Review

Integrating Next-Generation Dendritic Cell Vaccines into the Current Cancer Immunotherapy Landscape

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Cancer immunotherapy is experiencing a renaissance spearheaded by immune checkpoint inhibitors (ICIs). This has spurred interest in ‘upgrading’ existing immunotherapies that previously experienced only sporadic success, such as dendritic cells (DCs) vaccines. In this review, we discuss the major molecular, immunological, and clinical determinants of existing first- and second-generation DC vaccines. We also outline the future trends for next-generation DC vaccines and describe their major hallmarks and prerequisites necessary for high anticancer efficacy. In addition, using existing data we compare DC vaccines with ICIs targeting CTLA4, PD1, and PD-L1, and argue that in various contexts next-generation DC vaccines are ready to meet some challenges currently confronting ICIs, thereby raising the need to integrate DC vaccines in future combinatorial immunotherapy regimens.

Dendritic Cells As Tools for Anticancer Vaccination

Dendritic cells (DCs; see [Glossary](#)) are the most efficient antigen-presenting cells (APCs) of the innate immune system [1,2]. Conventionally, all APCs can present exogenously captured antigens as MHC II-associated peptides (to CD4⁺ T cells) and endogenous antigens as MHC I-associated peptides (to CD8⁺ T cells) [3,4]. However, DCs can accomplish both conventional presentation and cross-presentation (i.e., presenting exogenously captured antigens as MHC I-associated peptides), thereby eliciting more efficient CD4⁺ and CD8⁺ T-cell activity [3,4]. Productive activation of specific T cells not only requires a cognate antigen, called signal 1, but also costimulatory cues from ligands present on the APC surface (e.g., CD80, CD86, CD40; [Figure 1](#)) as well as proinflammatory cytokines, which constitute signal 2 and 3, respectively [5,6]. The full maturation of DCs is mandatory for the expression of molecules involved in signals 2–3 [7]. Signals 1–2–3 together ensure a **Type 1 immune polarization** of CD4⁺ T cells and efficient cytotoxic responses by CD8⁺ T cells [also termed as cytolytic T lymphocytes (CTLs)] [7]. By contrast, signal 1 delivered in absence of signal 2, and either absence of proinflammatory cytokines (signal 3) or presence of immunosuppressive cytokines, either elicits **type 2 immunity** (via **Th2 cells**) or immunosuppression (via **Tregs**) [3,8].

Clinically relevant tumors actively disrupt these signals in the microenvironment (e.g., by directly inducing DC dysfunction, loss of tumor antigens, and eliciting higher production of immunosuppressive cytokines [9]), thereby limiting productive immune responses [10]. These hurdles provided a rationale to produce vaccines based on patient-derived (autologous) DCs armed *ex vivo* with signals 1–2–3 to facilitate *in situ* antitumor T-cell immunity [7]. Of note, DC vaccines

Trends

The field of cancer immunotherapy is experiencing a renaissance spearheaded by ICIs and ACTs.

Applicability of ICIs/ACTs is restricted to only a subset of patients, due to limiting factors such as low tumoral T-cell infiltrates, low neoantigens burden, immunoresistant phenotypes, and autoimmune toxicities.

These limitations have spurred interest in ‘upgrading’ existing immunotherapies such as DC vaccines to meet current challenges.

Promising clinical ORRs and safety profile of current (second-generation) DC vaccines have raised interest in producing next-generation DC vaccines.

Next-generation DC vaccines currently aim to exploit specific DC subsets, naturally occurring DCs, cancer neoantigens, and/or ICD to improve CD8⁺ T cells-driven anticancer immunity.

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might be better than other anticancer vaccination approaches like whole tumor cell vaccines because they integrate several immunologically relevant signals required for the efficient induction of antigen-directed T-cell responses on a single cellular platform. The basic principle behind DC vaccines entails the isolation of autologous DCs from a patient followed by their *in vitro* 'loading' with appropriate source of tumor antigens (i.e., signal 1) and subsequent activation by defined 'maturation cocktails' (required to generate signals 2–3) [1,11,12]. These DC vaccines are then injected back into the patient at an appropriate site to facilitate their homing toward the nearest lymph node for priming the T cells with the antigens they engulfed *in vitro*, in presence of proper costimulation [13,14]. This priming facilitates T cell-driven antitumor immunity (full explanation of the DC vaccines concept is provided in Figure 1). Various molecular and immunological determinants, on the levels of cancer cells, DCs, or the patient/host/tumor (Figure 2, Key Figure), together regulate the efficacy of DC vaccines, for example, the nature and source of tumor antigens, the composition of DC maturation cocktails, the impact of tumor

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Figure 1. Principle behind Dendritic Cell (DC)-Based Cancer Immunotherapy. Autologous CD14⁺ monocytes derived from peripheral blood of cancer patients (through leukapheresis) are differentiated with GM-CSF and IL-4/IL-13 into immature DCs *in vitro* [27]. Thereafter immature DCs are supplied with tumor antigens, which can be derived from various sources [7], that is, whole autologous/allogeneic cancer cell lysates (induced to undergo cell death; require further antigen processing), recombinant antigenic peptides [derived from tumor-associated antigens (TAAs) or neoantigens; do not require antigen processing], RNA/DNA derived from cancer cells, recombinant RNA/DNA encoding defined tumor antigens, and DCs/tumor–fusion hybrids (provide stable antigen presentation). These antigen-loaded DCs are further matured/stimulated through 'maturation cocktails' [27] consisting of specific cytokines (predominantly including TNF/IL1β/IL6/PGE₂; although PGE₂ represents a double-edged sword since it not only induces DCs' competency for lymph nodes homing but also facilitates immunosuppressive Treg cells via IL12p70 suppression/IDO stimulation) [27]. PGE₂-related problems may be overcome through Toll-like receptor (TLR) agonists although these can induce IDO [27]. These factors facilitate DCs-based MHC I/MHC II-driven antigen crosspresentation, presence of appropriate costimulation (CD80/CD86/CD83/CD40), and upregulation of lymph node homing (via CCR7). Thereafter these fully mature immunogenic DCs are infused back into the patients where they migrate to the closest lymph node(s) and present the cancer antigens to CD4⁺/CD8⁺ T cells in presence of costimulation and T-cell stimulatory cytokines facilitating antitumor T-cell polarization, for example, Type 1/17 polarization (Th1/Th17) and cytotoxic T lymphocyte (CTL) activity. CpG, 5'-C-phosphate-G-3'; F/T, freeze/thawing; FLT3L, FMS-like tyrosine kinase 3 ligand; GM-CSF, granulocyte monocyte–colony stimulating factor; IL, interleukin; LPS, lipopolysaccharides; MHC, major histocompatibility complex; PGE₂, prostaglandin E₂; Poly (I:C), polyinosinic:polycytidylic acid; TNF, tumor necrosis factor.

