Review

Immune modulation by dendritic-cell-based cancer vaccines

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The interplay between host immunity and tumour cells has opened the possibility of targeting tumour cells by modulation of the human immune system. Cancer immunotherapy involves the treatment of a tumour by utilizing the recombinant human immune system components to target the pro-tumour microenvironment or by revitalizing the immune system with the ability to kill tumour cells by priming the immune cells with tumour antigens. In this review, current immunotherapy approaches to cancer with special focus on dendritic cell (DC)-based cancer vaccines are discussed. Some of the DC-based vaccines under clinical trials for various cancer types are highlighted. Establishing tumour immunity involves a plethora of immune components and pathways; hence, combining chemotherapy, radiation therapy and various arms of immunotherapy, after analysing the benefits of individual therapeutic agents, might be beneficial to the patient.

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1. Background

Harnessing the potential of the immune system to fight cancer relies on the delicate balance between anti-tumour and pro-tumour immunity. Understanding of the host immune responses to tumours and the evasion strategies employed by the tumour to escape attack by immune cells becomes essential to determine the treatment options specific to killing tumours with minimal toxicity to host cells. Immunotherapy drugs have been characterized into immune checkpoint inhibitors, monoclonal antibodies targeting tumour cells, cytokines and cancer vaccines. With their unique capability to link innate and adaptive immune responses, dendritic cells (DCs) are ideal antigen-presenting cells to mount an anti-tumour attack. DC-based vaccines have been shown to be safe and effective in numerous clinical trials. The success of DC vaccines depends on the successful uptake of an antigen from the tumour suppressive microenvironment and induction of long-lived anti-tumour response. Combining various immunotherapeutic approaches targeting different footsteps in the anti-tumour immunity pathways can lead to more efficient control tumours. This review focuses on cancer immunotherapy by highlighting DC-based platforms and combinational approaches to enhance the existing treatment options.

2. Interaction between host immune system and tumour

The potential link between the immune system and tumours was reported by William B Coley after he observed tumour

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regression upon systemic bacterial infections. Thereafter, extensive research in animal models has demonstrated the existence of tumour-specific antigens that are recognized by our immune system. This idea was strengthened when mice vaccinated with killed tumour cells prevented the relapse after being challenged with the original tumour, but not with any other tumour (Gross 1943). The role of innate and adaptive immunity in cancer immunosurveillance has been well established. Studies using RAG-2-deficient mice with inability to produce peripheral mature lymphocytes and additional follow-up studies using TCR-alpha- and TCR-delta-knockout mice identified gamma delta (γδ) T-cells and alpha beta (αβ) T-cells as the possible RAG-dependent lymphocytes playing an important role in anti-tumour immunity (Girardi *et al.* 2001, 2003). The effector functions of the anti-tumour cells involve IFNγ production and killing of the tumour cells. γδ T-cells act as an early source of IFNγ, which may then regulate the effector functions of tumour-induced CD4/CD8 T-cells, thus forming an important arm in cancer elimination (Gao *et al.* 2003). The immune system kills cancer cells by employing cytotoxic molecules like perforin and inducing expression of TNF-related apoptosis-inducing ligand (TRAIL) on immune cells primarily NK cells, dendritic cells and monocytes (van den Broek *et al.* 1996; Smyth *et al.* 2003). For developing successful cancer therapeutic drugs/vaccines, it is critical to understand the mechanism used by our immune system to distinguish between tumour cells and normal cells. CD4+/CD8+ T-cells recognize tumour antigens processed and presented to MHC II/MHC I molecules by antigen presenting cells of our immune system. Tumour antigens that show high specificity to the tumour include viral antigens produced in the tumour caused by viruses, antigens originated from point mutation in ubiquitously expressed genes and cancer-germline antigens like MAGE and BAGE that are expressed in many cancer types and in germline cells. On the other hand, peptides derived from proteins like wild-type p53 that are overexpressed in tumour cells show low specificity to the tumour (Barfoed *et al.* 2000; Vigneron 2015). Additionally, stress signals like NKG2D and uric acid also serve as recognition targets (Bauer *et al.* 1999; Shi *et al.* 2003).

Dunn *et al.* in 2004 projected immunoediting hypothesis as a new dimension in the cancer immunosurveillance theory. During the elimination of tumour cells by our immune system, some tumour cells withstand the immune attack, transforms further to form new variants and enter in equilibrium with our immune system. Subsequently, the tumour cells either endure in this phase or escape from all the immune pressures (Dunn *et al.* 2004). Cancer cells escape the immune attack by hampering the antigen processing and presentation pathways along with promoting pro-tumour immunosuppressive environment dominated by IL-10, TGF-β cytokines, inhibitors of T-cell responses like indoleamine 2,3-dioxygenase, galectin-1, immunosuppressive co-stimulatory ligands B7-H3, B7-H4 and non-classical HLAs (Marincola *et al.* 2000; Khong and Restifo 2002; Uyttenhove *et al.* 2003; Rubinstein *et al.* 2004; Fauci *et al.* 2012; da Silva *et al.* 2013). During the escape phase, tumour cells secrete soluble forms of stress ligands like NKG2D which block the NKG2D receptors on immune effector cells, thereby preventing the recognition of tumour cells (Groh *et al.* 2002). Studies have highlighted the up-regulation of immunosuppressive T-cell population like Treg cells and IL-13 producing NKT cells which further augments the proliferation of the tumour cells (Terabe and Berzofsky 2004). Tumour cells have also been reported to dysregulate the expression of immune system inhibitors like CTLA-4, PD-1 to achieve immune resistance (Pardoll 2012). Immunotherapy aims to channelize the ability of our immune system to recognize transformed malignant cells and utilize it to mount anti-tumour immunity. Potential goals for immunotherapy include eliminating the tumour or at least maintaining it in the equilibrium phase by modulating the host immunity.

