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REVIEW

Therapeutic cancer vaccines and combination immunotherapies involving vaccination

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Trang Nguyen[1]
Julie Urban[1]
Pawel Kalinski[1–5]

[1]Department of Surgery, [2]Department of Immunology, [3]Department of Microbiology and Infectious Disease, [4]Department of Bioengineering, University of Pittsburgh, [5]University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

Abstract: Recent US Food and Drug Administration approvals of Provenge® (sipuleucel-T) as the first cell-based cancer therapeutic factor and ipilimumab (Yervoy®/anticytotoxic T-lymphocyte antigen-4) as the first "checkpoint blocker" highlight recent advances in cancer immunotherapy. Positive results of the clinical trials evaluating additional checkpoint blocking agents (blockade of programmed death [PD]-1, and its ligands, PD-1 ligand 1 and 2) and of several types of cancer vaccines suggest that cancer immunotherapy may soon enter the center stage of comprehensive cancer care, supplementing surgery, radiation, and chemotherapy. This review discusses the current status of the clinical evaluation of different classes of therapeutic cancer vaccines and possible avenues for future development, focusing on enhancing the magnitude and quality of cancer-specific immunity by either the functional reprogramming of patients' endogenous dendritic cells or the use of ex vivo-manipulated dendritic cells as autologous cellular transplants. This review further discusses the available strategies aimed at promoting the entry of vaccination-induced T-cells into tumor tissues and prolonging their local antitumor activity. Finally, the recent improvements to the above three modalities for cancer immunotherapy (inducing tumor-specific T-cells, prolonging their persistence and functionality, and enhancing tumor homing of effector T-cells) and rationale for their combined application in order to achieve clinically effective anticancer responses are addressed.
Keywords: immunotherapy, cancer, vaccines

Introduction

Current comprehensive cancer care is centered on reducing the bulk of disease through surgery, chemotherapy, and radiation. Despite the increasing effectiveness of these cornerstones of treatment and high cure rates of multiple cancer forms, cancer remains a leading cause of death.[1] Recent breakthroughs in cancer immunotherapy have added several promising new therapies to the traditional armamentarium of oncology treatment regimens.

The strategy of utilizing the immune system in the treatment of cancer dates back to the 1890s and the work of William Coley.[2] Coley observed that some tumors regress in the setting of acute bacterial infection. He attempted to recapitulate this phenomenon by studying the injection of heat-inactivated *Streptococcus erysipelas* and *Serratia marcescens* (Coley's toxins) in cancer patients. The field of cancer immunology and immunotherapy has greatly advanced since Coley's initial studies, a time when little was known about the mechanisms underlying the antitumor effects of bacterial toxins. There is now a growing understanding of how the immune system identifies tumor cells and targets them for elimination. Just as important is the growing understanding

Correspondence: Pawel Kalinski
University of Pittsburgh and the University of Pittsburgh Cancer Institute, Hillman Cancer Center, UPCI Research Pavilion, Suite 1.46, 5117 Center Ave, Pittsburgh, PA 15213-1863, USA
Tel +1 412 623 7712
Fax +1 412 623 7709
Email kalinskip@upmc.edu

of how tumors can undermine the immune system's ability to recognize and eliminate cancer cells.

Briefly, an adaptive immune response against tumor cells is classically believed to be initiated when tissue-resident antigen-presenting cells, such as dendritic cells, take up and process tumor-specific or tumor-associated antigens, and present these antigens in the context of major histocompatibility complex (MHC) complexes to naïve T-cells in secondary lymphoid organs. Naïve T-cells can differentiate and expand into different classes of antigen-specific T-cells, including cluster of differentiation (CD)4$^+$ T helper cells and CD8$^+$ effector cytotoxic T lymphocytes (CTLs). At each step of this process, various signals shape whether an antitumor T-cell response will be produced, or conversely, an immunosuppressive and/or tolerogenic response will be made by such mediators as regulatory T-cells and myeloid-derived suppressor cells (reviewed by Palucka and Banchereau,[3] Chen and Mellman,[4] and Blattman and Greenberg[5]). Immunotherapies for cancer can target each or many of these steps to skew toward an antitumor response and away from an immunosuppressive response.

Cancer immunotherapies can be categorized as non-antigen-specific or antigen-specific therapies. Non-antigen-specific immunotherapies aim to either enhance the immune response in a general fashion or to decrease the immunosuppression present in the tumor environment. Non-antigen-specific therapies include cytokines and immune growth factors (eg, interferon (IFN)-α, interleukin [IL]-2, or granulocyte macrophage colony-stimulating factor), immunologic adjuvants (eg, Bacille Calmette-Guérin); Toll-like receptor (TLR)-3 agonists, such as poly-I:C (Rintatolimod, Ampligen®; Hemispherx Biopharma, Inc., Philadelphia, PA, USA) and poly-ICLC (Hiltonol®; Oncovir, Washington, DC, USA); TLR-4 agonists, such as monophosphoryl lipid A; the TLR-7 agonist, imiquimod; immune checkpoint blockers, eg, anticytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody;[6,7] and the programmed death-1 (PD-1) pathway agents, nivolumab and lambrolizumab.[8–11]

Compared with non-specific immunotherapies, antigen-specific therapies, such as therapeutic vaccines against cancer, aim to induce immune cells to target cancer cells that express a particular set of antigens. Different classes of cancer vaccines include peptide-based or protein-based vaccines, cancer cell-based vaccines, viral vector vaccines, DNA vaccines, messenger RNA vaccines, and carbohydrate vaccines.[12–19] In all cases, these vaccines involve two components, an antigen and an adjuvant, aimed at promoting local inflammation and the resulting immunization. Additionally, all of the above types of cancer vaccines rely on the patients' endogenous dendritic cells (DCs) for their uptake and effective antigen presentation to tumor-specific CD8$^+$ and CD4$^+$ T-cells.

Another category of cell-based cancer vaccines is use of patients' ex vivo-generated and tumor antigen-loaded DCs (or more precisely, autologous cellular therapeutics). This strategy limits the dependence of the immune system on patients' resident DCs, which have been shown to be defective in the advanced stages of cancer[3,20,21] or even redirected to differentiate toward myeloid-derived suppressor cells.[22,23] Regardless of whether endogenous or ex vivo-generated DCs are utilized for immunization, therapeutic cancer vaccines need to overcome several common challenges to induce immunity in the presence of established tumors and can benefit from recent developments in the area of DC biology.

