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DENDRITIC CELL VACCINE THERAPY FOR COLORECTAL CANCER

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Abstract

Colorectal cancer (CRC) remains a leading cause of cancer-related deaths in the United States despite an array of available treatment options. Current standard-of-care interventions for this malignancy include surgical resection, chemotherapy, and targeted therapies depending on the disease stage. Specifically, infusion of anti-vascular endothelial growth factor agents in combination with chemotherapy was an important development in improving the survival of patients with advanced colorectal cancer, while also helping give rise to other forms of anti-angiogenic therapies. Yet, one approach by which tumor angiogenesis may be further disrupted is through the administration of a dendritic cell (DC) vaccine targeting tumor-derived blood vessels, leading to cytotoxic immune responses that decrease tumor growth and synergize with other systemic therapies. Early generations of such vaccines exhibited protection against various forms of cancer in pre-clinical models, but clinical results have historically been disappointing. Sipuleucel-T (Provenge®) was the first, and to-date, only dendritic cell-based therapy to receive FDA approval after significantly increasing overall survival in prostate cancer patients. The unparalleled success of Sipuleucel-T has helped revitalize the clinical development of dendritic cell vaccines, which will be examined in this review. We also highlight the promise of these vaccines to instill anti-angiogenic immunity for individuals with advanced colorectal cancer.

Graphical abstract

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CONFLICT OF INTEREST

The authors have no competing interests to declare that would negatively influence the work.

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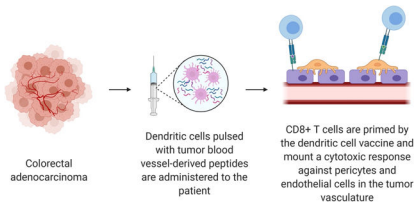

Keywords

tumor angiogenesis; immunotherapy; targeted cancer therapy; listeria monocytogenes; cytotoxic chemotherapy; Sipuleucel-T

INTRODUCTION

In the United States, over 100,000 new cases of colon cancer and 43,000 cases of rectal cancer will be diagnosed in 2020 [1]. There will also be approximately 53,200 deaths due to colorectal cancer (CRC), making the disease the third most common cause of cancer-related incidence and death in the country. Common risk factors for CRC include obesity, smoking, poor diet, genetic predisposition (e.g., Lynch syndrome, familial adenomatous polyposis), and other medical conditions such as chronic inflammatory bowel disorders. Yet, due in part to available screening methods (e.g., colonoscopy) that help detect/remove precancerous lesions, CRC incidence and mortality rates have seen dramatic declines in older adults[1, 2]. However, the incidence of this cancer type is increasing in individuals younger than the age of 55 [3].

CRC first develops in the mucosa of either the colon or rectum in the form of a noncancerous polyp (more commonly diagnosed as an adenoma) [4]. Adenomas typically grow slowly over time in the inner lining of the large intestine, but the risk of cancer increases as adenomas grow larger eventually penetrating the colon/rectal wall and accessing nearby tissues or underlying blood and lymphatic vessels that allows spread to the liver, lungs, or peritoneum [5]. Early-stage disease (Stage 0-II) that is confined to the bowel is usually treated with surgery only and results in an exceptional prognosis (90% 5-year survival rate) [6]. Unfortunately, a majority of patients present with either regional or metastatic disease (i.e., Stage III and beyond) that requires additional standard-of-care treatments such as chemotherapy and targeted drugs to help minimize cancer progression. Approximately 22% of CRC patients are found with metastases (designated mCRC) upon diagnosis, and 50–60% of individuals will develop metastases during the course of their disease [6, 7]. Despite rigorous medical interventions, 5-year survival rates for mCRC are only 14%, which clearly supports the need for new and more efficacious strategies once the cancer spreads beyond the large intestine [6].

Current standard-of-care treatments for stage IV colon cancer may include surgical resection of primary lesions and hepatic/pulmonary metastases (if possible) and systemic regimens that combine chemotherapy (e.g., fluorouracil-based) with targeted agents [8]. FDA-approved first-line targeted therapies include the more commonly used anti-epidermal growth factor receptor (EGFR) (cetuximab [Erbitux®], panitumumab [Vectibix®]) and anti-

vascular endothelial growth factor (VEGF) (bevacizumab [Avastin®]) antibodies [9]. However, cancer cells harboring mutations downstream of EGFR signaling such as in *KRAS*, *NRAS*, *BRAF*, or *PIK3CA*, will be unresponsive to EGFR inhibition, and treatment options must be adjusted accordingly [10–12]. In fact, CRC patients that do not exhibit wild-type *RAS*, will demonstrate reduced progression-free survival and overall survival following cetuximab or panitumumab treatment [13, 14]. In cases where cancer progresses following first-line treatment, approved second-line targeted options include ramucirumab (Cyramza®) (anti-VEGFR2 antibody), regorafenib (Stivarga®) (kinase inhibitor), or ziv⁻ aflibercept (Eylea® and Zaltrap®) (multiple angiogenic factor trap). The relative short-term successes of all of these treatment options for most advanced CRC patients have previously been reported [8, 15–17].

It is not currently clear whether VEGF abrogation is preferred to EGFR inhibition with first-line chemotherapy, but, historically, the addition of anti-VEGF agents to chemotherapy has provided increased survival to mCRC patients [18]. Bevacizumab, in particular, was the first angiogenic treatment to be approved by the FDA for the treatment of cancer and works by blocking VEGF interaction with cognate VEGF receptors (VEGFRs), helping prevent tumor growth by inhibiting tumor angiogenesis [19]. In one pivotal phase III trial, 813 previously untreated mCRC patients were randomly assigned to receive a fluorouracil-based chemotherapy regimen with/without bevacizumab [20]. The addition of VEGF blockade provided superior enhancements in median overall survival (20.3 months v. 15.6 months), median progression-free survival (10.6 months v. 6.2 months), and overall response rates (44.8% v. 34.8%). As a major development in the treatment of advanced CRC, bevacizumab has helped paved the way for expanding anti-angiogenic strategies against vascularized tumors such as colon cancer [21].

