median PFS of 2.3 months (Table I). As no clinical benefit in OS and PFS was observed in this trial, this DC-based vaccine was not advanced to a Phase III trial as monotherapy. Following that, the efficacy of this DC vaccine was assessed in combination with ICB therapy using avelumab (an anti-PD-L1 monoclonal antibody) against mismatch repair-proficient metastatic CRC in a Phase I/II multicenter clinical trial, GEMCAD 1602 (NCT03152565) (214). This clinical study demonstrated a median OS and PFS of 12.2 months and

Table I. Clinical studies on the efficacy of autologous tumor cell lysate-pulsed DC vaccines for mCRC.

First author/s, year	Study design	Combination therapy	Number and characteristics of patients	Primary endpoint	Clinical outcomes	NCT	(Refs.)
Español-Rego *et al*, 2023	• Phase I/II (completed in 2020) • Single-arm, non-randomized, open-label multicenter study with translational sub studies. • Control strategy: none	Antibody-ICB (avelumab)	• Screened patients: 28 • Treated patients: 19 • pMMR mCRC • Heavily pretreated with >2 chemotherapy regimens.	40% PFS at 6 months.	• Median OS: 12.2 months • Median PFS: 3.1 months • Two out of 19 patients (11%) were disease-free at 6 months. • Follow-up duration: ND • The treatment was well tolerated and safe.	NCT03152565	(214)
Caballero-Baños *et al*, 2016	• Phase II (completed in 2014) • Multi-arm, randomized, open-label single-center study • Control strategy: best supportive care alone	None	• Screened patients: 61 • Enrolled patients: 52 -Treated: 28 -Control: 24 • Progressive mCRC • Pretreated with >2 chemotherapy regimens. • Performance status: ECOG 0-2	PFS at 4 months.	• Median OS: 6.2 months (vs. 4.7 months in control arm) • 2-year OS: 7% (vs. 4% in control arm) • Median PFS: 2.7 months (vs. 2.3 months in control arm) • 4-month PFS: 15% (vs. 21% in control arm) • Follow-up duration: ND • The treatment was well tolerated and safe.	NCT01413295	(229)
Rodriguez *et al*, 2018	• Phase II • Multi-arm, randomized, open-label single-center study • Control strategy: Observation	None	• Screened patients: 19 • Enrolled patients: 15 - Treated: 8 - Control: 7 • mCRC patients who had undergone complete tumor resection.	Disease-free survival (DFS)	• Median DFS: 25.26 months (vs. 9.53 months in control arm) • Median follow-up duration: 42.58 months • The treatment was well tolerated and safe.	NCT01348256	(212)

Table I. Continued.

First author/s, year	Study design	Combination therapy	Number and characteristics of patients	Primary endpoint	Clinical outcomes	NCT	(Refs.)
Barth *et al*, 2010	• Multi-arm, randomized, blinded single-center clinical study • Control strategy: vaccination with DCs without CD40L activation	None	• Screened patients: 32 • Enrolled patients: 26 - Treated: 12 - Control: 14 • mCRC patients who had undergone complete tumor resection.	Immuno-genicity of the vaccine	• Tumor-specific immune responses were induced in 63% (15/24) of evaluable patients. • 5-year OS: 58% • 5-year recurrence-free survival (RFS): 38% • Follow-up duration: ≥5.5 years • The treatment was well tolerated and safe.		(230)
N/A	• Phase II (recruiting) • Single-arm, non-randomized, open-label single-center study • Control strategy: none	IL-2 treatment	• Estimated enrollment: 19 patients • mCRC patients who had undergone complete tumor resection.	Safety and immuno-genicity of the vaccine	Results are not yet available.	NCT02919644	
N/A	• Phase II (recruiting) • Single-arm, non-randomized, open-label multi-center study • Control strategy: none	Antibody-ICB and chemo-therapy (pembroli-zumab and Trifluridine/ Tipiracil plus bevacizumab)	• Estimated enrollment: 36 patients • Refractory pMMR or MSS mCRC	ORR	Results are not yet available.	NCT06522919	
N/A	• Phase III (unknown status) • Multi-arm, randomized, open-label multi-center study • Control strategy: chemotherapy alone	Chemo-therapy (modified FOLFOX-6)	• Estimated enrollment: 480 patients • mCRC	PFS	Results are not yet available.	NCT02503150	

DC, dendritic cell; mCRC, metastatic CRC; NCT, national clinical trial; ICB, immune checkpoint blockade; pMMR, mismatch repair-proficient; PFS, progression-free survival; ND, no data; ECOG, Eastern Cooperative Oncology Group; OS, overall survival; DFS, disease-free survival; RFS, recurrence-free survival; CBR, clinical benefit rate; MSS, microsatellite-stable; ORR, overall response rate; N/A, not available.