Key Figure

Schematic Summarization of Major Molecular and Immunological Hallmarks for Dendritic Cells (DCs)-Based Anticancer Immunotherapy

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Figure 2. This plot depicts the 13 molecular and immunological hallmarks or prerequisites that are most crucial for the efficacy of anticancer vaccination strategies (in various contexts) on the levels of cancer cells, DCs, and the host or tumor (color coded). Utilization of some prerequisites depends on context, for example, overall mutational load (surrogate for neoantigen load), loading of 'pure' TAAs (i.e., recombinant peptides), blockade of immune checkpoints, and usage of specific DC subsets may apply to some, but not all, cancer types. Of note, cancer cell (*ex vivo/in vitro*) and host/tumor-level (*in vivo/in situ*) determinants are applicable to all anticancer vaccines in general, whereas DCs-level determinants have dichotomous applicability, that is, *ex vivo* context for DC vaccines and *in situ* context for whole cancer cell vaccines. CTL, cytolytic T lymphocyte; DAMP, damage-associated molecular pattern; ICD, immunogenic cell death; PAMP, pathogen-associated molecular pattern; TAA, tumor-associated antigen; TLR, Toll-like receptor; TSA, tumor-specific antigen.

burden or the tumor immune microenvironment, the route/dose/scheduling of DC vaccination, and the overall immunological fitness of the patient. These determinants are properly introduced and described briefly in Table 1. Recently, the immunological knowledge gained about DCs, tumor antigens, and tumor cell DCs crosstalk has spawned new directions for creating 'next-generation' DC vaccines. In this review, we discuss the major features of the existing first- and second-generation DC vaccines. We also outline the future trends for next-generation DC vaccines and give particular attention to how these can be integrated into the current cancer immunotherapy landscape.

Glossary

Adoptive T-cell therapy (ACT):

consists of chimeric antigen receptor (CAR)-modified T cells (CAR-T cells), T-cell receptor-modified T cells, or TILs. ACT involves deriving T cells either from peripheral source (CAR-T cells) or within tumor (TILs), expanding them *ex vivo*, activating them and/or genetically modifying them to increase recognition of particular (or multiple) tumor antigens, and then infusing them back into the patients.

Dendritic cell (DC) subsets:

in humans, two main DC types exist, namely, myeloid DCs (mDCs; also called classical/conventional DCs) and plasmacytoid DCs (marked by BDCA2/4 and CD123). Here, human mDCs are further subdivided into BDCA1/CD1c⁺ DCs, BDCA3/CD141⁺ DCs, and CD16⁺ DCs. In mice, mDCs are subdivided as cDC1 (CD8α⁺/CD103⁺ conventional DCs; related to human BDCA3/CD141⁺ DCs) and cDC2 (CD11b⁺ conventional DCs; related to human BDCA1/CD1c⁺ DCs; Figure 3C) [85]. Monocyte-derived DCs may also represent a distinct subset. Murine/human DC subsets differ on several levels including MHC I/MHC II antigen presentation capacities and TLRs diversity [11].

Immune checkpoints:

immune checkpoints are essentially 'immunological brakes' that tumor cells exploit to escape from antigen-directed T cells [45,86]. Normally, immune checkpoints facilitate resolution of inflammation by exerting immunosuppressive effects and avoiding autoimmunity.

Immune checkpoints inhibitors (ICIs):

ICIs are mostly antibodies (but also small molecules) that target immune checkpoints to 'release the brakes' (like CTLA4/PD1/PD-L1) that form barrier to antitumor immunity [86]. Some emerging ICIs may target IDO1/TIM3/CD39/CD73/GITR/VISTA.

Immunogenic cell death (ICD):

cancer cells undergoing ICD liberate specific damage-associated molecular patterns (DAMPs) acting as danger signals and orchestrate immunostimulatory processes [like Type I interferon (IFN) response or CCL2/CXCL1/CXCL10 chemokines-based pathogen defense response-like 'altered-self' mimicry; Figure 3D] [43–45]. DAMPs liberated by ICD include surface calreticulin, surface-

Dendritic Cell Vaccines in the Current Cancer Immunotherapy Landscape

The field of cancer immunotherapy is currently experiencing a renaissance spearheaded by the success of **immune checkpoint inhibitors (ICIs)** against selected solid tumors and Hodgkin's lymphoma, as well as by recent development in **adoptive T-cell therapy (ACT)** [15]. Besides establishing the clinical applicability of cancer immunotherapy, ICIs/ACTs have introduced revisions in how oncologists assess patient responses to immunotherapy [16,17]. For instance, conventional clinical response criteria [e.g., Response Evaluation Criteria in Solid Tumors (RECIST)] have been reported to underestimate the therapeutic benefit of ICIs in ~15% of patients [16]. In fact, immune-related response criteria (irRC) demonstrated that positive patient responses are achieved by ICIs despite an initial RECIST-based negative outlook hallmarked by tumor 'pseudoprogression' [16]. However, it is also emerging that not all cancer patients respond to ICIs (applicable to at least ~50% patients [17,18]) due to immunoresistance mechanisms including, but not limited to, extremely low tumor-infiltrating lymphocytes (TILs; applicable to 10–70% patients depending on cancer type [18]), deregulation of **immune checkpoints**, loss of tumor antigens, monoclonality of response, mutations in interferon (IFN) signaling axes, or loss of sensitivity to IFN (see [Box 1](#) for full details) [13,17,19–22]. Besides, severe-to-fatal side effects due to uncontrolled autoimmune responses remain a problem for ICIs/ACTs [23]. This has amplified the need to assess alternative or additional immunotherapy options that can be therapeutically beneficial for the subset of patients not responding to ICIs/ACTs.

These trends have spurred interest in 'upgrading' existing immunotherapies that have previously failed to meet clinical expectations but still have the potential to confront various immuno-oncology challenges, and that may be used in combination with ICIs. On the top of this list is DC vaccines. In the past, remarkable efforts have been invested in developing DC vaccines with relatively modest clinical returns. **Objective response rates (ORRs)** to DC vaccines in cancer patients have rarely exceeded ~15%; however, it is worth noting that these estimates are based on the RECIST/WHO criteria [12]. Ironically, most DC vaccination clinical trials assessed RECIST/WHO-defined ORRs rather than overall survival (OS) or irRC-defined ORRs [12]. In assorted trials where OS was assessed, DC vaccines could shift OS by ~20% – the threshold for clinically meaningful improvement [12]. Whether the lack of irRC-like assessment is behind the underestimation of DC vaccines' efficacy is unclear. Nevertheless, while the present generation of DC vaccines is successful in increasing frequency/activity of TILs (in responding as well as few nonresponding patients), they appear to fail to sustain their accumulation and effector function in nonresponding patients [24,25]. In the subsequent sections, we discuss the different features of past, present, and future generations of anticancer DC vaccines.