TUMOR ANGIOGENESIS

Primary (avascular) tumors will generally grow only to a stable size of 1–2 mm^3 without a sufficient blood system to supply oxygen and nutrients and alleviate waste accumulation [22]. During the initial phases of cancer growth, there is a presumed balance between pro-angiogenic and anti-angiogenic factors, but when this balance is disrupted to favor angiogenesis (a threshold referred to as “the angiogenic switch”), the influence of pro-angiogenic factors is increased and tumor progression continues [23]. A defining trigger of the angiogenic switch is a lack of sufficient oxygen within the tumor microenvironment that results from unabated cancer cell growth [24, 25]. As a tumor expands, areas within the lesion too remote from blood vessel support (typically >100 μM) will experience hypoxia that disrupts (via hypoxia inducible factors [HIFs]) normal metabolic processes to instead drastically upregulate expression of key angiogenic molecules such as VEGF and platelet-derived growth factor [26]. Additionally, HIF-dependent angiogenesis can be prompted from genomic damage sustained by tumor cells. For example, mutations in tumor suppressor genes such as *P53* and *PTEN* are directly linked to HIF accumulation that works to promote VEGF expression [27, 28].

The continued development of a tumor lesion’s vasculature is reliant on a wealth of other soluble mediators (e.g., cytokines, chemokines, extracellular matrix remodeling factors, and

pro-angiogenic molecules) like fibroblast growth factor, angiopoietin-2, placenta growth factor, and matrix metalloproteases that are supplied by tumor cells and accessory cells such as cancer-associated fibroblasts and immune cells [29]. However, the continued overexpression of VEGF binds endothelial cell-derived VEGFR2 to initiate/sustain sprouting angiogenesis and vasculogenesis [30, 31]. Overall, the resultant vasculature within the tumor microenvironment is phenotypically distinct from blood vessels occurring in healthy tissues. Newly formed cancer-derived blood vessels may be deficient in a basement membrane and supportive cells such as pericytes and yield abnormalities in length/surface area that preclude archetypal blood vessel hierarchies in normal vascularized tissues [32, 33]. Functionally, this chaotic blood system demonstrates increased vascular permeability that sustains hypoxia and permits fluid leak into the surrounding tissue, exacerbating interstitial pressure [34]. Those tumor-retained cells that evolved during tumorigenesis will also continue to influence angiogenesis in order to further promote cancer progression and spread [35, 36].

Although passive infusions of anti-vascular immune agents (e.g., bevacizumab, ramucirumab) work to minimize such tumor-promoting networks and have provided mCRC patients short-term clinical relief, a conceptual therapeutic improvement relates to instituting sustained immune-related activity in the host through vaccination [37, 38] or adoptive cell therapy [39, 40]. Given their unique nature, tumor-derived blood vessels are immunogenic and can be specifically targeted by immune cells to induce tumor regression and institute immunologic memory to help prevent cancer recurrence [41, 42]. One such powerful immune cell inducer of anti-tumor immunity that also holds tremendous promise as an immunotherapeutic strategy is the dendritic cell (DC).

DENDRITIC CELLS

DCs are professional antigen presenting cells (APCs) that belong to the mononuclear phagocyte system and can activate naïve T cells against various host insults including cancer. Initially discovered in 1868 by Paul Langerhans, DCs received their name in 1973 upon the identification of cells in the mouse spleen displaying long cytoplasmic processes [43]. The various DC subsets first arise from a unique hematopoietic lineage in the bone marrow. Like other leukocytes, DCs develop from bone marrow-derived hematopoietic CD34+ stem cells and further differentiate from common myeloid progenitors, although a small fraction of DCs can arise from common lymphoid progenitor cells [44, 45]. Downstream of the common myeloid progenitors, macrophage/DC progenitor cells serve to provide the host a steady supply of monocytes, macrophages, and “classical” DCs. In particular to DC development, macrophage/DC progenitors give rise to common DC progenitor cells that expand into pre-DCs, which travel to lymphoid and non-lymphoid organs to further mature into functional DC subtypes based on intrinsic and/or external factors as clarified below [46, 47].

While residing in peripheral tissues, DCs sample the surrounding environment through receptor-mediated phagocytosis or macropinocytosis, and, following antigen uptake, migrate to draining lymph nodes by responding to the chemokines CCL19 (secreted by mature dendritic cells) or CCL21 (secreted by lymphatic vessel-derived endothelial cells) through

upregulation of CCR7 [48–51]. During their trafficking, antigen-bearing DCs assume a mature APC phenotype, which is marked by increased expression of surface-molecules such as major histocompatibility complex (MHC) class II and CD80/CD86 [52]. DCs then engage/present processed antigen to T cells in the lymph node paracortex via the MHC and provide the necessary costimulation and cytokine support for T cell activation and proliferation [53, 54] (outlined in Figure 1).

Mouse DCs have been well-classified based on the expression of defining markers such as CD4 and CD8α [55]. However, human DCs have only begun to reach a similar level of characterization in the last decade, with many details still missing pertaining to the phenotypic/functional similarities and differences between human and mouse DC populations. Although there may be uncertainty regarding the most appropriate way to taxonomically define DC lineages, several iterations of nomenclature have been proposed in recent years [56–58]. In particular, human DC subsets that have and continue to be extensively studied within the context of therapeutic DC vaccines are conventional DCs (cDCs), plasmacytoid DCs (pDCs), and monocyte-derived DCs (moDCs) (summarized in Table 1).