Challenges in therapeutic cancer vaccination

For a therapeutic cancer vaccine to be effective, it must be capable of inducing a high number of antigen-specific T-cells against an established tumor, which can migrate to the tumor and perform their effector functions at the tumor site (Figure 1). However, challenges are present for each of these three goals. The first challenge is achieving high numbers of antitumor T-cells when the vaccine is being administered in the presence of an ongoing, although dysfunctional, immune response. Due to the ongoing antitumor immune response, the vaccine-carrying antigen-presenting cells (using either endogenous DCs that have taken up vaccine-introduced antigens or ex vivo-generated tumor antigen-loaded DCs), may be recognized by the CD8$^+$ T-cells as "tumor".[24,25] Since this encounter occurs in the periphery, away from the immunosuppressive tumor microenvironment, the CD8$^+$ T-cells may be capable of eliminating the vaccine, and thus limiting the vaccine's effectiveness before it can induce an immune response.[3]

Additionally, there is a lack of the proinflammatory signals required to promote effective immune responses. These signals are replaced by tumor-induced immunosuppressive/anti-inflammatory signals predominating in cancer patients. Therefore, to achieve the goal of inducing high numbers of tumor-specific T-cells, the vaccine-carrying antigen-presenting cells must not only survive long enough to present antigen, but must also provide the inflammatory signals to drive effector cell functions.[3,26–28]

Unfortunately, the presence of high numbers of antigen-specific T-cells does not ensure an effective antitumor

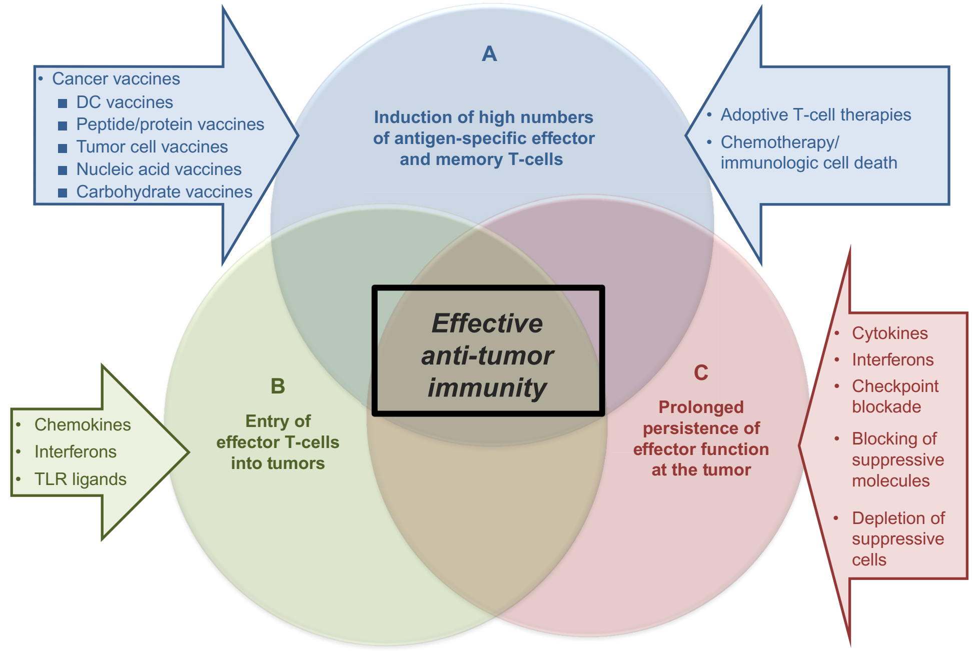

Figure 1 Elements of effective antitumor immunity.

Notes: Effective antitumor responses involve numerous features of immunity. These include (**A**) induction of high numbers of type 1 (cytotoxic/IFN-γ-producing) antigen-specific T-cells against an established tumor. This can be accomplished with various types of cancer vaccines. Other therapies that are not the focus of this review are adoptive T-cell therapies and certain chemotherapeutic agents that promote immunologic cell death.[137,138] Furthermore, the ability of vaccination-induced tumor-specific T-cells depends on the T-cells' ability to enter tumor tissues (**B**), which can be facilitated by manipulations aimed at local induction of effector cell (cytotoxic T lymphocyte, type 1 helper CD4+ T cell, natural killer)-attracting chemokines, especially when accompanied by suppression of factors that attract undesirable suppressive cells, such as myeloid-derived suppressor cells and regulatory T-cells. Finally, sustaining effector functions at the tumor site (**C**) can be supported by promoting effector T-cell activity and prolonging T-cell memory, which can be achieved with administration of cytokines such as IL-1, IL-7, IL-12, and IL-15 or IFNs, and by counteracting immunosuppressive mechanisms using checkpoint blockade of cytotoxic T-lymphocyte antigen-4 or the PD1-PDL1/2 pathway, blockers of immunosuppressive molecules like prostaglandin E2, indoleamine 2,3-dioxygenase, nitric oxide synthase, vascular endothelial growth factor, and transforming growth factor beta, or depletion of immunosuppressive cells such as regulatory T-cells and myeloid-derived suppressor cells.

Abbreviations: CD, cluster of differentiation; DC, dendritic cell; IFN, interferon; IL, interleukin; PD, programmed death-1; PDL, programmed death ligand; TLR, toll-like receptor.

response if these T-cells are unable to home to the tumor. In a normal infection scenario, where the immune response is targeting invading pathogens, the microorganisms and local tissue damage induce chemokines that recruit effector cells such as CTLs, type 1 helper CD4+ T-cells, or natural killer cells to the site of pathogen entry.[27,29] However, one of the immune evasion mechanisms evoked by tumors to support tumor growth and metastatic spread is downregulation of the chemokines that attract immune effector cells[28,29] and upregulation of chemokines that attract suppressor cells, such as regulatory T-cells,[30–32] suppressive plasmacytoid DCs,[33] and myeloid-derived suppressor cells.[34,35] Thus, a therapeutic vaccine needs to either induce T-cells that can respond to the spontaneously expressed tumor-associated chemokines or be administered as part of a combinatorial therapy with additional factors to alter the chemokine profile in the tumor microenvironment.[32,34]

Once high numbers of vaccine-induced tumor-specific T-cells have been generated and arrive at the tumor site, the T-cells must be capable of killing the tumor cells in order for the vaccine to be effective. Most types of cancer (including melanoma, ovarian, breast, renal, prostate, lung, and head and neck cancer) produce many factors, including IL-10, transforming growth factor beta, vascular endothelial growth factor, IL-6, and cyclooxygenase-2 products like prostaglandin E2, that contribute to immune dysfunction by suppressing the functions of endogenous or adoptively transferred DCs and T-cells.[21,36–38] These factors not only act to directly suppress DC and T-cell functions, but they can also promote cell-mediated immune suppression by enhancing the recruitment, expansion, and activation of regulatory T-cells and myeloid-derived suppressor cells.[30,31,39,40] While in some patients the high numbers of tumor-specific T-cells induced by the vaccine may be able to overcome the immunosuppressive tumor microenvironment, most therapeutic cancer vaccine strategies would greatly benefit from a combinatorial approach that alters the tumor to reduce immunosuppressive factors.