3. Cancer immunotherapy

Our understanding of cancer immunobiology gives us the opportunity to engineer our immune system to eliminate tumour cells. The mainstay of immunotherapy is to target cancer cells specifically and to induce a memory immune response to prevent relapse. The major challenge towards developing effective vaccines against tumour is the change in the tumour genetic makeup because of constant interaction with the host immune system. Passive immunotherapeutic approaches like immune checkpoint inhibitors target the mechanisms used by tumour cells to escape the immune attack, thereby reducing the pro-tumour immunosuppressive environment. Ipilimumab, an antibody against cytotoxic T-lymphocyte-associated protein (CTLA-4) which interferes with the co-stimulation required for T-cell activation, was the first immune checkpoint inhibitor to be approved for use in cancer treatment (Pentcheva-Hoang *et al.* 2014; Jeanbart and Swartz 2015). This was followed by approval of antibodies against programmed cell death protein (PD-1) (Nivolumab and Pembrozilumab) that down-regulates the signalling mediated by T-cell receptors (Sharma and Allison 2015). Median survival time of 10.1 months was observed in metastatic melanoma patients who received Ipilimumab alone as compared with survival time of 6.4 months in control group patients receiving gp100 peptide vaccine alone (Hodi *et al.* 2010). Other immunotherapies that have been approved by the FDA include recombinant cytokines like Proleukin (IL-2), monoclonal antibodies targeting cancer-associated proteins like Her2, EGFR, VEGF and CD20 (Mellman *et al.* 2011). The use of immune checkpoint inhibitors results in development of autoimmune reactions because of lack of specificity for the tumour cells (Attia

et al. 2005; Topalian *et al.* 2012). A study reported rare skin reactions, tumour mass liquefication with fatal outcomes, gastritis, aseptic meningitis and CNS inflammation as the immune-related adverse events (irAEs) in melanoma patients who received Ipilimumab (Voskens *et al.* 2013). Cytokine therapy like recombinant human IL-2 has also been associated with side effects like capillary leak syndrome (Weiss *et al.* 2011).

On the other hand, tumour-specific therapies like enriching tumour-infiltrating lymphocytes from patients with melanoma and adoptive transfer of the TILs after *in vitro* selection have shown objective response in 6 patients out of 13 (Dudley *et al.* 2002). Similar approaches along with multiple doses of aldesleukin (IL-2) showed interesting results with HPV-specific T-cells persistent in peripheral blood of cervical cancer patients for months (Stevanovic *et al.* 2015). Difficulty in obtaining autologous immunogenic tumour-specific lymphocytes in appropriate amount limits the use of this strategy. Hence, autologous T-cells expressing TCR or chimeric antigen receptor (CAR) are being exploited to improved efficacy of T-cell-based vaccines in clinical settings (Bonini and Mondino 2015). T-cells expressing NY-ESO-1-reactive T-cell receptor have shown survival benefits in patients with melanoma and synovial cell sarcoma (Zsiros *et al.* 2015). Employing allogenic T-cells is another approach but it poses a life-threatening risk for graft-versus-host disease (GVHD). Engineered T-cells expressing suicide genes like thymidine kinase from HSV are shown to control and resolve the GVHD in patients with hematological malignancies in allogenic settings (Ciceri *et al.* 2009). Allogeneic anti-CD19 CAR T-cells have been accounted to induce remission in 8 out of 20 patients with B-cell malignancies, and no case of acute GVHD was reported (Brudno *et al.* 2016).

Another immune system component that seems to be promising since the first published clinical report in 1995, are the dendritic cells (DCs) (Mukherji *et al.* 1995). DC-based vaccines aim to load DCs with tumour antigens *ex vivo* or *in vivo* followed by maturation of DCs that leads to their activation. Upon infusion into the patient, the activated DCs generate anti-tumour T-cell responses resulting from CD8+ effector T-cells.