Types of dendritic cells

cDCs specialize in antigen uptake and presentation to naïve T cells and are characterized by a CD11c+/CD123− phenotype [59]. cDCs in humans have been further divided into two larger groups (cDC1 and cDC2) based on mouse DC work and transcription factor dependence. Both subsets can be found in the blood, lymphoid, and non-lymphoid tissues [60] and are further defined by CD141+ that is analogous to mouse CD8a+/CD103+ DCs [61]. cDC1 development in the bone marrow is driven by IRF8, BATF3, ID2, and Flt3L [62]. cDC1s also have high expression of toll-like receptor (TLR) 3, TLR11, and TLR12 but lack TLR4 and TLR9 expression, which are observed in their mouse DC counterparts [63]. Functionally, cDC1s specialize in antigen cross-presentation to CD8+ T cells and production of IL-12 following migration to lymph nodes [64–67]. cDC2s are distinguished by CD1c+ expression and their development is dependent on IRF4, KLF4, and Notch2 [68]. The cDC2 subtype has recently been subdivided further into cDC2A and cDC2B cells as observed in mice. Initially, cDC2s were found to have varying levels of CD5 and were loosely divided into $CD5^{hi}$ and $CD5^{lo}$ populations [69], but cDC2As are more similar to cDC1s than their cDC2B counterparts, as evidenced by a higher expression of CD1c, HLA-DQ, and interferon regulatory factors. cDC2Bs more closely resemble monocytes, with higher expression of CD14, CD32, CD36, CD163, and MAFB [70]. In mice, cDC2As preferentially express TLR1, TLR5, and TLR7, whereas, cDC2Bs express TLR1, TLR2, TLR5, TLR6, and TLRs 7–9 [71]. Additionally, recent transcriptional analysis in mice has identified cDC2As as T-bet dependent while cDC2Bs rely on RORγt [71]. Generally, cDC2s are important for polarizing CD4+ T cells towards Th2, Th9, Th17, and Treg subsets [72]. An inflammatory cDC2 subset has also been identified in mice within the context of viral infections [73]. Inflammatory-cDC2s developed characteristics such as CD64 and IRF8 expression, which are usually associated with monocytes and cDC1s, respectively. It remains to be seen whether inflammatory-cDC2s are a distinct DC subset or an infection-driven

variant of pro-inflammatory cDC2Bs. Since the cDC2A and cDC2B stratification has only recently been recognized, further work is needed to validate these findings in humans [74].

pDCs are generally characterized by substantial production of type I interferons (IFNs) upon encountering nucleic acids from pathogens and a morphology similar to B cell-differentiated plasma cells [75]. pDCs traffic through the blood and accumulate in peripheral tissues experiencing inflammation [76], and, upon sensing pathogenic RNA and DNA (through TLR7 and TLR9, respectively), secrete IFN-β, most types of IFN-α, and type III IFN (among other cytokines and chemokines) to further direct/activate locoregional immune responses [77]. pDCs will also upregulate MHC class I/class II expression to directly engage T cells during periods of pathogen infection [78]. Like cDCs, pDCs are heavily dependent on Flt3L for development [79]. Progenitor cells in the bone marrow are destined to the pDC lineage by the transcription factor E2–2, which directs their differentiation via STAT3 [80]. pDCs have also been shown to develop from common DC and lymphoid progenitor cells, but recent work suggests pDCs derive predominately from an IL-7R+ lymphoid progenitor that requires exposure to IRF8 [81].

Monocytes are circulating leukocytes that provide innate immune responses, help modulate adaptive immunity, and support the maintenance of tissue homeostasis [82]. Monocytes are developmental precursors to both macrophages and moDCs, but they also perform effector functions in the blood [83]. Monocytes have been further subdivided into classical, nonclassical, and intermediate categories, although heterogeneity has been described even within these subtypes (reviewed in detail elsewhere [84, 85]). Classical monocytes are CD14++ CD16− and are recruited to areas of inflammation by CCR2 to respond to lipopolysaccharide and produce TNF-α/IL-1. Nonclassical monocytes (CD14 low/CD16++) primarily survey cells via CX3CR1 to maintain overall homeostasis by performing endothelium repair and removing cell debris [86]. Intermediate monocytes (CD14+/CD16+) display an inflammatory phenotype similar to classical monocytes but also express CX3CR1 as seen with the nonclassical subset [87]. Importantly, classical monocytes are capable of differentiating into moDCs, especially following recruitment to sites of inflammation that is controlled by the transcription factors MAFB and KLF4 [70]. These inflammatory-stimulated moDCs are typified by expression of HLA-DR+/CD11c+ and a combination of DC and macrophage markers (i.e., CD1c, CD1a, CD1b, FcεR1, CD206, CD14, and CD11b) [88]. Like the previously discussed DC subsets, moDCs can develop dendrites upon differentiation and stimulate T cells [89]. Although much remains to be determined about this DC subset in humans, particularly from a functional standpoint, moDCs can secrete inflammatory cytokines and induce Th17 polarization *in vitro* [90]. Lastly, DCs can be derived from isolated monocytes *in vitro* with the cytokines GM-CSF and IL-4 [91] (summarized in Figure 2). The discovery of this directed tissue culturing approach has had a tremendous influence on the field by providing a suitable supply of DCs for vaccine purposes in patients with cancer [92].

Dendritic cell vaccines for cancer

The initial success of DC vaccines to combat cancer became evident from pre-clinical studies in the late 1980s and early 1990s that demonstrated protective anti-tumor effects

afforded by tumor lysate-pulsed DCs [93, 94]. The translation of these experiments then culminated into clinical trials where autologous DCs were isolated from patients, modified *ex vivo* with antigen +/− maturation signals, and re-administrated. Pivotal clinical observations published in 1996 revealed that a series of infusions of antigen-specific DCs (initially obtained by leukapheresis from peripheral blood) were capable of generating anti-tumor immune responses as well as tumor regressions in a small cohort of patients with B-cell lymphoma [95]. A similar study in patients with stage IV melanoma exhibited that a MAGE-1 peptide-pulsed moDC vaccine could induce antigen-specific cytotoxic T cells, although no observable therapeutic benefit was demonstrated [96]. Importantly, these early studies helped establish that autologous DC vaccines were overwhelmingly safe following infusion in patients [97].