3.1 months, respectively; however, no objective responses were observed and only 2 out of 19 patients (11%) were disease-free at 6 months. These two clinical trials were recommended for early termination following interim analyses due to the lack of efficacy of the DC vaccine.

Furthermore, DC-based vaccines have demonstrated potential benefits in metastatic CRC patients with resectable liver metastases, an unmet clinical need due to the high risk of recurrence. In a Phase II randomized clinical trial (NCT01348256), autologous TCL-pulsed DC vaccine was shown to prolong median DFS by 15.73 months in metastatic CRC patients with liver metastases, compared with untreated patients who had a median DFS of 9.53 months (Table I) (212). Additionally, DC vaccines have been applied as adjuvant immunotherapy on metastatic CRC patients following complete surgical resection of metastases, aiming to prevent CRC recurrence. A previous clinical study demonstrated a marked improvement of RFS in 15 of 24 patients with metastases of metastatic CRC. This was achieved through the induction of a tumor-specific T-cell proliferative response by the autologous TCL-pulsed DC vaccine, in which a significant 45% increase in the 5-year RFS rate (63%) was observed relative to nonresponders (230). In this clinical study, out of 24 patients, eight remained alive and disease-free and four remained alive with recurrent disease for at least 5.5 years after DC vaccine treatment.

In addition, the efficacy of autologous DCs pulsed with allogeneic melanoma cell lysate was evaluated in 20 patients with advanced CRC expressing at least one of six *MAGE-A* genes (231). This Phase II clinical trial demonstrated a clinical benefit response rate of 40%; however, no complete responses were observed (Table II). The trial reported a median OS of 7.4 months and median PFS of 2.4 months. Among the patients with partial response (n=1) and stable disease (n=7), a median OS of 18.1 months was observed. Notably, this allogeneic melanoma cell lysate-pulsed DC vaccine prolonged the PFS of 25% of the patients (5/20) for more than 6 months, with two of these patients remained progression-free for over 27 and 37 months, respectively (231).

Apart from conventional stand-alone DCs, a combination immunotherapy consisting of an autologous TCL-pulsed DC vaccine and cytokine-induced killer cells (DC-CIK) was shown to markedly improve the survival of 27 patients with CRC, with a 5-year OS rate of 75% and a 5-year DFS rate of 66%, when compared with patients in the control group with a 5-year OS rate of 15% and 5-year DFS rate of 8% (232). This DC-CIK immunotherapy also resulted in a significant reduction in the risk of postoperative disease progression in CRC patients. Furthermore, autologous DC-CIK therapy combined with standard chemotherapy has been shown to be more effective than the control group (receiving standard chemotherapy alone) in markedly increasing the median OS by 4.5 and 5.13 months in refractory metastatic CRC patients without and with liver or extra-regional lymph node metastases, respectively (14.23 and 11.87 months in controls) and achieving a median PFS of 7.63 months (Table II) (233).

A recent ongoing Phase II multicenter clinical trial (NCT06522919) is evaluating the clinical efficacy of an innovative combination immunotherapy involving an TCL-pulsed DC vaccine in combination with ICB using pembrolizumab, followed by a maintenance chemotherapy with Trifluridine/Tipiracil and bevacizumab (known as CombiCoR-Vax) in treating refractory pMMR or MSS metastatic CRC. The innovative strategy of CombiCoR-Vax was inspired by a Phase III clinical trial (NCT04737187), which reported a significant improvement in OS and PFS in patients with refractory metastatic CRC after chemotherapy treatment with Trifluridine/Tipiracil plus bevacizumab (FTD/TPI + BEV) (234). In addition, an ongoing Phase II monocentric clinical trial (NCT02919644) is assessing the safety and immunological efficacy of an autologous TCL-pulsed DC vaccine (COREVAX-1), followed by a subcutaneous injection of IL-2 in patients with stage IV CRC after curative resection. A Phase III clinical trial on the safety and efficacy of an autologous DC vaccine loaded with autologous TCL in combination with a modified chemotherapy regimen of Oxaliplatin, 5-Fluorouracil and Leucovorin (mFOLFOX-6) has been proposed (NCT02503150), yet its current status remains unknown, with the last known update indicating that no participants had been recruited.