The Past, Present, and Future Generations of DC Vaccines: Experimental and Clinical Outlook

DC vaccines have been applied against various malignancies (>200 clinical trials [26]), with the four most targeted cancer types being melanoma (>1000 patients), prostate cancer (>750 patients), glioblastoma (GBM; >500 patients), and renal cell carcinoma (RCC; >250 patients) [7,12]. [Figure 3A](#) summarizes the most important historical landmarks in DC vaccine development. Accordingly, DC vaccines can be subdivided into three distinct 'generations' ([Figure 3B](#)). First- and second-generation DC vaccines will only be briefly discussed here. Instead, we will address in more detail the emergence of next-generation DC vaccines and the outstanding future challenges of this field.

First- and Second-Generation DC Vaccines

First-generation DC vaccines ([Figure 3B](#)) consisted of either patient-isolated natural DCs or ex vivo-generated monocyte-derived DCs (mo-DCs) that were not matured further [12,26,27], a

exposed/released heat shock proteins, secreted ATP, released HMGB1, and released nucleic acids [43–45]. These molecules together facilitate DC-based (immunogenic) phagocytosis of cell corpses and DC maturation [51]. These fully mature DCs, thereafter activate CD4⁺ T cells-driven CTLs/CD8⁺ T-cell responses against residual disease ([Figure 3D](#)) [44,45]. ICD inducers are subdivided based on the nature of endoplasmic reticulum (ER) stress-driven danger signaling, that is, Type I ICD inducers ('off-target' ER stress, e.g., anthracyclines, radiotherapy) and Type II ICD inducers ('on-target' ER stress, e.g., hypericin-based photodynamic therapy) [45].

Objective response rates: defined as the number/percentage of patients with a predefined quantitative reduction in tumor size for a minimum period. Response duration is typically measured from the time of initial response to palpable tumor progression.

Tregs: regulatory T cells makeup ~10% of CD4⁺ T cells; they play a crucial role in resolution of inflammation and immune homeostasis by negatively regulating autoreactive T-cell activity.

Type 1 immune polarization: this refers to a proinflammatory polarization characterized by phenotypic maturation/proliferation accompanied by production of immunostimulatory cytokines like IL12/IL6/IL1β/IFNα/IFNβ/IFNγ/tumor necrosis factor (e.g., Th1 cells, M1 macrophages).

Type 2 immune polarization: this refers to an anti-inflammatory polarization characterized by maintenance of immaturity or regulatory ligands accompanied by production of immunosuppressive cytokines like IL10/tumor growth factor-β/prostaglandin E₂ (e.g., Th2 cells, M2 macrophages).

Table 1. A Systematic Overview of the Main Molecular and Immunological Determinants of Anticancer DC Vaccines^a

Determinants	Features	Relevance or strategy for integration	Current challenges and future trends	Refs
Tumor antigens	Loading of tumor antigens on immature DCs is the first step toward DC vaccine production; Such antigens may include <ul style="list-style-type: none"> • Tumor-specific antigens (encoded by mutated genes/neoantigens, viral genes, or cancer germ-line genes) • TAAs (encoded by tissue-specific genes or by genes that are overexpressed in tumor cells as compared with normal cells) 	Antigens are loaded on DCs as synthetic antigenic peptides, antigen-encoding RNA/DNA, autologous or allogeneic whole tumor cell lysates, ^b tumor-derived whole RNA/DNA, or tumor-DCs hybrids <ul style="list-style-type: none"> • Antigenic peptides/defined RNA or DNA confer defined antigenicities (useful for cancer with known dominant antigens) • Whole tumor cell lysates/RNA/DNA exploit the entire collection of tumor antigens (useful for cancer types with antigenic heterogeneity/unknown antigens) 	Heterogeneity of antigen expression in the tumor and the stimulation of T cells with low avidity are important roadblocks for DC vaccines; pulsing DCs with neoantigens (i.e., mutant antigens resulting from somatic mutations with less susceptibility to central/peripheral tolerance mechanisms), if they are known, may correct the latter problem	[7,24,89–92]
DC maturation cocktail	DCs are activated through defined maturation cocktails, e.g., combination of proinflammatory cytokines (classically TNF + IL1 β + IL6 + PGE ₂) and TLR agonists; various combinations of cytokines/TLR agonists are utilized by different DC vaccine trials	More advanced strategies of DC maturation include (but are not limited to) <ul style="list-style-type: none"> • The α-type-1-DC-polarizing cocktail, TNF + IL1β + IFNγ + IFNα + poly(I:C) • TriMix-DCs electroporated with mRNA encoding constitutively active TLR4, CD40L, and CD70 	<ul style="list-style-type: none"> • Certain cytokines/TLR agonists (e.g., IL2, IFNα/γ, GM-CSF, bacterial toxoids), costimulatory ligand agonists (e.g., anti-4-1BB antibody), or oncolytic viruses can be coadministered with DC vaccines 	[7,12,26,27,33,48,93]
Tumor burden and immune microenvironment	<ul style="list-style-type: none"> • Tumor-induced immunosuppression is a direct function of tumor burden • Immunosuppressive TILs or tumor microenvironment predicts poor survival of DC-vaccinated patients 	<ul style="list-style-type: none"> • Tumor-burden reduction via surgery and radiotherapies/chemotherapies/targeted therapies creates favorable conditions for DC vaccines • DC vaccines exhibit higher clinical efficacy against minimum residual disease (i.e., adjuvant settings) rather than primary tumor/metastasis (i.e., primary setting) 	<ul style="list-style-type: none"> • Loss/decrease in MHC I expression or its components like β_2-microglobulin poses a major challenge • Systemic depletion or Type 1 immune polarization of TAMs, MDSCs, and Tregs is desirable 	[1,7,10,12,17,25,27]
Route of DC vaccination	Route of administration regulates DCs' access to lymph nodes	<ul style="list-style-type: none"> • Mostly administered intradermally near superficial lymph nodes • Ultrasound-guided intranodal administration • Intravenous administration 	<ul style="list-style-type: none"> • Intradermal injection allows only ~5% DCs to reach the lymph nodes, whereas intravenous injection routes them to nonpreferred locales, e.g., lungs, liver, spleen, bone marrow • Other routes like intranasal administration against mucosal tumors are also being tested 	[27,94]
Dosage of DC vaccines	DC dose positively associates with favorable prognosis	Minimal effective DC dose per vaccine remains unclear ^c (0.3–3 million DCs per vaccine is apparently sufficient)	Further research is required to delineate the minimal effective DC dose per vaccine	[7,45,54,66,86,95–97]
Scheduling of DC vaccines	Vaccines should be ideally administered in a repetitive manner to achieve maximal long-term efficacy	Administration should be as early as possible following tumor regression and before relapse/recurrence (or in concurrence with minimal metastatic disease)	Tregs-driven immunosuppression associated with multiple vaccinations is a potent challenge	[7,45,54,66,86,95–97]
Immunological fitness of patients	Immunological fitness is a classical determinant of vaccine efficacy; most markers for this are unclear but few are emerging, e.g., gut microbiome and genetic polymorphisms of immune receptors (e.g., TLRs);	<ul style="list-style-type: none"> • Patients with immunodeficiencies (natural or induced by steroidal/lymphoablative chemotherapy treatments) often fail to respond to DC vaccination • High incidence of MDSCs/Tregs predicts poor survival in DC-vaccinated patients 	Antibiotics, cyclophosphamide, or TLR agonists (amongst others) can modulate overall immunological fitness	[14,24,98]