4. DC biology

Dendritic cells are the immunological sentinels playing an important role in linking innate and adaptive immune response. DCs utilize phagocytosis, macropinocytosis or receptor-mediated uptake for internalizing antigens. Internalization of exogenous antigens by various pathways result in loading of tumour peptides to MHC Class II molecules; additionally, it can lead to cross-presentation of peptides to MHC I molecules by loading in endocytic compartments or TAP (transporter associated with antigen processing)-mediated transfer to endoplasmic reticulum. On the other hand, endogenous antigens are loaded on both MHC Class I and II molecules (Yewdell *et al.* 1999). Mature DCs express high levels of antigen presentation molecules (MHC I and MHC II) along with co-stimulatory molecules like B7-1/CD80, B7-2/CD86 leukocyte functional antigen (LFA-3/CD58) and intracellular adhesion molecule (ICAM-1/CD54), which facilitates interaction with lymphocytes and their stimulation. Maturation of DCs results in decreased capacity to uptake antigens accompanied by increased expression of MHC and co-stimulatory molecules (Stockwin *et al.* 2000). This highlights the tight regulation of display of cell surface markers by DCs during their life cycle.

Activation of DCs is followed by their migration to the lymphoid tissue where they interact with T-cells by virtue of high levels of surface MHC and co-stimulatory molecules. The outcome of the T-cell priming by DCs depends on the DC subtype; plasmacytoid-derived DC2 activates Th2 cells, whereas monocyte-derived DC1 activates Th1 cells when cultured in IL-3-supplemented media (Guermonprez *et al.* 2002). Ligation of CD40 on antigen presenting cells including DCs with CD40L on CD4+ T-cells is essential and adequate to prime CTLs (Schuurhuis *et al.* 2000). The CD8+ T-cells differentiated to CTLs generate secretory vesicles that, when released, cause lysis of neighbouring cells. The CD4+/CD8+ T-cells also differentiate into central memory and effector memory cells for proliferation and rapid effector function respectively (Pennock *et al.* 2013).

Activation of DCs results in the release of certain chemokines that attract new DC precursors and also activate NK cells. Activated NK cells are shown to kill immature DCs and help in inducing protective CD8+ T-cell responses (Mocikat *et al.* 2003; Ferlazzo and Munz 2004). Direct interaction between DCs and B-cells results in sending survival signal to B-cells that is CD40-dependent (Wykes and MacPherson 2000). Furthermore, DCs have also reported to induce B-cell proliferation and plasma cell differentiation through a B-cell activating factor, BAFF (O'Neill *et al.* 2004). Thus, with the multitude of ways to induce protective immunity, DCs offer the ideal candidate for cancer immunotherapy (figure 1).

5. Molecular mechanism of action of DC-based cancer vaccines

DC-based vaccines aim to load DCs with tumour antigens *ex vivo* or *in vivo* followed by maturation of DCs that leads to their activation. Upon infusion into the patient, the *ex vivo* mature DCs generate anti-tumour T-cell responses resulting from CD8+ effector T-cells (figure 2). Exogenous antigens, as discussed previously, prime CD8+ T-cells in addition to CD4+ T-cells by cross-presentation to MHC II. CTL

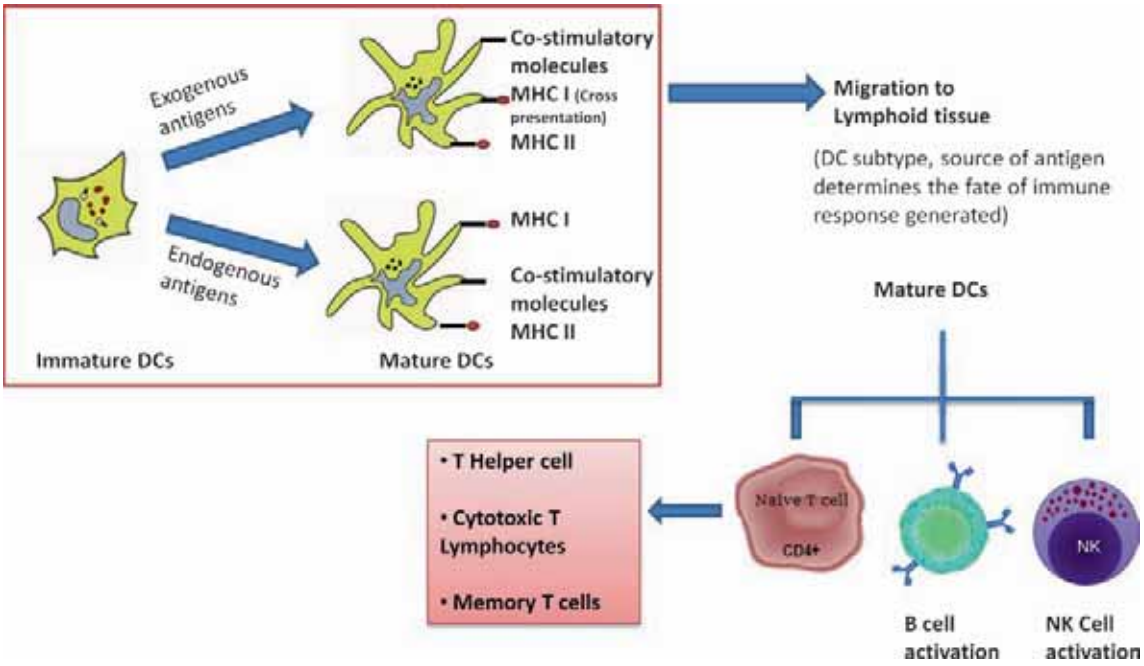

Figure 1. Immunobiology of DCs.

differentiation programme is initiated by naive CD8+ cells on encountering tumour-derived peptides presented by DCs. This is followed by expansion of T-cells, differentiation into memory CD8+ T-cells for generation of long-term T-cell responses and tumour-antigen-specific effector cells that secrete cytokines and mediate tumour cell lysis. CD4+ T-cells regulate the expansion of CTLs and induction of memory response. They also activate macrophages, further accentuating the overall anti-tumour response. Another important mechanism employed by T-cells for tumour cell lysis is, adherence of CD103 expressing CTLs to E-cadherin which leads to tumour rejection (Corthay *et al.* 2005).