To date, the only DC-associated therapeutic currently approved by the FDA is Sipuleucel-T (Provenge®). Approved in 2010, Sipuleucel-T is a preparation of autologous CD54+ APCs for the treatment of minimally symptomatic, hormone refractory prostate cancer [98]. Based on the preparation method, Sipuleucel-T is not strictly a DC vaccine, as other mononuclear cells are also present. However, following collection of peripheral blood mononuclear cells via leukapheresis, CD54+ cells are isolated by density gradient centrifugation and co-cultured with the recombinant protein PA2024, which consists of prostatic acid phosphatase fused with GM-CSF that provides APCs both antigen specificity and maturation potential [99]. Patients are provided at least 50 million antigen-pulsed CD54+ cells for each vaccine treatment that is provided up to 3 times over a period of 4 weeks. Preliminary characterizations of the vaccine product demonstrated enhanced APC and T cell activity through elaboration of activation-associated cytokines (e.g., IFN-γ, TNF-α) following autologous cell infusions [100] as well as antibody and T cell-specific responses against PA2024 [101]. In the pivotal stage III IMPACT trial, 512 men with metastatic castration-resistant prostate cancer were randomized to treatment with Sipuleucel-T or placebo and overall survival was assessed as the primary endpoint. IMPACT demonstrated a 4.1 month increase in the survival of patients treated with Sipuleucel-T over placebo as well as an increase in the long-term benefit of the therapy when assessing 3-year survival rates (31.7% for Sipuleucel-T versus 23.0% for placebo). Notably, the effects of Sipuleucel-T were consistent even in groups with adverse prognostic factors known to impact patient survival such as elevated PSA levels and bone metastases. As seen with earlier DC vaccine trials, few serious safety responses were observed, and the most common adverse events from Sipuleucel-T included elevated flu-like symptoms (i.e., chills and fever) within 1 day after infusion that was likely the result of cytokines released by activated immune cells [101]. It is important to note that there are puzzling aspects of the IMPACT trial such as the overall lack of effects on PSA level or time-to-tumor progression between the Sipuleucel-T and placebo groups. An independent review of internal FDA data (that did not become available until after Sipuleucel-T approval) explores these matters at length, but, briefly, post-hoc subgroup analyses revealed unexpected correlations between age and overall survival [102]. First, effects on median survival could only be seen in patients 65 years or older and seems to contrast with the long-standing principle that younger patients develop more robust immune responses following immunizations[103]. Second, patients over 65 in the placebo group saw shorter overall survival than expected when compared to placebo groups in trials with

similar enrollment restrictions. Finally, several major disparities in the processing/reinfusion of cells appeared to occur between the Sipuleucel-T and placebo-treated patients and included: [i] only a fraction of bulk processed cells were eventually delivered to placebo patients, [ii] placebo cells were not incubated with GM-CSF, and [iii] placebo cells were first incubated at 2–8 °C for 36–44 hrs that could have facilitated cell death prior to infusion. In all, the survival benefits of the IMPACT trial could potentially be a result of study design issues adversely impacting older placebo-treated patients. Yet, these alternative analyses/explanations have also been the subject of intense refute [104–106].

On the whole, though, these aforementioned DC vaccine clinical attempts have demonstrated a proof-of-principle that the DC platform can unleash potentially protective anti-cancer immune responses in patients. These trials have also helped spur additional work to enhance the therapeutic index of the DC vaccine approach for various forms of malignancies such as CRC [97].

Dendritic cell vaccine experience in the treatment of colorectal cancer

Currently, the majority of ongoing clinical trials investigating DC vaccines for CRC involve administrating DCs pulsed with autologous tumor lysates since this methodology has historically induced tumor-specific immune responses in patients [107, 108] (detailed in Table 2). Although this strategy ensures patients receive a personalized vaccine (by way of presenting unique tumor antigens), vaccine development is dependent on retrieval of an adequate amount of resected tumor that contains immunogenic material. Alternatively, DCs may be pulsed with exogenous tumor-associated peptides such carcinoembryonic antigen (CEA), which is expressed broadly by most colon cancer specimens [109]. Results of several early-phase clinical trials demonstrated that a CEA DC vaccine for CRC is effective at safely generating anti-CEA specific responses; however, overall survival or progression-free survival benefit has not been realized for a majority of patients [110–112]. An ongoing Phase I/II clinical trial is utilizing a DC vaccine loaded with CEA and the frameshift neoantigens caspase-5 and TFG-βRII in treating patients with microsatellite instability (MSI) CRC or as a preventative measure for germline mismatch repair (MMR) mutation carriers (Clinicaltrials.gov Identifier: NCT01885702). As Sipuleucel-T demonstrated improved efficacy in patients with reduced tumor burden, a preventative vaccine course may be an acceptable application for individuals at high-risk for developing CRC [113]. Relatedly, two clinical trials are exploring the usefulness of a DC vaccine to prevent relapse in either surgically resected stage I/II hypermutated or stage IV CRC where curative resection had been performed (NCT03730948, NCT02919644). In both scenarios, DC vaccines would be expected to inspire immune surveillance against microlesions that escaped initial detection.

It is clear that immune responses to CRC targets can be generated following DC vaccination, but improved clinical parameters (such as overall survival) are typically not observed in most patients. Obviously, this immunotherapeutic strategy holds tremendous potential (given the importance of DCs to fuel cytotoxic immune responses), but further research is required to enhance the approach for malignancies such as CRC. Major areas of continued development include identifying suitable patient subsets where DC vaccine treatment would

provide the most benefit, improving *ex vivo* culturing techniques and inoculation routes, determining synergistic therapies best suited for use alongside DC vaccination, and predicting/characterizing CRC-associated antigens (e.g., neoepitopes, vascular targets) for DC processing/presentation [114].

IMPROVING THE DC VACCINE PLATFORM

Dendritic cell subsets

Since blood-derived DC populations exist only in small numbers, moDCs have preferentially dominated the majority of DC vaccine clinical studies. There is evidence, though, that peripheral DCs (namely cDCs and pDCs) may be superior DC subsets for migration and antigen cross-presentation purposes, given their biological roles in the host (see the “Types of DCs” Section). A first-in-human trial of an allogeneic pDC line that was irradiated and loaded with melanoma antigens (MLANA, MAGEA3, PMEL, TYR) was able to significantly increase the number of antigen-specific T cells in 4 of 9 metastatic patients experiencing some period of stable disease up to 48 weeks [115]. While the small study size and prior treatment record obscure the seeming cause of clinical benefit, the pDC vaccine was safe and able to induce T cell expansion without causing neutralizing immune responses to the vaccine. A separate study comparing pDC and cDC2 vaccines in melanoma patients also revealed that pDCs produced higher levels of CXCR3/CCR5 ligands (promoting cytolytic immune cells) and attracted greater numbers of CD8+ T cells in skin biopsies while cDC2s expressed elevated levels of chemokines binding CXCR1/CXCR2 (yielding T cell priming properties) [116]. These findings suggest that a combined pDCs and cDC2 vaccine might effectively provide chemoattractive and anti-cancer properties to T cells.