Promise and challenges in therapeutic cancer vaccines: clinical trials

The development of clinically effective therapeutic cancer vaccines has been challenging. Currently, the only therapeutic cancer vaccine approved by the US Food and Drug Administration is sipuleucel-T, a treatment for metastatic androgen-independent prostate cancer that was approved in 2010.[41,42]

Sipuleucel-T consists of antigen-presenting cells that are activated ex vivo from autologous peripheral blood mononuclear cells by a fusion protein, PA2024, which is comprised of granulocyte macrophage colony-stimulating factor and prostatic acid phosphatase, a prostate adenocarcinoma-associated antigen.[41,43] In two randomized, double-blind, placebo-controlled multicenter Phase III trials, sipuleucel-T increased median survival by 4 months when compared with placebo.[43,44] Sipuleucel-T was administered in three doses at weeks 0, 2, and 4, each at 2 days following leukapheresis. In the D9901/D9902A trials of 225 patients with asymptomatic metastatic hormone-refractory prostate cancer randomized in a 2:1 ratio to treatment with sipuleucel-T or a control infusion, the primary objective was time to disease progression. While there was no statistically significant difference in time to progression (median 11.1 weeks with sipuleucel-T versus 9.7 weeks with control), there was a 33% reduction in risk of death with sipuleucel-T compared with control and a statistically significant difference in survival (median 23.2 months for sipuleucel-T versus 18.9 months for control, P=0.011).[44] In the IMPACT (Immunotherapy for Prostate Adenocarcinoma Treatment) study of 127 metastatic castration-resistant prostate cancer patients with the primary endpoint of overall survival, there was a 22% adjusted relative reduction in risk of death and a statistically significant increase in median survival of 4.1 months (median 25.8 months for sipuleucel-T versus 21.7 months for placebo, P=0.03), although there was no difference in disease progression.[43] Patients in the treatment group who had antibody titers of more than 400 against PA2024 had an increased survival compared with those who had titers of less than 400 ($P<0.001$).[43] Cumulative antigen-presenting cell activation measured by CD54 upregulation, antigen-presenting cell number, total nucleated cell number, and antigen-specific immune responses to PA2024 and/or prostatic acid phosphatase in the treatment group correlated with overall survival ($P<0.05$).[41]

The ClinicalTrials.gov registry gives an insight into upcoming cancer vaccines in development that show promise in improving outcomes.[45] A query of this website in November 2013 with a targeted search of Phase III and IV clinical trials with known statuses and "cancer" listed as the condition, "vaccine" as the intervention, and "survival" as the outcome measure, resulted in 42 studies. A summary of selected cancer-specific vaccines from this query is listed in Table 1 with additional information from publications and abstracts.[43,46–55]

In addition to the sipuleucel-T trials, a Phase III trial of a glycoprotein 100 peptide vaccine also posted positive results. In a randomized, multicenter trial, patients with advanced melanoma received IL-2 and glycoprotein 100:209–217 (210 M) peptide vaccination or IL-2 alone.[47] For the primary endpoint of clinical response, the IL-2 with vaccination group had a significantly higher response rate of 20% (complete response 11%, partial response 9%) versus a response rate of 10% in the IL-2 only group (complete response 2%, partial response 8%; P=0.05). Median progression-free survival was also significantly longer in the IL-2 with vaccination group (2.2 months) than in the IL-2 alone group (1.6 months; P=0.008). There was a trend of increased overall survival with the addition of vaccination to IL-2 (17.8 months) compared with IL-2 alone (11.1 months; P=0.06). It is important to note that this study was not powered to detect a difference in overall survival.

Several Phase III trials of therapeutic cancer vaccines are currently in progress. Another vaccine in Phase III trials is TG4010, a poxvirus vector vaccine encoding for the tumor-associated antigen Mucin-1 (MUC1) and IL-2, which is being investigated in non-small cell lung cancer (NSCLC).[49] IMA901, a multiple peptide vaccine for renal cell carcinoma, has also completed accrual for its Phase III study and its results are pending. The ten peptides for IMA901 were uniquely chosen using an antigen discovery platform that analyzed renal cell carcinoma tissue.[56] The HyperAcute® vaccines (NewLink Genetics, Ames, IA, USA) for pancreatic and NSCLC consist of allogeneic cancer cells that have been genetically modified to express murine α(1,3)galactosyl.[50,51]

ProstAtak™ (Advantagene Inc., Auburndale, MA, USA) and Prostvac®-V/F-TRICOM™ (Bavarian Nordic; Washington, DC, USA) are viral-based vaccines for prostate cancer. ProstAtak involves intratumoral injection of an adenovirus containing a Herpes virus thymidine kinase gene followed by valaciclovir. Prostvac-V/F-TRICOM is composed of recombinant vaccinia and fowlpox viral vectors that encode for prostate-specific antigen and TRICOM, a combination of three costimulatory molecules, LFA-3, B7.1, and intercellular adhesion molecule-1.[52]