Monocytes (CD14) and stem cells (CD34) are the cell types that can be used for generating and expanding DC population *ex vivo*. Generation of monocyte-derived DCs is a 7 day process, which involves culturing adherent population of PBMCs (peripheral blood mononuclear cells) in the presence of GM-CSF (granulocyte macrophage-colony stimulating factor) and IL-4, both of which are reported to differentiate CD14+ cells to a pure population of DCs (Stockwin *et al.* 2000). The non-adherent fraction of PBMCs (CD34+) is cultured for 12 days in the presence of TNF-α and GM-CSF to yield DCs. In some studies antibody-based separation technique is used for isolation of CD34+ cells (Syme and Gluck 2001). Comparative studies have suggested similar morphology, phenotypic characteristics, ability to uptake and present antigens of DCs derived from CD14+ and CD34+ cells. Additionally, both the DC populations exhibit comparable cytokine gene expression levels and produce equivalent CTL-induced interferon gamma (Triozzi and Aldrich 1997) (Siena *et al.* 1997; Ferlazzo *et al.* 1999). However, some studies reported significantly higher expression of co-stimulatory molecule B-7 in CD14+-derived DCs when compared to DCs manufactured from CD34+ cells.

Another critical component for the success of DC vaccine is the specific and efficient targeting of antigens to DCs for which *ex vivo* and *in situ* are the two major strategies being employed worldwide. The *ex vivo* approach involves loading of autologous DCs with antigens outside the host and *in situ* technique aims towards targeting DCs through DC-receptor-specific antibodies coupled with antigens/adjuvants. For, *ex vivo* loading of antigens, DCs can either be isolated directly or generated in the laboratory from precursors. Adoptive transfer of DCs require DCs to migrate to the lymph nodes after infusion; targeting DCs *in situ* will circumvent this problem (Kreutz *et al.* 2013). A critical finding from initial studies related to the *in situ* targeting of DCs was the importance of adjuvants to prevent the induced tolerance when antigens were targeted to DCs (Hawiger *et al.* 2001; Steinman and Banchereau 2007). Targeting DCs through DEC-205 receptor in the presence of adjuvants leads to induction of immunity against cancer and pathogens. Preliminary findings suggest disease stabilization in 13 out of 48 patients that received an *in situ* DC targeting vaccine (CDX-1401). CDX-1401 consists of tumour antigen NY-