TAA-pulsed DC vaccines for treatment of advanced CRC. The immunogenicity, safety and efficacy of autologous DC vaccines pulsed with TAAs in the form of peptides have been evaluated in several early-phase clinical studies. CEA has been primarily targeted as a TAA in this peptide-pulsed DC vaccine because of its overexpression in the serum of ~70% of CRC patients (235). Although CEA is the most commonly used tumor biomarker for CRC diagnosis, it is not specific to CRC as elevated CEA levels can result from other cancers such as pancreatic, breast, lung, liver, prostate and ovarian cancers, or non-cancerous conditions including pancreatitis, hepatitis, obstructive pulmonary disease, hypothyroidism, chronic kidney disease, smoking, chronic renal failure, Alzheimer's disease and ulcerative colitis (236,237).

Most clinical trials of CEA peptide-pulsed DC vaccines in advanced CRC were early-phase, focusing on safety and immunogenicity of the vaccines (Table II). These vaccines were safe and capable of stimulating potent CEA-specific T-cell responses in at least 70% of vaccinated patients (238-240). In a Phase I trial (NCT00228189), the immunogenicity of CEA mRNA-transfected DC vaccine in patients with resectable liver metastases of CRC was not found to be superior to DC vaccine pulsed with CEA peptide, in which no CEA-specific T-cell responses were observed in all five patients vaccinated with CEA mRNA-electroporated DCs (240). The efficacy of the CEA mRNA-pulsed DC vaccine treatment in metastatic CRC patients with liver metastases was also shown in a Phase II clinical study (241). Although the vaccination was well tolerated, out of 13 patients, nine patients experienced recurrence with a median time to recurrence (TTR) of 122 days and three patients remained disease-free for at least 16.7 months.

Among other TAAs, Wilms' tumor 1 (WT1) has been used to prime DCs against CRC. In a Phase I clinical study, WT1 peptide-pulsed DC vaccine induced durable WT1-specific cytotoxic T-cell responses lasting for at least two years in three patients with advanced CRC, with a calculated median DFS of 24.5 months and a median OS of 43.6 months (242).

Table II. Clinical studies on the efficacy of various DC-based vaccines for mCRC.

First author/s, year	Type of DC based vaccine	Study design	Combination therapy	Number and characteristics of patients	Primary endpoint	Clinical outcomes	(Refs.)
Toh *et al*, 2009	DC vaccine loaded with allogeneic melanoma cell lysate	• Phase II • Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Screened patients: 28 • Treated patients: 20 • mCRC	Clinical benefit rate (CBR)	• Median OS: 7.4 months • Median PFS: 2.4 months • CBR rate: 40% • Five patients (25%) experienced prolonged PFS of >6 months. • Two patients (10%) remained progression-free for >27 and >37 months, respectively. • Follow-up duration: ND • The treatment was well tolerated and safe.	(231)
Chang *et al*, 2024	Autologous DC cytokine-induced killer (DC-CIK) vaccine loaded with tumor cell lysate	• Multi-arm, non-randomized, single-center study • Control strategy: standard chemo-therapy alone	Chemo-therapy (Regorafenib)	• Enrolled patients: 54 - Treated: 27 - Control: 27 • Refractory mCRC • Heavily pretreated with >2 chemo-therapy regimens.	OS and PFS	• Median OS: 18.73 months (vs. 14.23 months in control arm) • Median PFS: 7.63 months (vs. 4.53 months in control arm) • Follow-up was until documented patient death or tumor progression. • The treatment was well tolerated and safe.	(233)
Liu *et al*, 2004	DC vaccine loaded with CEA peptide	• Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Treated patients: 10 • mCRC • Had failed first-line and second-line chemotherapy regimen.	Safety of the vaccine	• No grade II/III toxicity or autoimmune response was observed. • Two patients (20%) had stable disease for ≥12 weeks. • CEA-specific T-cell responses were observed in 7 out of 10 patients (70%). • The treatment was well tolerated and safe.	(238)

Table II. Continued.