Altogether, although underutilized for vaccine purposes, other peripheral DC subsets may provide alternative (or additional) strengths to the standard route of infusing patients moDCs for therapeutic use. However, based in part on current deficiencies in cell isolation and culturing techniques, major challenges to incorporating this method include purifying sufficient numbers of peripheral DCs for *ex vivo* maturation/expansion purposes, especially in patients who have previously received immunosuppressive chemotherapeutic regimens. For example, cDC1s hold great appeal for use as a DC vaccine due to an enhanced ability to cross-present exogenous antigen to CD8+ T cells. Unfortunately, the low percentage of cDC1s in circulation (approximately 0.03% of human peripheral blood mononuclear cells) and lack of an appropriate clinical-grade reagent for cell isolation have excluded their use in the clinic [117].

Inoculations

Even though Sipuleucel-T is administered intravenously other DC inoculation routes are being explored to potentially improve DC localization to lymph nodes for T cell activation. In mouse models, intradermally administered DC vaccines only result in the delivery of 2–4% injected material to tumor-draining lymph nodes intravenously administrated DCs largely traffic to vascularized organs such as the spleen, liver, and kidneys [118, 119]. A study comparing intranodal versus intradermal administrations in advanced melanoma patients also revealed that intranodal injections resulted in higher numbers of DCs migrating

to local lymph nodes, but intradermal injection provided a superior induction of T cells responding to tumor associated-antigens [120]. Additionally, intratumoral injection seems relatively efficacious since patients with metastatic disease exhibited an increased infiltration of CD8+ T cells in 5 out of 12 resected kidney tumors [121]. Despite the uncertainty of whether one injection scheme is superior overall, there is likely room for further optimization in this area. For example, the total number of DCs and timing of doses are likely critical for achieving maximal anti-tumor benefits in patients [122].

Combinations

A major factor, thus far, in the disappointing clinical performance of DC vaccines is an inability to overcome immunosuppressive properties of the tumor-microenvironment. Therefore, treatment combinations of DC vaccines with other immunomodulatory therapies is likely a positive way forward to achieve durable anti-tumor responses in patients. DC vaccines have frequently been administered alongside cyclophosphamide in order to suppress regulatory T cells [123–125], but success (in terms of patient responses) has still been limited, as regulatory T cells are not the only barrier to DC function within the tumor [92, 124, 126]. Other promising treatments that may have synergistic effects with DC vaccines include immune checkpoint inhibitors (ICIs), immunogenic cell death (ICD) inducers, and anti-angiogenic therapeutics.

The unprecedented success of ICIs has heralded a new era of immunotherapeutic promise for cancer in general. While clinical successes have been demonstrated with ICIs in select patients with tumors such as metastatic melanoma and MSI-high CRC, therapeutic efficacy as a single agent is still limited on a broader scale [127]. In the context of mCRC, FDA approval has only been granted for anti-PD-1 antibodies (nivolumab and pembrolizumab) in patients with MSI-high or MMR CRC subtypes [128, 129]. Yet, even with approved indications for immune checkpoint blockade, individuals may not respond, exhibit resistance (developed or inherent), or experience hyperprogression as a result of treatment [130]. In recent years, a crucial connection between ICIs and DCs has become evident, with the potential for these antibodies to boost the downstream effects of DC immunization. Vaccination with DCs loaded with autologous tumor lysates in 16 patients with metastatic melanoma revealed that patients with a significant increase in tumor-infiltrating CD8+ T cells also experienced upregulation of tumoral PD-L1 expression, indicating that concurrent PD-1/PD-L1 inhibition may improve immune-driven effects against immunosuppressive tumors [131, 132]. Conversely, timing of ICI administration severely alters immune responses to DC vaccines. A recent study of patients who received an autologous melanoma-specific DC vaccine reported that individuals provided immune checkpoint blockade after vaccine administration had considerably increased numbers of melanoma-specific CD8+ T cells in circulation, whereas, ICIs given prior to DC vaccination did not translate into improved cytotoxic T cell responses (by way of increased IFNy expression) [133]. PD-L1 abrogation may also instigate direct effects on DC function. For example, a patient sample analysis suggests that DCs could be suitable targets for anti-PD-L1 treatment by blocking PD-L1/B7.1 cis interactions on DCs, thus, freeing B7.1 to ligate CD28 and co-stimulate anti-tumor T cells [134]. Maturation of DCs with pro-inflammatory cytokines and TLR

ligands tends to upregulate PD-L1 surface expression, perhaps making DC vaccines themselves a prime target for ICIs during *ex vivo* manipulations [135, 136].

While certain anti-cancer cytotoxic therapies are known to be immunosuppressive, several chemotherapeutics (e.g., doxorubicin, fluorouracil, and oxaliplatin [137]) or physical interventions such as radiotherapy could be employed to promote ICD [138]. The discrete actions of ICD are generally engaged following target cell production of reactive oxygen species or endoplasmic reticulum stress that releases (usually hidden) internal components to the extracellular environment. These secreted or cell surface-expressed molecules then stimulate immune cell activity by interacting most prominently with pattern-recognition receptors on APCs [139, 140]. Some potential ICD pathways involve the surface appearance of calreticulin on dead/dying target cells, which encourages phagocytosis by APCs like DCs [141]. ATP may also be secreted from dying cells and serve as a “find me” signal for DC precursors [142]. Additionally, HMGB1 is released from cells in late stages of apoptosis and binds TLR4 on DCs [143, 144]. Ultimately, the ICD process can be harnessed (i.e., as an endogenous adjuvant) for DC vaccines since it creates an environment rich in inflammatory mediators that stimulates DC activation. As one example, in a preclinical study, mice received DCs loaded with doxorubicin-treated neuroblastoma tumor cell lysates, and, when given prophylactically, generated superior tumor protection versus DCs exposed to untreated neuroblastoma cell lysates [145]. Therapeutically, the DC/doxorubicin vaccine regimen was further augmented when a CXCR4 agonist was also delivered to mice.