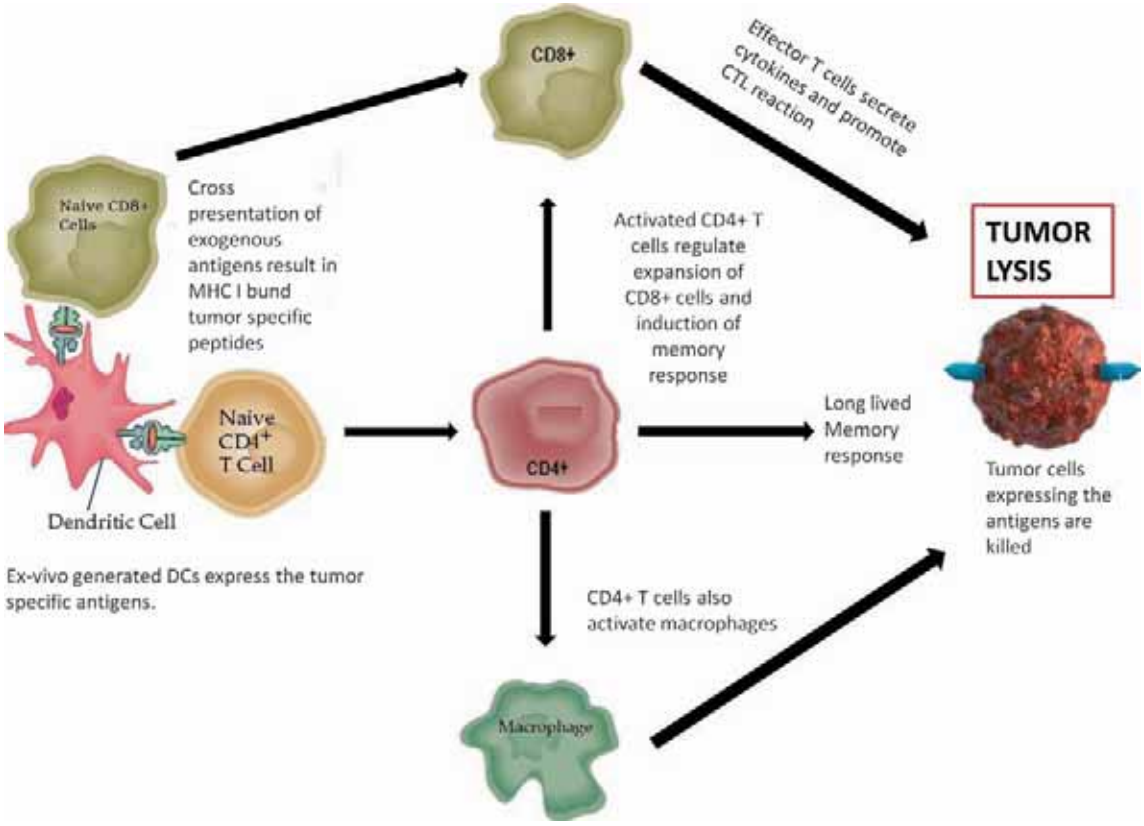

Figure 2. Molecular mechanism of DC-based cancer vaccines.

ESO-1 fused to anti-human DEC-205 antibody, which was administered along with adjuvants (TLR 7/8 agonist and TLR3 agonist) (Dhodapkar *et al.* 2014). Emerging approaches for *in situ* DC targeting includes using nanoparticles as the delivery vehicles for antigens and adjuvants, which have shown promising results in *in vitro* and *in vivo* studies.

6. DC-based cancer vaccines: *Ex vivo* approach

DCs are considered to be professional antigen presenting cells having the potential to activate B-cells, NK/NKT and T-cells, adding further to its anti-tumour potential. Since their discovery by Ralph Steinman, ways to utilize DCs in manipulation of the immune system to cure certain diseases like cancer has been the focus of research by various groups worldwide. After several pre-clinical and clinical trials, certain factors that determine the success of DC-based vaccines in cancer have been deciphered.

Immature DCs internalize antigens by phagocytosis, macropinocytosis and endocytosis. Uptake of foreign antigens and TLR signalling can subsequently result in DC maturation followed by migration to lymphoid organs (Banchereau and Steinman 1998). Several chemokine receptors like CXCR1, CCR1, CCR2, CCR5 and CCR6 are shown to be expressed by immature DCs, whereas maturation of DCs is characterized by altered expression levels of CCR6 and CCR7 (Dieu *et al.* 1998; Yanagihara *et al.* 1998; Sallusto *et al.* 1999). Maturation process further acquaints DC with the properties essential to present peptide-loaded MHC complexes to the cell surface and increased expression of co-stimulatory molecules which amplify T-cell receptor (TCR) signalling and support T-cell activation (Cella *et al.* 1997). Thus, maturation status of DCs in the vaccine is an important parameter to determine the migratory and T-cell stimulation properties of the DCs. To make use of the best anti-tumour potential of DCs, immature DCs are cultured with maturation stimuli following antigen uptake. A variety of factors can trigger maturation including double-stranded viral RNA, poly(I:C), bacterial-derived antigens (LPS, petidoglycan), ligation of certain cell surface receptors (CD40) and inflammatory cytokines (TNF-α, IL-1β, IFNγ) (Cella *et al.* 1999; Granucci *et al.* 1999; Bertho *et al.* 2005; He *et al.* 2007; Kim *et al.* 2008). Several studies have compared the efficiency of the different maturation stimuli alone and in combination. Rouas *et al.* (2004) showed that DCs matured using poly(I:C) retain their ability to secrete IL-12 in lymph nodes,

suggesting poly(I:C) as a low-cost and appropriate maturation stimuli for DC-based vaccines (Rouas *et al.* 2004).

While several clinical trials are ongoing in several institutions around the globe to use DC-based vaccines to induce anti-tumour immunity against various cancer types including ovarian cancer, prostate cancer, renal cell cancers, melanoma and glioma, it is important to understand which antigens or peptides will be most useful (Hirschowitz *et al.* 2004; Fay *et al.* 2006; Okada *et al.* 2011; Kandalaft *et al.* 2013; Bapsy *et al.* 2014; Prue *et al.* 2015). Summary of the first thousand patients who received dendritic cell vaccines by Ridgway in 2002 highlighted maximum number of trials to use peptide pulsed DCs (Ridgway 2003). Major challenges in developing peptide vaccines is the identification and selection of appropriate T-cell epitopes that are unique to the tumour to prevent development of immune tolerance and in a few cases autoimmunity. The peptide vaccines face a major limitation on the population coverage due to the MHC restriction. Therefore, there is a need to shift to personalized multiepitope vaccines where the source of tumour antigens can be whole tumour lysate, whole tumour RNA and apoptotic tumour cells. A meta-analysis of published immunotherapy trials by Neller *et al.* (2008) reported higher objective response rate in trials where whole tumour lysate was used as a source of antigen than trials utilizing defined tumour antigens (Neller *et al.* 2008).

Tumour cell vaccines can either use tumour antigens derived from patient's tumour sample or from established tumour cell lines. Using tissue biopsy sample from patients as a source of antigens offers advantage of having unique patient's specific tumour-associated antigens, but faces the limitation of availability of sufficient tumour sample from the patient.