Table 1 Selected Phase III trials of cancer-specific vaccines

	Type	Name	Sponsor/manufacturer	Description	Cancer	Trial ID (status)	Results, if published
Favorable results							
Completed	APC based	Sipuleucel-T[43] (Provenge®)	Dendreon Corporation, Seattle, WA, USA	Autologous DC precursors activated with PAP-GM-CSF fusion protein	Prostate cancer	NCT00065442 NCT00005947 NCT01133704	4.1 months increased median survival and relative reduction of 22% in risk of death compared with placebo.
	Peptide	Gp100:209–217 (210M)[47]	National Cancer Institute, Washington, DC, USA	Gp100 antigen in montanide ISA-51 adjuvant	Melanoma	NCT00019682	Overall clinical response and median PFS was significantly higher than IL-2 alone. Not powered to detect difference in OS.
Final results not yet available							
Completed	Peptide	–	National Cancer Institute, Washington, DC, USA	Tyrosinase:368–376 (370D), gp100:209–217 (210M), MART-1:27–35 multipeptide vaccine	Melanoma	NCT01989572	Full results anticipated in 2014 with preliminary results presented demonstrating that vaccination did not reach endpoints for OS and DFS.[55]
Recruiting	Dendritic cell	AGS-003	Argos Therapeutics, Durhan, NC, USA	Autologous tumor RNA-loaded DCs	Renal cell carcinoma	NCT01582672	
		DCVax®-L	Northwest Biotherapeutics, Bethesda, MD, USA	Autologous tumor lysate-loaded DCs	GBM	NCT00045968	
	Whole cell	Algenpantucel-L[50] (HyperAcute®-Pancreas)	NewLink Genetics, Ames, IA, USA	Allogeneic pancreatic cancer cell transfected to express murine α(1,3)galactosyl	Pancreatic cancer	NCT01836432 (recruiting) NCT01072981 (Active, not recruiting)	
		Tergenpantucel-L[51] (HyperAcute®-Lung)	NewLink Genetics, Ames, IA, USA	Allogeneic NSCLC cells genetically engineered to express α(1,3)galactosyl	NSCLC	NCT01774578	
	Viral vector	TG4010[49] (MVA-MUC1-IL2)	Transgene, Illkirch Graffenstaden, France	Recombinant modified Vaccinia strain with MUC1 and IL2 coding sequences	NSCLC	NCT01383148	
		Prostvac®-V/F-TRICOM™[52]	Bavarian Nordic, Inc., Washington, DC, USA	Vaccinia and fowlpox recombinant viral vectors encoding for PSA and TRICOM (costimulatory molecules LFA-3, B7.1 and ICAM-1)	Prostate cancer	NCT01322490	
		ProstAtak™	Advantagene, Inc., Auburndale, MA, USA	Adenovirus containing Herpes virus thymidine kinase gene with valacyclovir treatment	Prostate cancer	NCT01436968	
	Peptide	Rindopepimut (CDX-110)	Celldex Therapeutics, Hampton, NJ, USA	EGFRvIII peptide conjugated with KLH	Glioblastoma	NCT01480479	
		E75 peptide acetate (NeuVax)	Galena Biopharma, Portland, OR, USA	Her2/neu peptide vaccine	Breast cancer	NCT01479244	

(*Continued*)

Table 1 (*Continued*)

	Type	Name	Sponsor/manufacturer	Description	Cancer	Trial ID (status)	Results, if published
Active, not recruiting	Whole cell	M-Vax	AVAX Technologies, Philadelphia, PA, USA	Autologous, hapten-modified melanoma cell	Melanoma	NCT00477906	
	Protein	CimaVax-EGF	Bioven, London, UK	Recombinant human rEGF-P64K/montanide ISA 51 vaccine	NSCLC	NCT01444118	
	Peptide	IMA901	Immatics Biotechnologies GmbH, Tuebingen, Germany	Multipeptide vaccine	Renal cell carcinoma	NCT01265901	
	Shed Antigen	POL-103A	Polynoma, San Diego, CA, USA	Polyvalent, shed antigen vaccine from allogeneic and xenogenic cell lines	Melanoma	NCT01546571	
Negative results							
Active, not recruiting	Whole cell	Belagenpumatucel-L[53] (Lucanix™)	NovaRx Corporation, San Diego, CA, USA	TGF-β2 antisense gene-modified allogeneic tumor cell	NSCLC	NCT00676507	Median OS of 20.3 months with vaccine was not statistically significant compared with placebo. Significantly higher median OS with vaccine for patients within 12 weeks of chemotherapy completion (*P*=0.036).
	Protein	GSK1572932A[57,59]	GlaxoSmith Kline, Philadelphia, PA, USA	Recombinant MAGE-A3 protein vaccine with AS15 immunostimulant	NSCLC	NCT00480025	Study stopped early due to failure to increase the primary endpoint of disease-free survival.
Completed	Peptide	MDX-1379[46]	Bristol-Myers Squibb, New York, NY, USA	Gp100 melanoma peptide vaccine	Melanoma	NCT00094653	Median OS with vaccine alone was significantly less than ipilimumab plus vaccine or ipilimumab alone.
		GV1001[48]	Royal Liverpool University Hospital, Liverpool, UK	Telomerase peptide vaccine	Pancreatic cancer	NCT00425360	Addition of vaccine to gemcitabine and capecitabine did not improve OS.
Recruiting		Tecemotide[54] (Stimuvax®/L-BLP25)	Merck KGaA, Darmstadt, Germany	MUC1 peptide vaccine	NSCLC	NCT01015443 (recruiting) NCT00409188 (active, not recruiting)	Median OS was not significantly increased with L-BLP25 compared to placebo. Predefined subgroup of patients with concurrent chemoradiotherapy had increased median OS of 30.8 months versus 20.6 months with placebo (*P*=0.016).

Abbreviations: APC, antigen presenting cell; AS15, adjuvant system 15; DC, dendritic cell; DFS, disease-free survival; EGF, epidermal growth factor; EGFRvIII, epidermal growth factor receptor vIII; GBM, glioblastoma multiforme; Gp, glycoprotein; gp100:209-217 (210M), modified peptide (amino acids 209-217) with a methionine substitution at position 210; Her2, human epidermal growth factor receptor 2; ICAM-1, intercellular adhesion molecule 1; IL2, interleukin-2; KLH, keyhole limpet hemocyanin; LFA-1, lymphocyte function-associated antigen 1; LFA-3, leukocyte function-associated antigen-3; MAGE-A3, melanoma associated antigen-3; MART-1, melanoma antigen recognized by T-cells 1; MUC1, Mucin-1; NSCLC, non-small cell lung cancer; OS, overall survival; PAP-GM-CSF, prostatic acid phosphatase-granulocyte/macrophage colony-stimulating factor; PFS, progression-free survival; PSA, prostate specific antigen; rEGF, recombinant human epidermal growth factor; TGF, transforming growth factor; Tryosinase:368-376 (370D), modified peptide (amino acids 368-376) with an aspartic acid substitution at position 370.