Lastly, tumor-derived blood vessels may serve to inhibit the collective effects of DC vaccines. Sustained tumor-produced VEGF can mediate detrimental effects to DC function through mechanisms that include inducing PD-L1 expression on myeloid DCs, impairing mature DC mobility, and suppressing expression of MHC class II and other costimulatory molecules [146–150]. Although anti-angiogenic agents such as VEGF-specific antibodies (e.g., bevacizumab) and small molecule drugs (e.g., axitinib, dasatinib, sunitinib) have been approved for some time, resistance to these monotherapies develops quickly in patients due to tumor blood vessels adopting compensatory reliance on other growth factors [151]. Yet, VEGF blockade in combination with other drugs such as chemotherapy [152], ICIs [153], or mTOR inhibitors [154] have shown improved anti-tumor effects. One possible explanation for such synergy involves the “vascular normalization” hypothesis, which proposes that in addition to causing limited vascular destruction, anti-angiogenic drugs transform the chaotic tumor vasculature into a more normal arrangement that allows co-applied drugs to effectively distribute and function throughout the tumor [155]. Ultimately, while DC vaccines alone may have a limited ability to catalyze T cell infiltration and immune cell cytotoxicity within the tumor microenvironment based on the aberrant and immunosuppressive properties of the tumor lesion, vascular normalization strategies could help unleash the ability of DCs to induce superior anti-tumor immunity by restoring blood flow dynamics and minimizing immune-defeating properties like hypoxia, acidosis, and downregulation of leukocyte adhesion molecules (e.g., ICAM-1, VCAM-1, E-selectin, and CD34) [156–159]. To further support this concept, in mouse models of melanoma, DC vaccines were capable of instituting superior antigen-specific tumor protection when animals were first sensitized to anti-angiogenic drugs like axitinib or dasatinib [160, 161].

Vascular dendritic cell targets

The relative clinical success of bevacizumab (and other FDA approved anti-angiogenic agents) has helped attract attention to furthering the development of immunotherapeutic strategies such as vaccines that target the tumor vasculature. In many cases, DC-inspired immune responses against the underlying endothelium (by way of CD8+ T cell cytotoxicity, for example) would be favored to induce poly-specific immunity and immunological memory (Figure 1).

To maintain vaccine safety, the ideal vascular target is one that is overexpressed within the tumor but not found or only evident at low levels on healthy endothelium to avoid disruptions to normal physiological processes such as wound healing [162]. Abrogating tumor-derived VEGF/VEGFR dynamics has been extensively studied in the clinic, particularly from a standpoint of infusing CRC patients blocking antibodies such as bevacizumab (anti-VEGF) or ramucirumab (anti-VEGFR2). Patients may experience dose-dependent hypertension following antibody infusions but such issues can be clinically manageable [163]. Since VEGF maintains roles in physiological angiogenesis, a vaccine-inspired immune response against VEGF/VEGFR also raises concerns about adverse events occurring outside of the tumor microenvironment. In one scenario, a vaccine consisting of recombinant human VEGF in combination with an adjuvant derived from *Neisseria meningitides* was assessed in a multi-center phase I clinical trial (CENTAURO-2) [154]. Dosing schemes resulted in 75% seroconversion in patients exhibiting anti-VEGF blocking antibody responses while still maintaining an acceptable safety profile. The placental endothelial cell vaccine ValloVax™ has also been utilized as a whole cell vaccine to immunize patients against naturally occurring blood vessel components [164]. Early clinical experience indicates that all patients vaccinated elicited enhanced antibody responses against vascular antigens such as VEGFR1, VEGFR2, CD105, and FGFR without mediating abnormal safety responses [165]. Ultimately, such examples give some degree of assurance that immunologic responses can be generated in individuals against self-vascular targets without inspiring unmanageable off-target toxicities in healthy tissues.

In relation to engaging DC activity, DNA and peptide-based vaccines have also been formulated against tumor-derived angiogenic factors such as bFGF, FGFR-1, avB3, angiomotin, CD105, survivin, Robo4, Tie-2, EGFR, HP59, PDGFRβ, TEM1, and TEM8 [166]. The goal of a tumor associated peptide vaccine is to conform MHC class I or II binding requirements so that an APC like a DC will present the administered peptide and activate CD8+ T cells or CD4+ T cells, respectively, against target cells. DNA vaccines function in a similar manner by essentially directing the expression/presentation of targets of interest in cells such as DCs upon plasmid DNA uptake [167]. Unfortunately, peptide and DNA vaccines, overall, have notoriously fallen flat in clinical trials, despite preclinical successes [167–169]. However, one encouraging DC preparative approach utilizes the unique properties of the intracellular bacterium *Listeria monocytogenes* (Lm) to direct DC antigen-specificity. Lm is a gram-positive bacterium that readily infects APCs, through expression of phospholipases and cytolysins, and can escape phagolysomal destruction and gain entry to the cytosol where it replicates prior to infecting a nearby cell [170, 171]. Once in the cytosol, the bacterium facilitates proteasomal processing, MHC Class I presentation,

and robust induction of cytotoxic T lymphocyte responses against protein antigens that it secretes [172, 173]. As such, Lm-based therapeutic vaccines have been designed to express and secrete tumor associated-antigens of interest, and, upon DC infection, induce DC maturation and antigen presentation for CD8+ T cell activation, even at mucosal surfaces [174–176]. Due to the ability to break immunologic tolerance to self-antigens, Lm-based vaccines are ideal for targeting both tumors directly and the tumor-associated vasculature [177–180]. In fact, Lm-based vaccines targeting vasculature-associated antigens such as VEGFR2, HMWMAA, and CD105 have been able to generate both protective and therapeutic cytotoxic T lymphocyte responses in pre-clinical models of melanoma and breast cancer [179, 180]. The general clinical outlook for Lm-based vaccines is promising with numerous studies demonstrating significant efficacy in addition to high tolerability and safety in patients [181]. While Lm-based vaccines have advanced to phase III clinical trials, recent publication of phase II clinical trial results demonstrated that cervical cancer patients receiving an Lm-based vaccine targeting HPV16 E7 had clinically relevant tumor responses and prolonged survival in a select population, outperforming historical standards [182]. Further, the anti-tumor efficacy of Lm-based vaccines is enhanced when administered in combination with other interventions such as ICIs and radiation, suggesting a promising future for this therapy on improving patient survival [183–185].