7. Various *ex vivo* DC-based modalities tested clinically

Palucka and others summarized clinical trials testing *ex vivo* DCs-based vaccination for various cancer types including melanoma, glioma, renal cell carcinoma, multiple myeloma and colon cancer (Palucka and Banchereau 2012). A phase 1 study involving autologous DCs pulsed with tumour lysate in conjunction with tumour necrosis factor and keyhole limpet hemocyanin (KLH) showed tumour regression in 1 out of 10 liver cancer patients and with KLH-specific delayed-type hypersensitivity in 7 patients (Iwashita *et al.* 2003). *Ex vivo* loading of DCs with MAGE-3 peptide displayed promising results in a pilot study for bladder cancer (Nishiyama *et al.* 2001). A preliminary study involving HER-2/neu overexpressing ductal carcinoma *in situ* (DCIS) revealed tumour regression upon administration of DC vaccine targeting HER-2/neu. This offers a possible role of DC-based vaccine in control of development of invasive breast cancer (Sharma *et al.* 2012).

Several sponsoring companies are developing DC-based vaccines and running trials to check their clinical response. Here, we summarize few of such vaccine candidates for various cancer types (table 1).

7.1 *Ovarian cancer*

CVac™ manufactured by Prima Biomed consists of monocyte-derived dendritic cells loaded with mucin 1 protein, a protein which is abnormally expressed by many epithelial tumours including ovarian cancer. MUC1, VNTR recombinant protein is conjugated to oxidized mannan. CVac has shown to prolonged survival by 10.3 months in patients with ovarian cancer and has shown improved progression-free survival when given as maintenance therapy to patients with second clinical remission (Mitchell *et al.* 2014; Gray *et al.* 2016). Another DC-based vaccine utilizing the defined antigen platform is Vaccell by Tella, where DCs differentiated from patient's PBMCs are incubated with MHC-I restricted Wilms tumourgene 1 peptide antigens (WT1). The median survival time for 56 patients with recurrent ovarian cancer was reported to be 14.5 months after the vaccination (Kobayashi *et al.* 2014). Whole tumour lysate pulsed DCs are also being evaluated for ovarian cancer treatment by Sotio. The product DCVAC/OvCa is currently in Phase I/II study (*J. Clin. Oncol.* **32**:5s 2014).

7.2 *Prostate cancer*

Sipuleucel-T has been approved by FDA for treatment of asymptomatic or minimally symptomatic metastatic castrate-resistant (hormone refractory) prostate cancer. DC precursors are loaded *ex-vivo* with recombinant PAP (prostatic acid phosphatase) fused to GM-CSF (Small *et al.* 2006; So-Rosillo and Small 2006). DCVAC/PCa by Sotio is also a DC-based approach that utilizes killed PSA-positive prostate cancer cell line (LNCaP) and is under phase III clinical trial. DCVAC/PCa has shown to induce PSA-specific T-cell responses and down-regulation of Treg cells, thus improving the OS in patients (Podrazil *et al.* 2015).

7.3 *Glioblastoma*

DCVax-L manufactured by Northwest Biotherapeutics is an active autologous DC therapy for patients with glioblastoma utilizing whole tumour lysate prepared from tissue biopsy sample of the patient as the source of antigens. Newly diagnosed glioblastoma multiforme patients who received DCVax-L showed OS of 36 months that increased to 48 months for 33% of the patients. Further, benefits of DC therapy can vary for different sub groups of GBM patients. DCVax-L has entered phase III clinical trial with 312 subjects (Polyzoidis and Ashkan 2014).

7.4 *Non-small cell lung cancer*

Products that have entered phase II clinical trial for non-small cell lung cancer (NSCLC) are Vaccell and MelCancerVac

Table 1. List of *ex vivo* DC-based vaccine candidates for various cancer types

Name	Composition of DCs	Study size and efficiency	Reference
		Ovarian cancer	
Cvac	DCs targeting MUC-1 glycoprotein	N=29 Patients in second clinical remission showed overall survival benefit of 26 months	Gray *et al.* 2016
DCVac/OvCa	DCs pulsed with whole tumour lysate		*J. Clin. Oncol.* **32** 2014 (suppl; abstr TPS3134)
Vaccell	DCs pulsed with Wilms tumorgene 1 peptide	N=56 Median Survival time 14.5 months	Kobayashi *et al.* 2014
		Prostate cancer	
Provenge	DCs pulsed with recombinant PAP	N=82 Median survival time 25.9 months	Small *et al.* 2006
DCVac/Pca	DCs pulsed with killed tumour cells	N=25 Median Overall Survival 11.8 months	Podrazil *et al.* 2015
		Lung cancer (NSCLC)	
MelCancerVac	DCs pulsed with allogenic melanoma cell lysate	N=22 5 patients showed unexpected prolonged survival	Engell-Noerregaard *et al.* 2013
Vaccell	DCs pulsed with Wilms tumorgene 1 peptide		*http://www.sotio.com/clinical-trials/lung-cancer*
		Glioblastoma	
DCVax-L	DCs pulsed with whole tumour lysate	N=22 Mean Overall survival for recurrent GBM 9.5 to 35.9 months	Polyzoidis and Ashkan 2014
		Renal cell carcinoma	
AGS-003	DCs pulsed with whole tumour RNA	N=21 Median Oversall survival 30.2 months from patient registration	Amin *et al.* 2015
		Metastatic melanoma	
Eltrapundencel-T	DCs pulsed with autologous tumour cell line	N=20 95% Overall survival at 13.8 months	Dillman *et al.* 2004
		Pancreatic cancer	
Vaccell	DCs pulsed with Wilms tumorgene 1peptide	N=10 WT1 peptide specific immune response in 6 patients	Mayanagi *et al.* 2015
		Colorectal cancer	
MelCancerVac	DCs pulsed with allogenic melanoma cell lysate	N=20 Median overall progression-free survival was 2.4 months	Toh *et al.* 2009
		Solid malignancies	
APCEDEN	DCs pulsed with whole tumour lysate	N=38 ORR as per RECIST 28.9% Overall survival was 397 days	Bapsy *et al.* 2014