A few Phase III trials have failed to meet their primary endpoints, and highlight the difficulties of cancer vaccine development. One of the largest studies in NSCLC, the MAGRIT (MAGE-A3 as Adjuvant NSCLC Immunotherapy) trial, which utilized a melanoma-associated antigen 3 (MAGE-A3) protein vaccine, was stopped in early 2014 after failing to increase the primary endpoint of disease-free survival in MAGE-A3-positive patients overall or MAGE-A3-positive patients without chemotherapy treatment, compared with control.[57–59] This was following a double-blind, randomized, placebo-controlled Phase II study that showed clinical activity, with all treated patients developing anti-MAGE-A3 antibodies and with a pretreatment 84-gene expression signature being associated with increased disease-free response.[60,61] However, the subsequent Phase III trial was not able to determine a subpopulation of gene signature-positive patients who would benefit from treatment since there was an insufficient treatment effect.[59]

Belagenpumatucel-L, an allogeneic genetically modified NSCLC tumor cell vaccine, showed a trend toward increased median survival but this did not reach statistical significance.[53] However, the subgroup of patients who received vaccination within 12 weeks of chemotherapy had a statistically significant improvement, and the study is continuing in this subgroup of patients. Similarly, tecemotide, a MUC1 peptide vaccine for NSCLC, failed to demonstrate a statistically significant difference in overall survival compared with placebo, but a significant increase in median overall survival in the subgroup of patients who had concurrent chemoradiation has led to plans for a randomized trial of tecemotide with concurrent chemoradiation in stage III NSCLC patients.[54]

One of the largest studies in metastatic melanoma was MMAIT-IV (Malignant Melanoma Active Immunotherapy Trial for Stage IV Disease), an international, multicenter, randomized, double-blind Phase III trial in 1,656 stage III and IV patients of an allogeneic whole melanoma cell vaccine, Canvaxin™ (CancerVax Corporation, Carlsbad, CA, USA) with a Bacille Calmette-Guérin adjuvant, compared with placebo plus Bacille Calmette-Guérin, that was closed early after interim analysis showed a low probability of demonstrating a significant increase in survival in the Canvaxin with Bacille Calmette-Guérin arm.[62] Although the trial had negative results, an ancillary study of pretreatment and post-treatment circulating tumor cell biomarkers for melanoma antigen recognized by T-cells 1 (MART1), MAGE-A3, and paired box 3 (PAX3) from patients in the MMAIT-IV trial was able to demonstrate that pretreatment and serial circulating tumor cell levels were significantly associated with decreased disease-free survival and overall survival.[63]

Another large melanoma vaccine study, the randomized Phase III trial of adjuvant ganglioside (GM2) conjugated to Keyhole Limpet hemocyanin (KLH) admixed with adjuvant QS-21 (GM2-KLH/QS-21) vaccine versus observation in 1,314 stage II melanoma patients, was terminated after the second interim analysis due to failure to increase recurrence-free survival and a trend toward increased overall survival in the observation arm, which was also confirmed on final analysis after a median follow-up of 4 years.[64]

A challenge in evaluating therapeutic cancer vaccines is appropriate patient selection. While clinical trials of new oncologic therapies are traditionally first tested in patients with advanced cancers who have failed multiple treatment regimens, vaccines may be more effective when the disease burden is low.[65] Another challenge in trial design and evaluation is that the kinetics of tumor growth rates for vaccine therapy differ from those of traditional chemotherapy and radiotherapy.[66] Compared with these directly cytotoxic therapies in which the treatment response occurs immediately following their administration and the tumor growth rate often returns to pretreatment levels following termination of treatment, positive responses to vaccine therapy may begin months after treatment, with a potentially prolonged treatment effect persisting long after administration.[67] Therefore, the intermediate endpoint of progression-free survival based on the commonly used Response Evaluation Criteria in Solid Tumors or World Health Organization criteria has very limited value in vaccine therapies, and more relevant immunologic endpoints are needed.[66–68] A common phenomenon with immunotherapy trials is that overall survival may improve without a change in progression-free survival.[43,46,69] In fact, there may even be a treatment response after initial progression or tumor growth.[67] In result, the recently formulated immune-related response criteria[67] are better predictors of prolonged overall survival of patients treated with immunotherapy than the classical response criteria used to evaluate the effectiveness of chemotherapeutic agents (Response Evaluation Criteria In Solid Tumors [RECIST] and World Health Organization).[67]

Finally, another important trial design consideration is immunologic selection and response monitoring of patients. Pretreatment markers would help to determine which patients would benefit the most from vaccine treatment but this work is still in its infancy.[70] The discovery of markers to monitor immune responses that correlate with clinical outcomes is still in development. Current biomarkers to evaluate the immune response focus on CTL antigen recognition and the humoral response. Markers shown to correlate with clinical outcome include antigen-specific T-cell response based on IFN-γ enzyme-linked ImmunoSpot (ELISPOT) assays, cytokine expression levels, and reduction in regulatory T-cells.[41,71–73]

Furthermore, two clinical trials involving DC vaccines indicated a role of DC-produced IL-12p70 as a predictive marker of the clinical benefit of vaccination.[74,75]

Avenues for improved immunization: exploiting the biology of dendritic cells

The primary aim of cancer vaccines is to generate a CTL response against cancer cells.[76] An important advantage of therapeutic immunizations, compared with traditional cancer treatments, is that the treatment effect is typically durable due to the induction of long-lived effector memory and central memory T-cells, which can persist for prolonged periods after administration of the vaccine. The second advantage is the very high selectivity of the immune response in targeting tumor cells, while not damaging healthy tissue. As mentioned before, several strategies, such as protein or DNA vaccines, utilize a patient's endogenous DCs at the injection site for uptake and presentation of tumor antigens, but the observed dysfunction of DCs in cancer patients due to tumor-related suppressive factors may limit the effectiveness of these vaccines, which rely on endogenous DCs for antigen uptake.[37,77–79] Therefore, the use of ex vivo-generated DC vaccines is an attractive option for circumventing this issue, enabling DCs to mature in the absence of tumor-related immunosuppression and allowing more control of the DC maturation process to direct the nature of the immune response.