Similar to Sipuleucel-T, directly infusing *ex vivo* matured DCs into patients likely holds the best route for securing immunity against tumor-derived targets but requires the necessary infrastructure to deal with cell isolation, maturation/expansion, and infusion. In pre-clinical models, adoptive therapy of DC vaccines encoding the tumor blood vessel antigens DLK1, EphA2, HBB, NRP1, PDGFRB, RGS5, or TEM1 resulted in the regression of MC38 or B16 subcutaneous tumors in HLA-A2 transgenic mice. CD8+ T cells were specifically invoked against tumor-derived blood vessel antigens that resulted in long-term inhibition of tumor growth. Importantly, no adverse immune responses were observed against healthy vascularized tissues or impairment to wound healing [186]. This strategy has also translated to the clinical for treatment of patients with melanoma (NCT01876212) or breast (NCT02479230) cancer and is awaiting further action.

CONCLUSIONS

Despite the number of mCRC treatments coming to market over the last two decades, the disease still remains deadly in its later stages. DC vaccines have historically performed poorly in clinical trials for cancer, but renewed interest in this immunotherapeutic strategy has been sparked by the relative success of Sipuleucel-T for prostate cancer and advent of immunomodulatory agents that may synergistically improve DC function. However, further research advancements are required in order to establish DC vaccines as a clinically efficacious approach for advanced CRC. Typical vaccine characteristics such as DC subtype, administration, timing, and dosage still require heavy research investment. Additionally, reasonable improvements in DC-elicited immune responses could be expected through rational combinations that might include immune checkpoint blockade and/or anti-angiogenic therapies. Lastly, tumor blood vessel-derived antigens represent an exciting area for DC vaccination purposes in order to trigger cytotoxic CD8+ T cell responses against the underlying blood vessel network of CRC. As bevacizumab has helped pave the way for anti-

angiogenic treatments against the disease, further success is possible by instilling durable and broad T cell responses against the tumor vasculature.

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Abbreviations used:

APC	antigen presenting cell
CEA	carcinoembryonic antigen
CRC	colorectal cancer
cDC	conventional DC
DC	dendritic cell
EGFR	epidermal growth factor receptor
HIF	hypoxia inducible factor
ICI	immune checkpoint inhibitor
ICD	immunogenic cell death
IFN	interferon
Lm	Listeria monocytogenes
MHC	major histocompatibility complex
mCRC	metastatic CRC
MSI	microsatellite instability
MMR	mismatch repair
moDC	monocyte-derived DC
pDC	plasmacytoid DC
TLR	toll-like receptor
VEGF	vascular endothelial growth factor
VEGFR	VEGFR receptor

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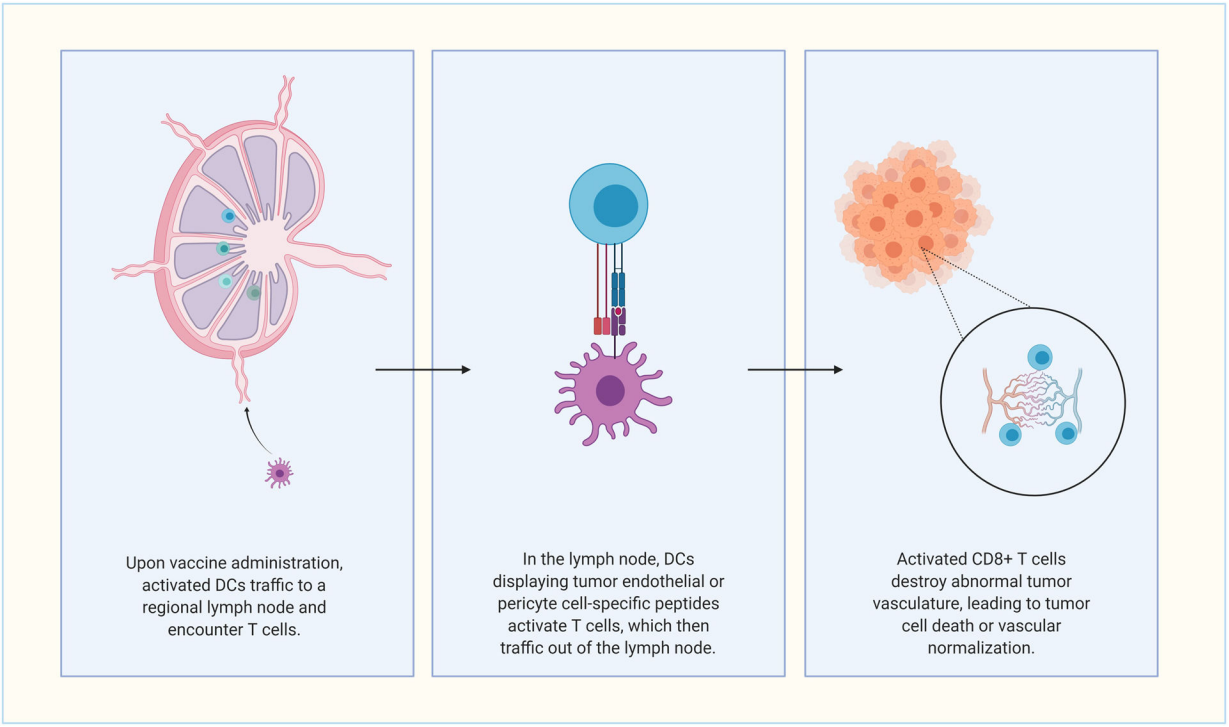

Figure 1.