produced by Tella and Dandrit Biotech respectively. Vaccell comprises of DCs from patients pulsed with MHC-I restricted Wilms tumorgene 1 peptide antigens (WT1) (*http://www.sotio.com/clinical-trials/lung-cancer*). MelCancerVac preparation

includes DCs loaded with allogenic melanoma cell lysate containing antigens like MAGE-A, that is known to be expressed in about 40% of NSCLC cell lines. Phase I/II trial has indicated positive response among patients vaccinated with MelCancerVac and the product is under phase IIb trial (Engell-Noerregaard *et al.* 2013).

7.5 *Renal cell carcinoma*

AGS-003, a product from Argos Therapeutics, is prepared by *ex vivo* loading of DCs with RNA amplified from patient's tumour along with synthetic CD40L RNA. AGS-003 in combination with sunitinib resulted in clinical benefit in 62% of the patients, with OS of at least 4.5 years in 33% patients (Amin *et al.* 2015).

7.6 *Other cancer types*

Eltrapuldencel-T uses irradiated cells from autologous tumour cell lines as source of tumour antigens for patients with melanoma. Phase I/II trial results indicated 95% OS at a median follow-up of 13.8 months (Dillman *et al.* 2004). Vaccell by Tella, resulted in induction of anti-tumour immunity in pancreatic cancer patients with non-liver metastasis in a Pilot Phase I study (Mayanagi *et al.* 2015). Phase II study using MelCancerVac resulted in 40% clinical response rate in MAGE positive colorectal cancer patients (Toh *et al.* 2009). Autologous DCs loaded with whole tumour lysate followed by maturation of DCs using poly(I:C) constitute APCEDEN®, which has shown survival benefits of over 200 days and Objective response rate of 29% in advanced stage refractory solid malignancies (Bapsy *et al.* 2014).

8. Combinatorial approach to cancer treatment

Immunotherapy using monoclonal antibodies against tumours, immune checkpoint inhibitors and small molecules like TKIs impedes tumour growth and progression by interfering with oncosignalling and host immune responses. Whereas, active cancer immunotherapy including *ex-vivo* loading of immature DCs with defined or undefined tumour antigens augments the immune system capacity to fight cancer. Autologous DCs as vaccine candidates are safer and improve the quality of life of cancer patients compared with conventional chemotherapy and have earlier been used for cancers that progressed after chemotherapy. Thus, utilizing strengths of various kinds of immunotherapeutic approaches and conventional methods can help limit their individual weaknesses and improve the clinical outcome (summarized in figure 3).

Antigen presenting cells like DCs play an important role in developing anti-tumour immunity, the first step towards which is uptake of antigens. Cetuximab is a neutralizing antibody against EGFR and is being evaluated for treatment of pancreatic cancer (Krempien *et al.* 2005). Moreover, Cetuximab is reported to facilitate DC priming to activate anti-tumour response by enhancing expression of MHC-II molecules and co-stimulatory molecules (Vanneman and Dranoff 2012). Tumour cells when exposed to anti-cancer drugs like 5-Fluorouracil and Cetuximab showed increased tumour antigen expression and enhanced uptake of tumour cells by DCs (Correale *et al.* 2012). Thus, in generation of *ex vivo* DC vaccines, co-incubation with Cetuximab might be beneficial. The next process in generating anti-tumour immunity is recognizing and attacking tumour cells. Cytotoxic therapies like ionizing radiation and anthracyclines cause immunogenic cell death that leads to release of danger signals, thus facilitating recognition by immune cells (Zitvogel *et al.* 2011). Drugs like Bortezomib and Vemurafenib can sensitize the tumour cells for CD8+ mediated killing (Shi *et al.* 2008; Boni *et al.* 2010). Bortezomib is a proteasome inhibitor which when used in combination with a DNA vaccine against E7 antigen of *Human papillomavirus* has shown to increase apoptosis of tumour cells, making them more susceptible to specific immune responses (Tseng *et al.* 2008). HSP90 inhibitors are also reported to increase expression of NKG2D ligand in multiple myeloma and Hodgkin's lymphoma, thereby executing an anti-tumour effect (Boll *et al.* 2009; Fionda *et al.* 2009).