^aAbbreviations: CD, cluster of differentiation; GM-CSF, granulocyte-macrophage colony stimulating factor; IL, interleukin; MDSC, myeloid-derived suppressor cells; PGE, prostaglandin; TAM, tumor-associated macrophages; TNF, tumor necrosis factor.

^bAutologous lysates are usually preferred over allogeneic lysates due to their higher immunological efficacy [18,24,91].

^cInterestingly, a computational model proposed, ~85 antigen-presenting DCs to be sufficient for eliciting nodal T cell responses [95].

Box 1. Major Molecular Resistance Mechanisms against Immune Checkpoint Therapy

Deregulation of tumor susceptibility to immune checkpoints: This acquired resistance mechanism occurring in response to treatment with a particular ICI entails most commonly the upregulation of alternative immune checkpoints, thus rendering the tumor resistant to that ICI; for example, resistance to anti-PD1 therapy may occur in lung cancer due to upregulation of an alternative immune checkpoint, T-cell immunoglobulin mucin-3 (TIM-3) [19].

Loss of tumor antigens: A tumor microenvironment can select for cancer cells that have, through epigenetic or post-translational means, downregulated the expression of major tumor antigens recognized by T cells, thereby paving way for a 'cold' tumor phenotype on TILs level.

Loss of sensitivity to interferons (IFNs): Chronic exposure of a tumor to IFNs (both Type I and Type II IFNs) can cause upregulation of ligands for multiple T-cell inhibitory receptors, thereby giving rise to immune resistance rather than immune-susceptible tumor phenotype [13].

Low TILs: Based on the density of TILs, tumors can be classified as 'hot' (i.e., consisting of immunogenic immune microenvironment marked by high TILs), 'cold' (i.e., consisting of nonimmunogenic immune microenvironment marked by low TILs), or somewhere in between [20]. The density of TILs reflects the degree to which the immune system is recognizing and reacting against a tumor. Thus, cancer patients with 'cold' tumors/low TILs are likely to respond poorly to immunotherapy [20]. Beyond direct immunosuppression, TILs may also be excluded from a tumor due to stromal or vessel dysregulation.

Monoclonality of immune response: An effective anticancer immune response should be normally driven by various populations of T-cell clones with affinity toward different types of cancer antigens [21]. Monoclonality or predominance of T-cell clones directed toward one or few antigens can eventually limit the efficacy of immunotherapy if a tumor is successful in losing the expression of these antigens or restricting immune access to them [17].

Mutations in IFN signaling axes: Genomic defects in IFN- γ pathway-associated genes have been found in patients not responding to ICIs, suggesting that tumors with loss of IFN- γ signaling are resistant to immunotherapy [22].

major reason behind their failure [26]. These DCs were loaded with either recombinant/synthetic antigenic peptides [tumor-associated antigens (TAAs) derived, Table 1] or with tumor cell lysates prepared through accidental necrosis [via freezing and thawing (F/T) or mechanical disruption] [26]. Despite their limited success against RCC, melanoma, and non-Hodgkin's lymphoma (tumor regression rate of only 3.3% in patients [26,28,29]), first-generation DC vaccines established the safety and feasibility of DC vaccines [12,26,27].

Second-generation DC vaccines (Figure 3B) [7,27] used mo-DCs that were fully matured via maturation cocktails (Figure 1, Table 1); and tumor antigens that consisted of recombinant/synthetic antigenic peptides and tumor cell lysates prepared via physical or mechanical disruption (e.g., necrosis) alone or followed by additional treatments (e.g., with irradiation like UV, X rays, or γ rays and/or heat shock) ensuring 100% cancer cell death, that is, avitalization and eliciting some immunogenic (side)effects [30]. Second-generation DC vaccines have mostly (but not always) employed antigenic peptides derived from tumor antigens such as melanoma-differentiation antigens (e.g., MAGE antigens, MLANA, gp100), Wilms tumor 1 (WT1), and NY-ESO-1 [31,32]. Besides this, other means of antigen loading have also been implemented, for example, RNA/DNA transfection and cancer cell-DCs (hybrid) fusions (Table 1) [7,33]. Of note, evidence (across 173 clinical trials) indicates that 1711 patients treated with tumor cell lysates-based vaccines exhibit higher ORRs (8.1%) than 1733 patients who received antigenic peptides-based vaccines (ORR, 3.6%) – an observation providing some consensus on suitable tumor antigen source [18,24,34]. Second-generation DC vaccines have performed better in the clinic than first generation, by achieving ORRs in the 8–15% range, depending on the cancer type [12]. In addition, >50% cancer patients treated with second-generation DC vaccines experienced antigen-specific CTL activity or positive natural killer (NK) cell responses [35,36]. Furthermore, second-generation DC vaccines have increased median OS by ~20% in many (but not all) studies [12].

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Figure 3. A Timeline of Major Historical Events in Dendritic Cell (DC) Vaccine's Development, and Their Classification into Different Generations.

(A) DC vaccines' development commences from their discovery in 1973 when Zanvil Cohn and Ralph Steinman described them as rare murine splenocytes with

(Figure legend continued on the bottom of the next page.)