First author/s, year	Type of DC based vaccine	Study design	Combination therapy	Number and characteristics of patients	Primary endpoint	Clinical outcomes	(Refs.)
Lesterhuis *et al*, 2006	DC vaccine loaded with CEA peptide	• Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Treated patients: 10 • mCRC with resectable liver metastases	Immunogenicity of the vaccine	• CEA-specific T-cell responses were induced in 7 out of 10 patients (70%). • Median follow-up duration: 14 months • The treatment was well tolerated and safe.	(239)
Sakakibara *et al*, 2011	DC vaccine loaded with CEA peptide	• Phase I/II • Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Treated patients: 10 • mCRC	Safety and tolerability of the vaccine	• Among 8 evaluable patients, one achieved stable disease lasting 2 months from treatment initiation, while the remaining 7 had progressive disease. • Median follow-up duration: ND • The treatment was well tolerated and safe.	(244)
Shimodaira *et al*, 2015	DC vaccine loaded with WT1 peptide	• Phase I • Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Treated patients: 3 • mCRC	Safety and immunogenicity of the vaccine	• In the 3 patients, OS was 43.6, 32.4 and 76.9 months and DFS was 34, 24.5 and 22.2 months, respectively. • Calculated median OS: 43.6 months • Calculated median PFS: 24.5 months • Median follow-up duration: ND • The treatment was well tolerated and safe.	(242)
Lesterhuis *et al*, 2010	DC vaccine loaded with CEA peptide or mRNA	• Phase I (completed in 2010) • Multi-arm, non-	None	• Treated patients: 16 - Peptide group: 11 - mRNA group: 5 • mCRC with resectable	Immunogenicity of the vaccine	• CEA-specific T-cell responses were observed in 8 out of 11 patients in the peptide group.	(240)

Table II. Continued.

First author/s, year	Type of DC based vaccine	Study design	Combination therapy	Number and characteristics of patients	Primary endpoint	Clinical outcomes	(Refs.)
		randomized, open-label single-center study • Control strategy: none		liver metastases		• No CEA-specific T-cell response was detected in the mRNA group. • Median PFS of peptide group: 18 months • Median PFS of mRNA group: 26 months • Follow-up duration: ND • The treatment was well tolerated and safe.	
Morse *et al*, 2003	DC vaccine loaded with CEA mRNA	• Phase II • Single-arm, non-randomized, open-label single-center study • Control strategy: none	None	• Treated patients: 13 • mCRC with resectable liver metastases • Had undergone complete tumor resection.	Safety and feasibility	• Nine of 13 patients experienced disease recurrence. • Median time to recurrence (TTR): 122 days • Follow-up duration: ND • The treatment was well tolerated and safe.	(241)
Liu *et al*, 2016	DC vaccine loaded with recombinant human CEA protein	• Phase I • Single-arm, non-randomized, open-label single-center study • Control strategy: none	IL-2 treatment	• Treated patients: 12 • mCRC • Had failed standard chemotherapy.	Safety	• CBR rate: 16.7% • Out of 12 patients, 2 achieved stable disease lasting >98 days from treatment initiation, while the remaining 10 patients had progressive disease. • Follow-up duration: ND • The treatment was well tolerated and safe.	(243)

DC, dendritic cell; mCRC, metastatic CRC; NCT, national clinical trial; CBR, clinical benefit rate; OS, overall survival; PFS, progression-free survival; ND, no data; CEA, carcinoembryonic antigen; WT1, Wilms' tumor 1; DFS, disease-free survival; TTR, time to recurrence.

Furthermore, vaccination with autologous DCs pulsed with recombinant human CEA protein in combination with tetanus toxoid adjuvant and subsequent IL-2 treatment in a Phase I clinical trial (NCT00154713), showed a safe profile but limited clinical benefits in 12 patients with metastatic CRC (243). Similar modest clinical efficacy was also observed in a Phase I/II clinical study of autologous DCs loaded with CEA peptide in eight patients with stage IV CRC (244). Taken together, it appears likely that pulsing DCs with TAA in the form of peptides provides improved antigen-specific T-cell responses than mRNA- or protein-based approaches; however, further clinical studies are necessary to clarify the clinical impact of peptide-pulsed DC vaccines in CRC.