Effective induction of an antigen-specific T-cell response requires delivery of at least four types of signals (Figure 2) by DCs, each of which can be optimized to improve the cancer vaccine.[80] The first signal (signal 1) is the presentation of processed antigen in the context of MHC molecules by DCs to naïve T-cells via the T-cell receptor.[81] One of the key characteristics of DCs that makes these cells a unique tool for cancer vaccination is their ability to take up different forms of antigens, process them, and then cross-present these antigens to naïve $CD4^+$ and $CD8^+$

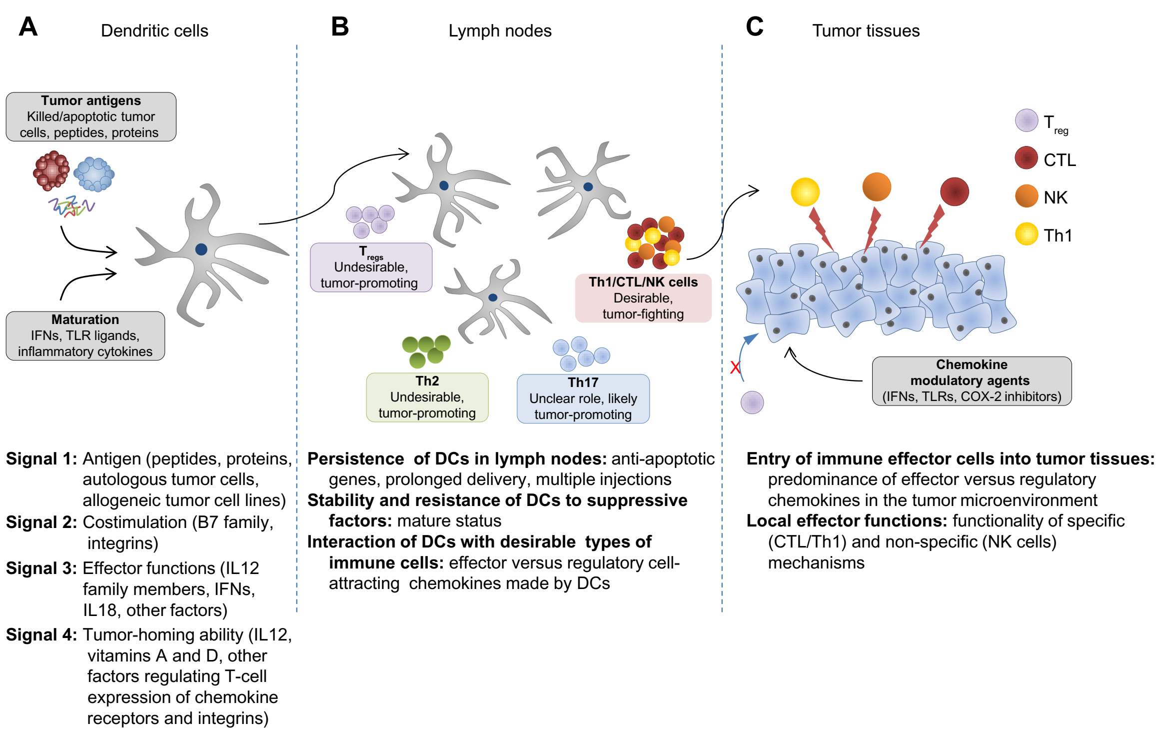

Figure 2 Four types of DC-mediated signals regulating the magnitude and quality of tumor-specific T-cell responses.

Notes: (**A**) An effective cancer vaccine needs to promote delivery of four types of signals to T-cells. DC-delivered antigenic (signal 1) and costimulatory (signal 2) signals are required for T-cell activation and expansion. Signal 3 (polarization of effector mechanisms of immune responses) drives the type of differentiation of T-cells (ie, type 1 cell-mediated response or type 2 humoral response). Signal 4 imprints the tumor-homing ability of T-cells by regulating the profile of chemokine receptor expression on activated T-cells. (**B**) Additional requirements of vaccine stimulated DCs include the ability to migrate to and persist in draining lymph nodes and preferentially interact with desirable types of immune cells (CTL, Th1, and NK cells, rather than MDSCs and regulatory T-cells). (**C**) Activated effector cells need to migrate to the tumor tissue and overcome the immunosuppressive mechanisms of the tumor environment in order to have sustained antitumor activity.

Abbreviations: COX2, cyclooxygenase-2; CTL, cytotoxic T lymphocyte; DC, dendritic cell; IFN, interferon; IL, interleukin; MDSCs, myeloid derived suppressor cells; TLR, toll-like receptor; Th1, type 1 helper; Th2, type 2 helper; Th17, type 17 helper; T_{regs}, regulatory T-cells.

T-cells. This broadens the source of antigens that can be used in vaccines to include not only peptides (which are MHC-restricted and limited to known, well characterized tumor antigens and thus only applicable to patients who express the appropriate MHC haplotype and have tumors that express the specific antigen), but also recombinant proteins, tumor lysates, or whole tumor cells from either autologous or allogeneic sources. The use of proteins or whole cell sources of antigen increases the ability to prime immune responses to undefined patient-specific tumor antigens. Various methods of processing tumor cells for loading have been studied, such as freeze-thaw lysates, irradiation, and oxidation of tumor cells, to enhance the uptake and cross-presentation of whole tumor cells by DCs.[79,82–84] Of note, loading DCs with apoptotic cells was shown to be more effective in stimulating CTLs compared with loading with necrotic cells.[85]

Signal 2 involves costimulatory signals that amplify the T-cell receptor signal and prolong the MHC:T-cell receptor interaction to ensure T-cell activation. This amplification signal is provided by B7 family molecules, such as CD80 and CD86, that bind to CD28 on the T-cell.[86,87] The MHC:T-cell receptor and CD28:CD80/CD86 interactions are stabilized by integrins, notably leukocyte function-associated antigen-1 (LFA-1): intercellular adhesion molecule-1 interactions, so that the cell-cell interactions are not prematurely terminated, resulting in incomplete activation.[88] The absence of costimulation during antigen presentation by DCs can induce $CD8^+$ T-cell tolerance to the antigen.[89] The molecules involved in costimulation are upregulated upon DC maturation, when the DC also gains the ability to respond to the lymph node-homing chemokines CCL (chemokine [CC motif ligand]-19 and CCL22) by upregulating CC chemokine receptor type 7 (CCR7).[90,91] The first generation of DC vaccines utilized immature or partially matured DCs capable of cross-presentation of antigens but deficient in costimulatory and lymph node-homing abilities.[86] This led to protocols for DC maturation producing the "second generation" of DC vaccines that are able to provide both signals 1 and 2. In these protocols, the DCs are matured using either monocyte-conditioned medium[92] or a cytokine cocktail consisting of IL-6, IL1β, tumor necrosis factor alpha, and prostaglandin E2.[93] While these maturation strategies induce upregulation of costimulatory molecules and CCR7, and have enhanced immunogenicity in vitro and in vivo in healthy volunteers, their initial promise diminished in a randomized multicenter Phase III trial for advanced melanoma when less than 5% of patients receiving the vaccine demonstrated a clinical response and there was no impact on overall survival.[94]