Of note, Sipuleucel-T, also known as Provenge, which is the only cellular vaccination product currently approved by US FDA for use in (prostate) cancer patients, can be arbitrarily positioned at the intersection between the first- and second-generation DC vaccines. This is because it did not employ a defined DC ‘maturation cocktail’ (as is typical for second-generation DC vaccines, [Table 1](#)) but rather relied on activation achieved by a fusion protein (PA2024) consisting of prostate antigen, prostatic acid phosphatase, fused to granulocyte–macrophage colony stimulating factor [\[37\]](#). Moreover, Sipuleucel-T consisted of autologous peripheral blood mononuclear cells, which included but were not limited to DCs. Sipuleucel-T has been able to prolong the OS of prostate cancer patients in some clinical trials, but it has largely failed to affect the time to disease progression [\[37\]](#).

Next-Generation DC Vaccines

Clinical improvements from first- to second-generation DC vaccines spurred interest in advancing toward next-generation DC vaccines ([Figure 3B](#)). A major effort focuses on the nature and source of DCs. For instance, the use of specific subsets of naturally occurring DCs is being employed since they confer two major advantages over mo-DCs (i.e., better functionality and reduction in culturing time and costs) [\[7\]](#). Utilization of patient-derived DC subsets has been made possible by some current technologies (e.g., antibody-coated magnetic beads) that allow rapid and enumerated isolation of natural DCs (>10 million DCs) [\[38,39\]](#). Of note, the superior functionality of this next-generation DC vaccine approach originates from the use of more defined DC subsets specialized in MHC-I/II-based antigen presentation and eliciting CTL responses ([Figure 3C](#)) [\[28\]](#). For instance, plasmacytoid DCs are better at Type I IFN responses, whereas myeloid DCs (mDCs) are better in uptake of dead/dying cells and antigen presentation [\[40,41\]](#). Moreover, the use of BDCA1/CD1c⁺ mDCs (equivalent to murine cDC2) and BDCA3/CD141⁺ mDCs (equivalent to murine cDC1), two DC subsets displaying high MHC-based antigen presentation capacity [\[11\]](#), would allow highly potent CTLs expansion and CD103⁺CD8⁺ memory T cells’ generation, respectively [\[26\]](#) ([Figure 3C](#)).

Initial clinical trials show that next-generation DC vaccines using naturally occurring DC subsets are not only safe and feasible but may also exhibit promising clinical efficacy [\[38,39,42\]](#). For instance, a clinical study employing naturally occurring, autologous, plasmacytoid DCs loaded with TAA peptides found that several melanoma patients administered with this vaccine experienced antigen-specific CD4⁺/CD8⁺ T-cell responses and exhibited a measurable IFN signature [\[38\]](#). Similarly, another clinical study from the same investigators found that DC vaccines based on naturally occurring, autologous, CD1c⁺ mDCs induced long-term progression-free survival (12–35 months) in 4/14 melanoma patients [\[39\]](#). One crucial point that is currently unclear from these clinical studies pertains to the superiority of a particular DC subset over the others. This is decisive because, for instance, a recent study showed that murine cDC1 and cDC2 differentially activate CD8⁺ T cells and Th17 cells, respectively [\[85\]](#). On the level of vaccination, cDC1- and cDC2-based vaccines elicited antitumor CTLs and reduction in macrophages/myeloid cells–based immunosuppression within the tumor, respectively [\[85\]](#). In a nutshell, different DC subsets across human and mouse species differ in terms of their MHC-I/II-based antigen presentation capabilities ([Figure 3C](#)). Thus, it

distinctive treelike morphology (dendritic derived from the Greek word for tree, i.e., *dendreon*) [\[27\]](#). Several discoveries outlining the highly specialized role of DCs as antigen-presenting cells coupled with the discovery of human cancer antigens paved way for exploiting DCs for cancer immunotherapy [\[7,87,88\]](#). The first clinical trial with DC vaccines was published in 1996 followed by initiation of numerous clinical studies across many different cancer types [\[88\]](#), including Phase III clinical trials in renal cell carcinoma/prostate cancer/glioblastoma/melanoma [\[27,89\]](#). These studies outlined the safety, efficacy, and immunological or T-cell responses associated with DC vaccines [\[7,89\]](#). The field of DC vaccines received a major boost in 2010, when Sipuleucel-T (Provenge from Dendron Corporation) became the first cellular immunotherapy approved by US FDA [\[27\]](#). Currently, next-generation DC vaccines are being created with some in early clinical phase. (B–D) Considering the historical development phases of DC vaccines, they can be convincingly divided into first-, second-, and next-generation DC vaccines (B) [currently being explored on the lines of different DCs subsets [\[11\]](#) (C) and immunogenic cell death (ICD; D) in cancer cells] based on developments on cancer cells/DCs levels. CRT, calreticulin; F/T, freezing and thawing; HMGB1, high-mobility group box-1; HSP, heat shock proteins; IFN, interferon; P2, purinergic-2; TAA, tumor-associated antigen; TLR, toll-like receptors.

would be necessary, in near future, to initiate a multiarm clinical trial in cancer patients that compares the efficacy of DC vaccines based on different DC subsets to irrevocably establish the DC subset with highest capacity of eliciting TAA-specific T-cell responses associated with prolonged patient survival.

It is clear that implementing the increased knowledge of DC biology has largely dominated and guided the transitions between different generations of DC vaccines, whereas poor attention has been paid to the mechanisms of cancer cell death. By contrast, in recent years, the immunogenic potential of dead/dying cells has been emerging as a crucial definer of the anticancer vaccination effect [43–45]. In addition, in last decades, the dogmatic view of necrosis being immunogenic and apoptosis being immunosuppressive has changed [43,44]. In fact, accidental (i.e., genetically uncontrolled) necrosis can be poorly immunogenic [46]. By contrast, some instances of regulated cell death, being necroptosis or apoptosis, can be immunogenic [47,48].

In particular, the molecular mechanisms underlying the induction of an immunogenic modality of apoptosis, that is, **immunogenic cell death (ICD; Figure 3D)** [43–45] have been characterized in depth, recently. Cancer cells undergoing ICD exhibit high immunogenic potential owing to ordered exposure/release of a diversity of danger signals. These include – but are not limited to – surface-exposed ‘eat me’ signals that engage phagocytic receptors, ‘find me’ signals that facilitate recruitment of immune cells and other factors, which act as innate immune stimulators (Figure 3D for more details) [44,45]. ICD-based anticancer vaccines are superior to F/T or mechanical necrosis-based vaccines [46,49]. In fact, we recently demonstrated the high, danger signals-dependent efficacy of ICD-based next-generation DC vaccines against GBM [46]. ICD-based DC vaccine synergized with the standard-of-care chemotherapy to cause a Treg-to-Th1/Th17/CTLs shift in brain immune microenvironment, leading to CTLs-driven anti-GBM immunity [46]. Interestingly, the ICD-derived Toll-like receptor (TLR) agonist HMGB1, along with other danger signals, was crucial for DC vaccine’s efficacy despite the presence of a TLR agonist in the DC maturation cocktail [46]. This highlights the vital role of endogenous danger signals for efficient DC-driven immunological responses.