Proposed trial framework for DC vaccines in metastatic CRC. It is notable that utilizing antigen sources derived from TCL and *ex vivo* activation of DCs with these TAAs and TSAs represents an improved approach, as observed particularly in TCL-pulsed DC vaccine treatments for GBM and metastatic melanoma (141,211,215,216). In CRC treatment, the clinical progress and benefits of DC-based vaccines primed with TCLs are greater than those of peptide-pulsed DC vaccines; however, their clinical outcomes remain suboptimal, when applied as monotherapy.

A practical clinical trial framework for assessing the effectiveness of DC vaccines in humans is of utmost importance. It would be ideal to evaluate the safety and efficacy of autologous with TCL-pulsed DC vaccine in an early-phase clinical trial involving patients with metastatic CRC after complete resection of metastases or patients with high-risk stage III CRC following standard adjuvant chemotherapy. This allows the effectiveness of DC vaccines to be assessed among cancer patients with minimal residual disease or clinically disease-free after standard adjuvant chemotherapy, thereby creating a condition that is favored for immunotherapy and where any benefits from the DC vaccine treatment would be adjunctive to standard chemotherapy. In particular, a single-arm, open-label Phase I/II trial can be conducted to assess safety, feasibility and preliminary efficacy, with safety as the primary endpoint and OS and PFS as secondary endpoints. Furthermore, utilization of autologous TCL as an antigen to prime DCs represents a promising strategy for the generation of potent DC vaccines (245). Pulsing DCs with autologous TCL prepared from fresh tumor tissues as soon as possible (typically within a few hours) after surgical resection is preferred, as this preserves optimal antigen quality.

Additionally, it is worthwhile to evaluate the efficacy of TCL-pulsed DC vaccines in combination with other immunotherapies, especially ICB therapy using the combination of nivolumab and ipilimumab, which has been approved by FDA following evidence of significant clinical benefits (227,228). The efficacy of DC vaccines in combination therapy warrants assessment in a multi-arm, randomized, controlled Phase II trial, with DFS as the primary endpoint and OS as the secondary endpoint. Overall, the clinical trial framework shall be carefully designed and conducted in accordance to the study objectives, in order to achieve appropriate patient selection with a clearly defined inclusion and exclusion criteria of the targeted population, clinically meaningful primary and secondary endpoints and standardized protocols in DC vaccines preparation to ensure reliable and consistent clinical outcomes.

9. Challenges and limitations of DC-based vaccines in colorectal cancer immunotherapy

The immunogenicity and safety of DC-based vaccines in treating CRC have been evidenced in numerous clinical studies; however, their clinical outcomes, especially when applied as monotherapy, remain suboptimal in the majority of patients. These limitations can be ascribed to several major challenges associated with DC-based vaccines for CRC, including tumor heterogeneity, an immunosuppressive TME and suboptimal T-cell activation. Tumor heterogeneity in CRC remains a major challenge that limits the effectiveness of DC vaccines, particularly when the antigen loaded onto the DCs is a neoantigen, such as TAA or TSA, which is delivered in the form of a recombinant protein or peptide. Neoantigen-based DC vaccines are currently considered one of the most promising approaches for cancer therapy as demonstrated by the success of Sipuleucel-T, the first FDA-approved DC-based vaccine for mCRPC. However, the identification of immunogenic neoantigens that are uniformly expressed across all tumor cells is laborious, as it requires deeper sequencing such as single-cell sequencing and Ribo-seq (246-248) and advanced bioinformatics, including the use of deep learning algorithms for enhancing the precision of *in silico* neoantigen prediction (249,250), to address the high false-positive rate of candidate neoantigens and low accuracy related with current prediction tools. Additionally, proteogenomics, which integrates genomic data with advanced mass spectrometry-based proteomics, has emerged as a promising novel strategy for discovering and identifying neoantigens in solid tumors (251,252).