Signal 3 is the DC-produced cytokine profile that skews the type of immune response generated (ie, type 1 cell-mediated versus type 2 humoral responses), and provides survival and differentiation signals to naïve T-cells. A prototypical example of a signal 3 cytokine that promotes cell-mediated immunity is IL-12p70,[95] which is produced by DCs when they are matured in the presence of IFN-γ, a cytokine produced by activated natural killer cells at the site of infection, and in the absence of the chronic inflammatory cytokine, prostaglandin E2. One possible factor in the negative results of the clinical trials using "second generation" DCs is the use of prostaglandin E2-containing maturation cocktails, since prostaglandin E2 has subsequently been shown to have a deleterious effect on IL-12p70.[95–97]

In order to generate mature DCs with high costimulatory molecules and lymph node-homing ability, as well as high IL-12-producing capacity to promote the desirable cell-mediated immunity, a "third generation" of DCs was generated.[73,74] The "third generation" DCs are generally matured in conditions mimicking viral infection, which predominantly drives cell-mediated immunity. Some of the strategies to mature DCs are: to coculture immature DCs with other immune cells, such as IL-18 activated natural killer cells[98] or memory $CD8^+$ T-cells;[25,99] to mature with conditioned medium from activated CTLs;[100,101] or to use cytokine cocktails that include viral-mimicking TLR ligands.[102–107] Each of these strategies generate DCs that are "type 1 polarized" (DC1), possessing not only high antigen cross-presentation and costimulatory abilities, but also a superior ability to secrete IL12 for up to 48 hours after interaction with CD40L-expressing $CD4^+$ T-cells.[102,103,108–110] Additional inclusion of IFN-α to a "type 1 polarizing" cytokine cocktail consisting of IFN-γ, IL1β, tumor necrosis factor alpha, and poly-I:C enhanced the expression of the lymph node homing chemokine receptor CCR7.[111–113] These αDC1s also preferentially produce chemokines that promote migration of naïve, memory, and effector T-cells, but show reduced expression of chemokines that promote immunosuppressive cell recruitment, further enhancing the ability of αDC1s to interact and prime strong antitumor immune responses.[111–113] A recent clinical trial utilizing αDC1 vaccines and an alternative type of "type 1 polarized DCs" induced by the combination of CD40L and IFN-γ demonstrated that the ability of DC1 vaccines to produce high IL-12p70 levels was the strongest predictor of prolonged progression-free survival in vaccinated patients.[74,75]

The last type of signal (signal 4) delivered to T-cells during priming interactions with DCs results in programming of specific chemokine receptor expression on activated T-cells that directs them to specific tissues.[86] In vitro and ex vivo studies have demonstrated that different DC subsets isolated from various tissues can modulate the chemokine expression profile on activated T-cells, thereby directing T-cells back to the tissues of DC origin.[114,115] This differential chemokine expression programming is not limited to DCs developed in various tissues in vivo, but also extends to ex vivo-generated, cytokine-matured DCs. A comparison of $CD8^+$ T-cells from melanoma patients sensitized ex vivo by either prostaglandin E2-matured DCs (second generation) or type 1 polarized DC1s (third generation) demonstrated different chemokine expression on the activated $CD8^+$ T-cells.[97] Specifically, T-cells sensitized by DC1s had higher expression of CCR5 and CXC chemokine receptor 3 (CXCR3), two chemokine receptors involved in peripheral homing to the skin and entry into melanoma and other tumors, compared with T-cells sensitized by prostaglandin E2-matured DCs.[111,112,114,116]

Helping vaccination-induced T-cells to work: conditioning tumor microenvironments for effective CTL entry and function

Future developments in cancer immunotherapy research will likely focus on the challenges that vaccine-induced CTLs encounter in reaching the tumor microenvironment and performing their antitumor cytotoxic functions. Areas of current investigation in changing the tumor milieu include promoting CTL entry via chemokine modulation, inhibiting immune checkpoints that block CTL effector function, and decreasing immunosuppressive cells, such as regulatory T-cells and myeloid-derived suppressor cells.

Chemokine modulation aims to shift the balance of the tumor environment toward expression of effector T-cell attracting chemokines, and away from regulatory T-cell attracting chemokines.[32] Tumor infiltration of certain immune cells such as CTLs, type 1 helper $CD4^+$ T-cells, DCs, and M1 macrophages has positive prognostic value, while infiltration by regulatory T-cells, type 2 helper $CD4^+$ T-cells, myeloid-derived suppressor cells, and M2 macrophages is associated with poor outcomes.[117–120] There are currently several monoclonal antibodies and small molecule inhibitors targeting various chemokine receptors in clinical trials.[121] Our group has also shown that ex vivo treatment of tumor tissue with type 1 IFNs, a TLR-3 ligand, and a cyclo-oxygenase-2 inhibitor increased the production of the effector T-cell attracting chemokines, CCL5 and CXCL10, while decreasing the production of regulatory T-cells attracting chemokine CCL22.[32]

Combining vaccines with agents that reduce the levels of immunosuppressive cells (such as myeloid-derived suppressor cells and regulatory T-cells) has also been an attractive strategy. Low-dose cyclophosphamide has been used extensively for its ability to suppress regulatory T-cells since it is inexpensive and easily obtained.[122] A randomized Phase II study of the renal cell cancer peptide vaccine, IMA901, demonstrated that a single cyclophosphamide dose was effective in reducing the number of regulatory T-cells, and that among patients who were immune responders, those treated with cyclophosphamide had increased survival.[56] Another Phase I/II trial of a multipeptide-loaded DC vaccine in advanced ovarian cancer showed a trend toward increased survival with the addition of cyclophosphamide treatment.[123] Other combination strategies to reduce regulatory T-cells in vaccine trials have included anti-CD25 monoclonal antibodies and a CD25 targeting immunotoxin.[124,125] Preliminary data from an ongoing randomized DC vaccine trial targeting myeloid-derived suppressor cells using all-trans-retinoic acid show that the treatment arm receiving all-trans-retinoic acid and vaccination had an improved immune response compared with vaccination alone.[126] Other inhibitors of immunosuppressive targets shown to correlate with decreased survival, such as prostaglandin E2, indoleamine 2,3-dioxygenase, and nitric oxide synthase, are also potential targets for combinatorial therapy with cancer vaccines.[127–130]