Importantly, a clinical study showed that ICD-based DC vaccines exhibited clinical/immunological responses in 6/18 indolent B-cell lymphoma patients such that cancer cells of responders underwent better ICD (i.e., exposed/released higher damage-associated molecular patterns) than nonresponders [50]. Of note, ICD is prone to contextual failures depending on factors such as ICD-inducer types (**Type I vs. Type II ICD**), cancer types, or mechanisms compromising danger/immunogenic signaling (e.g., cancer cell-intrinsic low expression or defects in trafficking/exposure of danger signals, dysfunction of immunogenic phagocytosis, defects in danger signal sensing on DC level, such as loss-of-function polymorphism in *TLR4*) [14,51]. Hence, proper preselection of ICD inducers tailored to specific cancer types, and if possible the patient’s genetic background, is a crucial parameter for hitting the right ‘susceptibility zone’. Moreover, chemical ICD inducers (like anthracyclines) are not desirable for production of DC vaccines compared with physical ICD inducers (hypericin-photodynamic therapy, high hydrostatic pressure) because they either leave residual (active) drug concentrations behind or may exert cytotoxicity against DCs.

Apart from the aforementioned major strategies, the emerging relevance of neoantigens (i.e., tumor-specific antigens largely derived from nonsynonymous single-nucleotide variations) in cancer immunotherapy has spurred interest in producing neoantigens-based next-generation DC vaccines [52,53]. In fact, a recent study found that neoantigen-loaded DCs generate potent MHC class I-restricted neoantigen-specific T cells (even *de novo*) in three melanoma patients,

although survival advantage was not clear [54]. Interestingly, some recent studies, utilizing antigen-based vaccination approach in absence of the autologous DCs, suggested that neoantigens-based vaccines may work in melanoma patients [55]. Nevertheless, it is not clear whether neoantigens are superior to TAAs/tumor cell lysates. Potential drawbacks of this strategy include high frequency of neoantigens being restricted to few cancer types [7] and patient-to-patient neoantigen heterogeneity, making broad vaccine production difficult. Moreover, there is some concern that neoantigen-specific T cells generated against a mutated tumor protein via these approaches may also crossreact with the nonmutated version of that protein, thereby eliciting autoimmunity [56]. Nevertheless, more clinical insights are required to understand the potential efficacy of neoantigens-based DC vaccines.

Last but not least, although not encompassing DC vaccines in *stricto sensu*, but rather the more general ‘anticancer vaccines’ category, it is worth noting that advances have also been made to activate DCs at the systemic level. Recently, intravenously administered RNA lipoplexes encoding neoantigens or TAAs have been shown to be efficiently captured *in situ* by DCs, thereby driving their maturation and consequent antigen-specific T-cell responses, causing efficient tumor rejection [57].

Next-Generation DC Vaccines: Best Combinatorial Partner for Immune Checkpoint Inhibitors?

ICIs have autonomously achieved high clinical ORRs in cancer patients (see Table S1 in the Supplemental Information online [58–74]), making it imperative to delineate the positioning of next-generation DC vaccines within the future oncological paradigms.

Neoantigen-directed T cells are particularly active in tumors regressing following ICI treatment [45,53]. Neoantigen generation is a highly stochastic process with low occurrence of immunologically relevant neoantigens (so-called neoantigen lottery) [75]. However, higher genomic mutational burden results in higher neoantigen burden, which increases the probability of creating immunogenic neoantigens [53]. This caused the emergence of overall genomic mutational burden as a (surrogate) predictive biomarker of positive ICI responsiveness [53]. In fact, solid tumors with higher mutational load, such as melanoma or lung cancer, or overrepresentation of mutational lesions (e.g., microsatellite instability) have higher TILs [76] and are relatively superior responders to ICI treatment [17,53,75]. For example, if we compare the clinical ORRs achieved by ICI treatment against various tumor types (presented in Table S1 [58–74]) with respective tumoral mutational burdens [77], it is indeed visible that cancer types with higher mutational burden respond better to ICIs (Figure 4A) – a fact well-established in the literature [53,76].

However, neoantigen’s burden is also possibly a limiting factor since a subset-of-patients with low neoantigen’s burden may not respond to ICIs [20,75]. This creates an immunotherapy void that needs to be filled by finding an appropriate combinatorial counterpart for ICIs that can increase TILs. Based on the available data trends, we propose that next-generation DC vaccines can fill this void (Figure 4). For instance, recently a meta-analysis documented the average ORRs achieved by DC vaccines across all published clinical trials related to melanoma (8.5%), prostate cancer (7.1%), GBM (15.6%), and RCC (11.5%) [12]. If we compare these ORRs achieved by DC vaccines with respective tumoral mutational burdens, a discordant scenario emerges (Figure 4A). Here, on the one hand, the efficacy of DC vaccines seems to be similar for two cancer types with very different mutational burdens, that is, melanoma and prostate cancer (Figure 4A). Yet, on the other hand, two cancer types that respond most favorably to DC vaccines (i.e., GBM and RCC) also exhibit low tumoral mutational burdens (Figure 4A). Overall, this implies that, unlike ICIs, mutational burden may not be a reliable predictive biomarker for the efficacy of DC vaccines.

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Figure 4. The Status Quo for Dendritic Cell (DC) Vaccines in the Current, Immune Checkpoint Inhibitor (ICI)-Dominated, Cancer Immunotherapy Landscape. (A) A graphical representation of published objective response-rates (ORRs) clinically achieved by ICIs that have been averaged by pooling multiple studies for some cancer types (see Table S1) or average ORRs achieved by DC vaccines (derived from a published meta-analysis [12]) against indicated cancer types, versus published (representative) median somatic mutational burdens of corresponding The Cancer Genome Atlas tumor types depicted below the ORRs graphs (derived from [77]). (B) ORRs-based (arbitrary) comparison of efficacy of conventional chemotherapy/radiotherapy, targeted therapy, cytokine therapy, DC vaccines, and specific ICIs against melanoma, glioblastoma, and renal cell carcinoma (RCC). ACTs, adoptive T-cell therapy; CTLA4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cells; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; OV, ovarian cancer; PD1, programmed death-1; PDL1, programmed death-ligand 1; RCC, renal cell carcinoma; TCR, T-cell receptors.