The TME in CRC can suppress anti-tumor immune responses by inhibiting the proper maturation and function of DCs through immunosuppressive mediators, including the secretion of soluble factors such as VEGF, TGF-β, IL-6, IL-10 and PGE_2, the production of β-catenin and ROS and the induction of immune checkpoint expression of PD-L1 on DCs (187). As well as this, the metabolic composition of the TME, such as lipid peroxide byproducts, can lead to lipid accumulation in DCs that blocks antigen cross-presentation, thus reducing T-cell activation by DCs (118). In the CRC TME, cancer cells actively recruit and promote the activity of immune-suppressing cells such as Tregs, especially those with high expression levels of forkhead box P3 and MDSCs, which further promote immunosuppression within the TME and thus allow cancer cells to evade immune surveillance (187,253). Furthermore, cancer cells can downregulate or lose MHC class I molecules to avoid recognition by tumor antigens presented by MHC class I molecules, thus inhibiting T cell-mediated immune response (254).

In the TME, upregulated expression of immune checkpoint molecule PD-L1 on tumor cells and DCs, especially the type I cDCs upon antigen uptake, can dampen the anti-tumor response of PD-1-positive T cells and thus prevent tumor cells from being killed by CTLs (255). The CRC TME can induce immune-tolerant mature DCs enriched in

immunoregulatory molecules (mregDCs), a novel DC subset found in the TME (256), via the expression of the TAM family receptor tyrosine kinases such as TYRO3, AXL and MER on apoptotic tumor cells following phagocytosis by cDCs, thus leading to tumor immune escape (187). Previous studies have shown that high-mobility group box 1 protein (HMGB1) and mucin 2 produced by CRC cells could induce apoptosis of DCs (257,258), causing the suppression of anti-tumor immunity and immune evasion due to the substantial loss of DCs in the TME.

To achieve effective and specific anti-tumor responses, *ex vivo*-activated DCs pulsed with tumor antigens must migrate to the LNs and prime T cells. However, most antigen-loaded DCs are unable to reach the LNs (259), which poses a challenge to conventional DC-based therapeutics. The migration of antigen-loaded DCs into the T-cell zones of LNs is limited because the majority of DCs remain at the intradermal injection site, where they are eliminated by macrophages within 48 h (259). Overexpression of TGF-β in the TME inhibits the expression of the chemokine receptor CCR7 on DCs, which is essential for directing migration of DCs to LNs, thereby preventing homing of DCs to TDLNs (260,261).

Another challenge in DC-based vaccine application lies in the technical limitations of DC-based vaccine production. These include generating monocyte-derived DCs at optimal amounts, achieving optimal loading of tumor antigens on DCs and ensuring efficient delivery of antigen-loaded DCs to the targeted tumor cells. Scaling up the production of personalized DC-based vaccines from laboratory research to large-scale manufacturing remains a major obstacle, as the *ex vivo* generation and culture of autologous antigen-loaded DC vaccines are operationally complex, costly and require GMP-compliant facilities, constituting one of the limitations to their widespread clinical use in cancer treatment.

10. Future perspectives and strategies for overcoming the limitations

In view of the immunosuppression on the function of DCs and the limited number of activated DCs in the CRC TME, DC-based vaccines could be an ideal approach to overcome this challenge through the *ex vivo* generation of activated DCs loaded with tumor antigens at higher cell numbers. The antigen-loaded DCs, which lack PD-L1 expression on their cell surface, are then re-infused into the patient to train and activate T cells to specifically target and destroy cancer cells. Pulsing DCs with TCL derived from a patient's own tumor is an advantageous strategy to address the challenge of tumor heterogeneity, as the DCs will be loaded with a broad repertoire of TAAs, including patient-specific TSAs, which is crucial given CRC's heterogeneity. This, in turn, allows for comprehensive immune activation, leading to improved tumor recognition and immune targeting. To promote enhanced tumor-specific immunity via pulsing DCs with TAAs, other potential tumor biomarkers for CRC, such as carbohydrate antigen, tissue polypeptide-specific antigen and tumor-associated glycoprotein 72, can be targeted as single or multiple TAAs, particularly in the form of peptides (235,262). Nevertheless, artificial intelligence (AI)-driven identification of efficacious neoantigens for CRC from experimental and *in silico* data can streamline the design of personalized, potent DC-based vaccine immunotherapy (263,264).