In contrast with combinatorial therapies that reverse immunosuppressive cells, cancer vaccines may be combined with cytokine treatments that promote effector T-cell activity and prolong T-cell memory (see Figure 1C). IL-7, IL-15, IL-21, and IL-27 are similar to IL-2 as part of the common gamma chain cytokine receptor family.[131] IL-7 has a role in development, homeostasis, and survival of T-cells and B-cells.[132,133] Administration of recombinant IL-7 to cancer patients has been shown to be safe and to rapidly expand circulating CD4 and CD8 cells that express CD127, but not regulatory T-cells.[134] IL-15 has a role in T-cell and natural killer cell activation and proliferation and maintenance of memory T-cell responses.[135,136] Early phase clinical trials utilizing IL-15 for cancer treatment are ongoing or recently completed, but without published results as yet.[136] IL-21 is produced by activated $CD4^+$ T-cells and natural killer T-cells, and contributes to antitumor immunity by its induction and activation of $CD8^+$ T-cells, natural killer cells, and natural killer T-cells.[131,137,138] Early Phase I and II studies have shown encouraging results in metastatic melanoma and metastatic renal cell carcinoma.[139–142] IL-27 is produced by antigen-presenting cells and can enhance $CD8^+$ T-cell and natural killer cell activation, but development of IL-27 as a therapeutic is still in preclinical stages.[143,144]

The approval by the US Food and Drug Administration of ipilimumab for metastatic melanoma in 2010 signaled a change in the landscape of cancer therapies. Ipilimumab (MDX-010, Yervoy®; Bristol-Myers Squibb, New York, NY, USA) is a fully human monoclonal antibody against CTLA-4, and a homologue of CD28 with greater affinity to B7 molecules which outcompetes CD28 binding, effectually preventing the costimulatory signal 2.[11] Anti-CTLA-4 antibodies block this inhibitory interaction or immune checkpoint and restore signal 2 for T-cell activation. In a randomized, double-blind, three-arm Phase III trial comparing ipilimumab with and without a glycoprotein 100 vaccine (MDX-1379) with vaccination alone in patients with metastatic melanoma, subjects in the ipilimumab treatment groups were found to have a significantly higher median survival compared with those receiving vaccination alone (10 months versus 6.4 months).[46] The failure of the vaccination arms in the Phase III study to improve overall survival was unexpected, but it is possible that this resulted from the application of a single-epitope glycoprotein 100 peptide vaccine. A similar glycoprotein 100 vaccine did not show an improvement in survival, although that study was only powered to detect a difference in progression-free survival and not overall survival.[47] Furthermore, the original Phase III study had ipilimumab and vaccination administration occurring concurrently, whereas there is more recent evidence from a murine model that sequential therapy of vaccination followed by anti-CTLA-4 antibody was superior to the anti-CTLA-4 antibody when administered first.[145] Some of the early preclinical studies of ipilimumab indeed focused on using it in combination with cell-based cancer vaccines, and other anti-CTLA-4/vaccine combinations are in clinical trials.[11,146–148] A recent Phase II study comparing ipilimumab alone or in combination with GM-CSF-secreting whole cell vaccine showed a higher survival rate when ipilimumab was combined with vaccine.[146]

Another actively studied immune checkpoint receptor is PD-1 (CD279).[149] PD-1 and its ligands, PD-1 ligand 1 and 2 (PDL1 and PDL2), are expressed on more cell types than CTLA-4. PD-1 expression can be induced not only on activated T-cells, but also on B-cells and natural killer cells, while PDL1 and PDL2 can be upregulated on tumor cells, antigen-presenting cells, and other cells in inflammatory conditions. Several clinical trials of anti-PD-1 and anti-PDL1 antibodies have shown durable response rates.[6,150–152] While studies using combinatorial PD pathway agents and vaccine therapy are not as advanced as those with anti-CTLA-4 agents, there is promising preclinical and early clinical trial data suggesting that the dual combination or even the triple combination with anti-CTLA-4/PD pathway blockade/vaccination therapy will have increased clinical benefit by further enhancing the antigen-specific T-cell response from vaccination and decreasing regulatory T-cells.[153–157]

An effective combinatorial vaccine therapy will likely need to address three goals: building a robust antigen-specific CTL response; altering the tumor microenvironment to allow CTL infiltration and reduce migration of regulatory T-cells and myeloid-derived suppressor cells; and counteracting CTL inhibitory mechanisms such as immune checkpoints that lead to immunosuppression (Figure 1). An encouraging study using a combination of a peptide vaccine, anti-PD-1 antibody, and low-dose cyclophosphamide in a murine tumor model demonstrated that this combination of drugs synergized in increasing survival and reducing tumor burden.[158] One of the concerns about optimal application of complex immunotherapies is determination of the optimal sequence and duration of application of each of the components. It also needs to be determined how to optimally incorporate immunotherapy, different forms of which can either suppress or enhance both the induction of immune responses and the susceptibility of cancer tissues to immune attack.

Conclusion

Several of the new cancer vaccines have recently shown promise in prolonging patient survival. The next era of vaccine development is likely to involve both continued improvement of the vaccines themselves as well as combinatorial application of vaccines with agents that target the tumor microenvironment to promote entry of vaccination-induced cells, while eliminating local predominance of suppressive cells, and amplifying and prolonging the duration of the effector phase of antitumor immunity at tumor sites. The development of optimized immunotherapies for advanced cancer will also benefit from identification of the most relevant laboratory correlates of clinical effectiveness and integration of immunotherapy with other elements of comprehensive cancer care.

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Disclosure

Alpha-type 1 polarized DCs, one of the types of DC discussed in this paper, are a topic of a recently issued US patent. None of the authors receives any form of support or remuneration related to that intellectual property. The authors report no other conflicts of interest in this work.

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