General overview of directing dendritic cell vaccines against colorectal cancer-derived blood vessels. Abbreviation used: dendritic cell (DC). Created with Biorender.com.

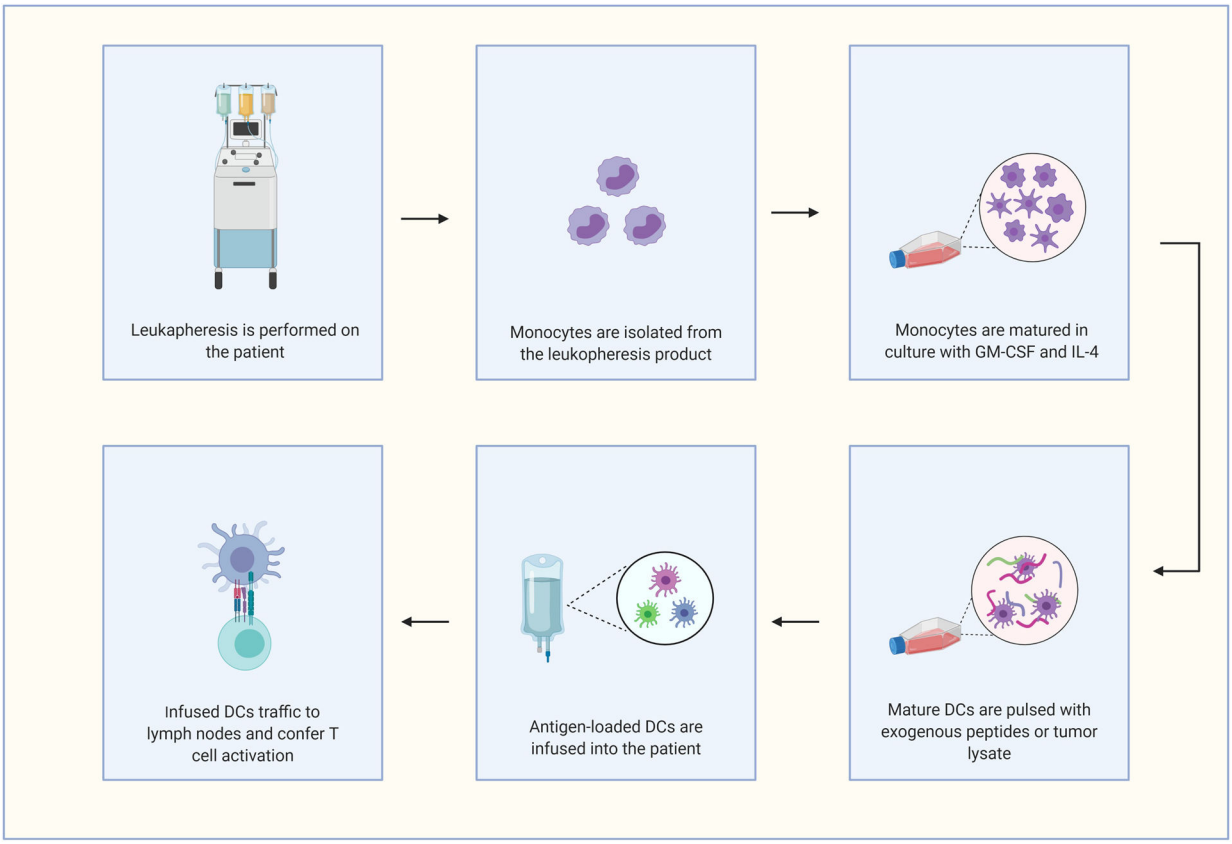

Figure 2.

Standard *ex vivo* approach for generating dendritic cell vaccines for patient infusion. Abbreviation used: dendritic cell (DC). Created with Biorender.com.

Table 1.

Human dendritic cell subsets

DC type	Major function	Selective markers	Transcription factor dependence
cDC			
cDC1	Antigen cross-presentation to CD8+ T cells	CD11c, Clec9, CD141	IRF8, BATF3, ID2,
cDC2A	CD4+ T cell polarization; antiinflammatory phenotype	CD1c, CLEC4A	IRF4, KLF4, Notch2
cDC2B	CD4+ T cell polarization; pro-inflammatory phenotype	CD14, CD32, CD36, CD163, CLEC10A	RORγT, ZEB2
pDC			
	Type I IFN production upon encountering pathogens	CD123, BDCA-2, BDCA-4	Flt3L, E2–2, STAT3
moDC			
	T cell stimulation; inflammatory cytokine secretion	CD11c, CD1c, CD1a, CD1b, FcεRl, CD206, CD14, CD11b	MAFB, KLF4

Abbreviations used: conventional DC (cDC), dendritic cell (DC), plasmacytoid DC (pDC), monocyte-derived DC (moDC), interferon (IFN)

Table 2.

Representative colorectal cancer clinical trials utilizing dendritic cell vaccines

Clinicaltrials.gov identifier (Estimated completion year)	Phase	CRC indication	Treatment
NCT01885702 (2020)	I/II	Adjuvant DC vaccine for MSI-positive CRC/ preventative DC vaccine for germline MMR-gene mutation carriers	Autologous DC vaccine loaded with CEA, and frameshift-derived neoantigens
NCT02503150 (2020)	III	Metastatic CRC with no previous therapy for metastatic lesions	Autologous DC vaccine loaded with autologous tumor lysate in combination with modified FOLFOX-6
NCT03152565 (2020)	I/II	MSS metastatic CRC treated with at least two forms of chemotherapy	Autologous DC vaccine in combination with avelumab
NCT03730948 (2021)	I	Surgically resected stage I and II hypermutated CRC	Autologous DC vaccine with mutated peptides
NCT02919644 (2024)	II	Curative resection of stage IV CRC	Autologous DC vaccine with autologous tumor lysate followed by IL-2 injection

Abbreviations used: carcinoembryonic antigen (CEA), colorectal cancer (CRC), dendritic cell (DC), mismatch repair (MMR), microsatellite instability (MSI), microsatellite stable (MSS)