Of note, to a certain extent, GBM could be exceptionally susceptible to DC vaccines as compared with some other cancer types (Figure 4A). In fact, we have recently shown in preclinical settings that while GBM is readily susceptible to next-generation DC vaccines [46], it fails to strongly respond to ICIs targeting CTLA4, PD1, or IDO1 [77]. Moreover, most clinical

predictive biomarkers of ICI responsiveness forecast ICI-resistant phenotype in adult human GBM tumors (as compared with ICI-responsive cancer types such as melanoma and lung cancer) [77].

Furthermore, if we compare the overall clinical ORRs landscape for melanoma, GBM, and RCC, an interesting picture emerges (Figure 4B). For decades, the ORRs achieved by chemotherapy (mainly dacarbazine) or radiotherapy/chemotherapy (mainly radiotherapy + temozolomide) regimens against melanoma and GBM, respectively, remained stagnant around or below 10% [12,78]. For melanoma, DC vaccines could not breach this 10% ORR ‘barrier’ [12], which was eventually achieved by ICIs (see Table S1) [67]. However, for GBM, the 10% ORR ‘barrier’ has already been breached by the current, second-generation DC vaccines (15.6% ORR; Figure 4B) [12], a breakthrough initially achieved by anti-CTLA4 therapy for melanoma [67,70]. Conversely the scenario is different for RCC. As opposed to melanoma and GBM, standard-of-care cytokine therapies alone (IFN α , interleukin-2), targeted therapies alone (sunitinib, pazopanib), or combinations thereof (bevacizumab + IFN) have achieved promising ORRs (10–30%) against RCC [79]. DC vaccines have performed fine against RCC (average ORR, 11.5%) when compared with cytokine therapies (ORR, 10–15%) but relatively poorly when compared with targeted therapies (ORR, 20–30%) [12,79]. By contrast, while ICIs have achieved very promising ORRs in RCC patients, these have been relatively less compared with melanoma (Figure 4B) [80].

These analyses indicate the importance of DC vaccines for at least some (but not all) tumors with low mutational burden. This also makes biological sense, since tumors with low mutational burden often have low TILs, a defect that DC vaccines typically aim to correct [7,46]. Overall, the aforementioned analyses also show that each cancer type may exhibit unique patterns of susceptibility to different kinds of immunotherapies. Consequently, a single immunotherapeutic agent may not be globally applicable across all cancer patients. Henceforth, the application of next-generation DC vaccines in combination with ICIs could be highly desirable for majority of patients of some cancer types (e.g., GBM) or a subset of patients of other cancer types (e.g., melanoma or RCC patients with low or negligible TILs).

Clinical Reality for Next-Generation DC Vaccines: Biomarker-Driven Combinatorial Adjuvant Treatment

The niche for next-generation DC vaccines involves cancer patients prone to recurrence despite chemotherapy/radiotherapy/targeted therapy or ICIs/ACT. Such patients will have to be delineated after tumor debulking/regression in the primary setting. In Figure 5, we outline a putative strategy for integrating DC vaccines into future cancer immunotherapy approaches. We believe that the choice as well as the success of immunotherapy (including DC vaccines) will be heavily driven by specific or broad predictive biomarkers that can reliably differentiate putative responders and nonresponders [17]. The choice of these biomarkers would be in turn guided by the ever expanding knowledge on immune-resistance mechanisms [17] uncovered in respective nonresponding patients (illustrative examples are indicated in Figure 5). Such predictive biomarkers could be detected on the level of peripheral blood (e.g., frequency of memory T cells, exosomes-specific markers) or tumor (e.g., immunoscore, TILs-related meta-gene/protein signatures, immune-checkpoint status) [81,82]. We elaborate the combinatorial cancer immunotherapy strategy integrating next-generation DC vaccines in the following section.

Once a clinically diagnosable tumor has formed, it mainly represents a mass of highly immunoevasive cancer cells that have undergone immunoediting and successfully escaped cancer immunosurveillance (Figure 5). In this primary setting, tumor regression, resetting of immunosuppressive microenvironment, and/or induction of antitumor immunity is achievable through a

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Figure 5. Integrating Next-Generation Dendritic Cell (DC) Vaccines into Highly Efficacious, Biomarkers-Driven, Combinatorial Regimens against Cancer. Neoplastic cells usually undergo the process of cancer immunosurveillance consisting of cancer immunoediting, that is, initial immune-mediated elimination of neoplastic (recently transformed) clones (this is the point when the 'first' peak of antitumor immunity is reached), followed by their equilibrium with immune-resistant clones, ultimately resulting in escape of these latter clones, while the immune-susceptible ones are progressively eliminated accompanied by a 'global' suppression of antitumor immunity. Immune-resistant cancer cells that have escaped from immunosurveillance form the clinically relevant tumors that are usually surgically resected upon clinical detection. Following surgery, the residual disease is usually treated with conventional therapies. Some patients may either receive conventional therapy in combinatorial regimens with ICIs/ACTs or receive ICIs/ACTs after failing to respond to the former (i.e., nonresponders). In cases where ICIs are first-line therapies, some patients do not receive conventional therapies first, hence they are labeled 'nonrecipients'. Application of ICIs/ACTs can be either biomarker driven or sometimes a standard of care. Patients who do not respond to these earlier regimens or receive conventional therapies but not ICIs/ACTs, and relapse (nonrecipients in that context), can be routed for combinatorial regimens involving next-generation DC vaccines (i.e., adjuvant settings) and other highly efficacious immunotherapies or targeted therapies whose rationale for combination can be biomarkers driven. Of note, specific biomarkers may define the presence of certain broad antitumor immunity-resistance mechanisms. Following the 'spikes' in antitumor immunity achieved by ICD/ICIs/ACTs, these DC vaccines-based combinatorial regimens, if administered repeatedly, can help maintain periodic increases in antitumor immunity, thereby prolonging patient survival. ACT, adoptive T-cell therapy; ICIs, immune checkpoint inhibitors.