Furthermore, the emerging approach of nanovaccines using *in situ* antigen-capture strategies (AC-NVs) has been developed and applied to overcome the limitations of DC-based vaccine immunotherapy, such as impaired functions of DCs caused by the immunosuppressive CRC TME. AC-NVs offer personalized and targeted activation of anti-tumor immunity without the need for pre-identification and synthesis of TAAs or neoantigens. AC-NVs enable the direct capturing of tumor-derived antigens, which are released from dying tumor cells, inside the cancer patient's body via the application of nanoparticles engineered with antigen-binding components. Subsequently, the AC-NVs transport the captured TAAs to TDLNs and deliver the TAAs to DCs, thus initiating the maturation of immature DCs into functional DCs, which in turn induce and activate tumor-specific T-cell responses. Following that, 'antigen libraries' can be built by the AC-NVs at the tumor site (265).

As the TAAs released from immunogenic cell death of tumor cells are rapidly cleared by efferocytosis mediated by MER proto-oncogene tyrosine kinase (MerTK) recognition of phosphatidylserine on apoptotic tumor cells, nanovaccines mU@OMVs, consisting of the MerTK inhibitor UNC2025 encapsulated within bacterial outer-membrane vesicles (OMVs), have been applied to overcome this issue (266). Phagocytosis of mU@OMVs by tumor-associated macrophages (TAMs) subsequently inhibited MerTK phosphorylation, thus delaying the clearance of apoptotic tumor cells. The inhibition of MerTK signaling led to tumor cell apoptosis and secondary necrosis, that further increased the abundance of TAAs and created *in situ* 'antigen libraries' that were then captured by the maleimide groups attached to the surface of mU@OMVs. This localized TAA enrichment enhanced the activation of DCs and provoked robust anti-tumor T-cell responses. The application of biomimetic nanovaccines using tumor cell membrane-coated nanoparticles has been shown to effectively induce intense anti-tumor immune responses through enhanced broad antigen repertoire presentation to DCs (267,268). Additionally, biomimetic nanovaccines coated with cancer cell membrane have been demonstrated to synergistically potentiate more effective anti-tumor immunity when combined with an ICB cocktail consisting of anti-CTLA4 and anti-PD1 (269).

DC-based vaccines can be combined with other cancer therapies, especially ICB immunotherapy using FDA-approved monoclonal antibodies such as pembrolizumab, nivolumab and ipilimumab (228), to achieve enhanced cell-mediated immune responses and optimal clinical benefits in cancer therapy. Additionally, emerging next-generation nanotechnology, including the use of nanoparticles and potential future nanorobots, can markedly enhance the efficacy of DC-based cancer vaccines by improving antigen delivery, promoting DC maturation and migration to LNs and delivering adjuvants that create a stronger anti-tumor immune response, leading to more effective T-cell activation and improved therapeutic outcomes (270). Although the limited number of activated DCs in the TDLNs is still sufficient to induce antigen-specific anti-tumor responses (259), augmenting DC numbers in

the TDLNs to an optimal level is essential for enhancing anti-tumor immune responses. Nanorobots within a specific size range of 20-45 nm, such as DNA origami-based constructs, can efficiently drain to LNs (271,272). Thus, nanorobots represent a promising application to promote the migration of antigen-loaded DCs to LNs, where DCs can interact with T cells and initiate the immune response more effectively.

11. Conclusion

The unique ability of DC-based cancer vaccines in initiating and shaping tumor-specific cell-mediated immune responses makes them a compelling avenue for CRC immunotherapy. Despite the safety, feasibility and immunogenic potential of DC-based vaccines for advanced CRC, their clinical efficacies have been inconsistent and generally modest when applied as monotherapy. Hence, further clinical research to determine the optimal combination therapies with DC-based vaccines is warranted to improve the effectiveness of DC vaccines against CRC. While the challenges in generating an effective DC-based CRC vaccine remain, addressed partly through combination immunotherapy with ICBs, the additional integration of emerging nanotechnology as well as the application of AI in neoantigen selection pave the way for development of more potent DC-based cancer vaccines for advanced CRC, transforming them from experimental therapy to clinically viable therapeutic cancer vaccines in the era of precision medicine.

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Authors' contributions

CLP and SKT conceptualized the review manuscript. LYP and SAH conducted literature search. LYP and SAH drafted the original manuscript. LYP, SAH, CLP, SKT, RM, CYW and KSP reviewed and edited the manuscript. CLP and SKT supervised this work. Data authentication is not applicable. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to enhance the readability and language of the manuscript and to generate some images. The authors revised and edited all AI-assisted content and take full responsibility for the final version.

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