first-line (standard-of-care) therapeutic regimen consisting of conventional therapies (chemotherapy/radiotherapy/targeted therapies), ICIs, ACT, or a combination thereof (Figure 5). Choice of conventional therapies can be guided by their ability to induce immunogenic (side-)effect like abscopal effect (e.g., fractionated radiotherapy) and/or ICD (e.g., anthracyclines) [45], although setting demanding multiple cycles of (systemic) chemotherapy can be detrimental. For instance, chemotherapy-induced cancer cell death may fail to induce T cell-driven immunity due to severe lymphoablation caused by multiple cycles of chemotherapy [46,83]. Properly executed conventional treatments will either result in patients with no residual disease (creating long-term survivors/responders) or minimum residual disease (that does not yet represent clinical recurrence) with amenability toward immunotherapy. Simultaneously, various biomarkers such as mutational burden, immune-checkpoint expression patterns, antigen landscape of the tumor, and degree of TILs (and their functional markers) can guide appropriate treatment combinations and/or sequence for primary immunotherapy. For example, deciding (i) between preference toward ICIs or ACT as first-line therapy (hence

nonrecipients for conventional therapy, Figure 5) or second-line therapy (i.e., nonresponders to conventional therapy, Figure 5); or (ii) whether ICIs/ACT ought to be administered in concurrent combination with conventional therapies. Biomarkers can also help decide whether to skip ICIs or ACT entirely (nonrecipients, Figure 5) owing to prediction of nonresponsiveness or severe autoimmunity.

Patients who either fail to respond to the aforementioned first-/second-line therapy options (nonresponders, Figure 5) or are deemed unsuitable for ICIs/ACT (i.e., nonrecipients, Figure 5), due to various resistance mechanisms including (but not limited to) low TILs, low immunogenic potential of cancer cells (in terms of danger signaling or MHC defects), or low antigenicity (Box 1), would be ideal for adjuvant application of next-generation DC vaccines (Figure 5). However, beyond these broad resistance mechanisms, there can also be specific resistance mechanisms that cannot be overcome by next-generation DC vaccines alone. Such a scenario would demand combining DC vaccines with other immunotherapies and/or targeted therapies wherein the exact combination to be pursued would be guided by specific predictive biomarkers (Figure 5). Such biomarkers would be determined by known immune-resistance mechanisms (preclinically and clinically validated; Figure 5). To this end, next-generation DC vaccines can be combined with emerging ICIs (in case of tumors overexpressing assorted, context-dependent, immune checkpoints), oncolytic viruses (e.g., owing to defects in Type I IFN signaling), ACT (in cases where ACT was not already given in primary setting, ACT can be especially vital for late-stage cancer patients who exhibit severe tumoral/systemic T cell dysfunction [84]), and/or emerging targeted therapies (Figure 5). Considering the fact that most antitumor T cells have low avidity T-cell receptors against various TAAs [45], we believe that the dosages of DC vaccines and other corresponding combinatorial therapies will have to be administered repeatedly to sustain long-term antitumor immunity (Figure 5).

Targeted therapies to be combined with DC vaccines can include (but may not be limited to) (i) therapies targeting oncogenic signaling proteins (e.g., BRAF^{V600E} inhibitors, anti-Her2 antibodies, tyrosine kinase inhibitors with some immunogenic properties such as sunitinib/imatinib) and (ii) therapies depleting specific immune cell types, for example, anti-CD25 antibodies against Tregs or cyclooxygenase-2/arginase inhibitors against myeloid-derived suppressor cells [7,27]. Interestingly, several clinical trials or studies are already exploiting some of the aforementioned combinations (although not exactly with next-generation DC vaccines), for example, combination of chemotherapy + DC vaccines with cyclooxygenase-2 inhibitors or ACT has shown promise in melanoma/lung cancer clinical trials [7]. Similarly, combination of DC vaccines with anti-CTLA4 ICI has been able to achieve 38% tumor response rate and 51% 6-month disease control rate [33]. Currently, there are a number of clinical studies (according to the clinical trials database, www.clinicaltrials.gov) that are studying the combination of DC vaccines with anti-PD1 ICIs or CAR-T cells/TILs-based ACT in cancer patients.

Concluding Remarks

Depending on the context (e.g., cancer type, antigen heterogeneity/immunogenicity, tumor immune microenvironment, general immunological fitness), DC vaccines require various molecular or immunological hallmarks as prerequisites to enforce efficient anticancer activity. The positive clinical response rates and safety profile of existing DC vaccines set up a solid foundation for next-generation DC vaccines to progress and address some of the challenges currently facing the immuno-oncology field. Next-generation DC vaccines can be integrated in an adjuvant combinatorial treatment setting at least for some cancer types such as GBM or RCC. While several challenges still need to be overcome (see Outstanding Questions), some lucid directions for creating next-generation DC vaccines are available. Based on current concepts, the most efficacious next-generation DC vaccines could include loading specific naturally occurring DC subsets with cancer cells undergoing ICD. Besides biological progress,

Outstanding Questions

Can next-generation DC vaccines overcome the clinical and immunological problems that plagued the second-generation DC vaccines? Will next-generation DC vaccines (applied in adjuvant combinatorial-settings) show clinical success in patients not responding to ICIs/ACTs?

Will usage of immune-related patient clinical response criteria improve the clinical outlook of DC vaccines?

Are neoantigen-based DC vaccines superior to TAAs/lysates-based DC vaccines?

In which format (whole cell lysate vs. RNA/DNA transfection vs. DC cancer cell fusion) should the tumor material be loaded in DCs?

Is it better to simply use whole cell lysates rather than multiantigenic peptides-loaded DC vaccines?

Which route of administration and dose schedule are desirable for DC vaccines?

Can induced pluripotent stem cell-derived DCs be explored as superior options for DC vaccine production?

What is the exact diversity and biology of T-cell responses generated by DC vaccines on the level of antigen specificity, T-cell receptors' avidity, and susceptibility to central tolerance mechanisms?

Some patients (1–50% depending on cancer type) are susceptible to ICIs/ACTs-induced severe or fatal (autoimmune) toxicities. Considering the impressive clinical safety profile of DC vaccines, will these high-risk patients respond to DC vaccines?

Do different human racial/ethnic groups (e.g., Caucasians vs. Indians vs. Africans) derive different benefits from DC vaccines?

Has the overuse of few murine models (especially with C57BL/6 background) for testing DC vaccines skewed the preclinical data?

Will humanized mice models or other animal models (e.g., Rhesus

technological innovations that ease regulation-compliant manufacturing hurdles are also necessary to increase affordability of DC vaccines for patients, especially in combination with already costly ICIs and given the current strains on health-care systems [17].

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Supplemental Information

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Macaques or canines) improve clinical translation of DC vaccines?

Can augmenting immunological fitness (e.g., on the level of gut microbiome composition or Type I/II/III IFN responses) of cancer patients help increase responsiveness to DC vaccines?

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