Therapeutic vaccines for cancer should be capable of inducing the memory response which would require a long-lasting activation of T-cells and their differentiation into memory T-cells. Clinically used as immunosuppressant, mTOR inhibitors have exhibited their T-cell stimulatory roles in mouse model of lymphocytic choriomeningitis viral infection (Araki *et al.* 2009, 2011). Additionally, inhibitor of WNT signalling pathway component GSK3β are also reported to differentiate T-cells into long-lasting memory like T-cells (Gattinoni *et al.* 2011).

An important step towards establishment of a successful T-cell response is the evasion of immunosuppressive microenvironment. *In vitro* and *in vivo* studies have highlighted the potential of chemotherapeutic drugs like cyclophosphamide, gemcitabine and 5-Fluorouracil to selectively kill myeloid-derived suppressor cells (Suzuki *et al.* 2005; Apetoh *et al.* 2011; Ghiringhelli and Apetoh 2015). Immune checkpoint inhibitors like anti-CTLA-4, anti-PD-1 and Tim-3 are well reported to release the brakes put on our immune system by restoration of the T-cell effector function (Bakacs *et al.* 2012). Administration of irradiated autologous tumour cells followed by anti-CTLA-4 antibody induced anti-tumour immunity with no toxicity in metastatic melanoma patients (Le *et al.* 2013). Use of CTLA-4 antibody in conjunction with DC-based vaccine exhibits convincing results

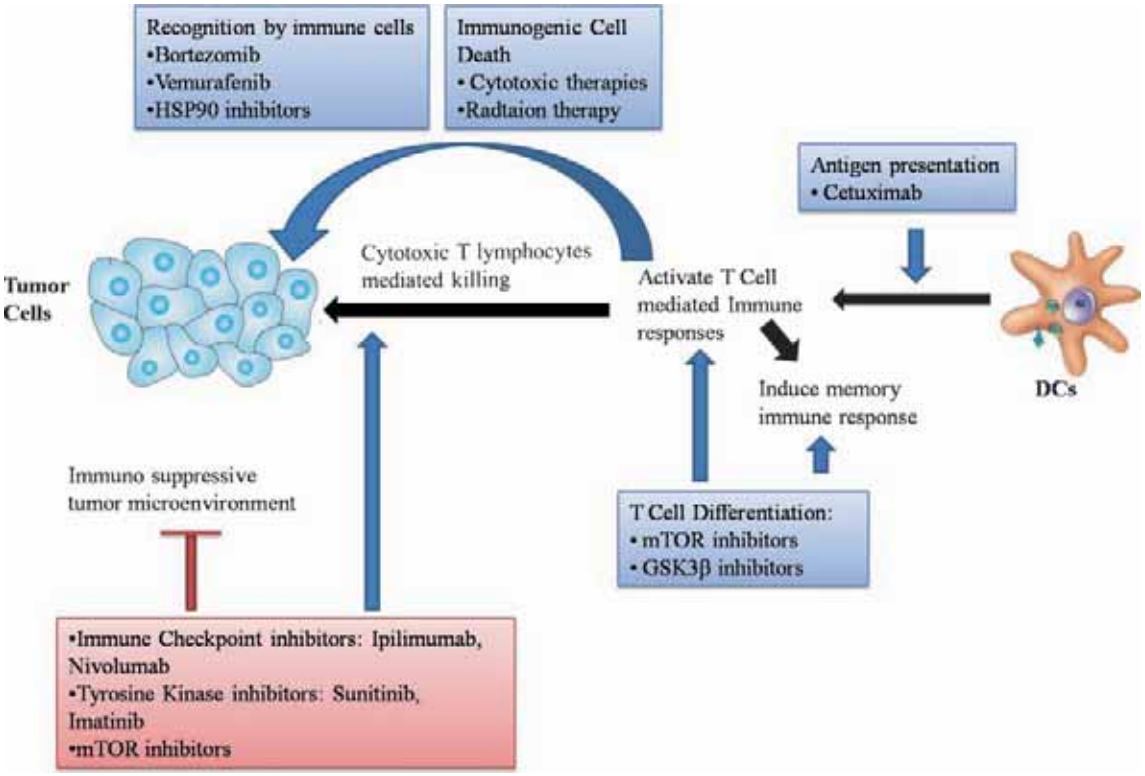

Figure 3. Combinational approach to cancer. The effector immune functions activated by DC-based vaccines (shown in black arrows) can be improved by other therapies that support different pathways (shown in blue arrows) leading to establishment of anti-tumour immunity.

in phase I clinical study in melanoma patients (Ribas *et al.* 2009). Further, tyrosine kinase inhibitors are increasingly being used in cancer treatment. Sunitinib targets VEGF receptor and has shown to suppress MDSC and Tregs population, thereby increasing IFNγ production in mouse models (Ozao-Choy *et al.* 2009). AGS-003, a DC-based vaccine in combination with Sunitinib, is under phase III trial in renal cell carcinoma patients (Amin *et al.* 2015). Imatinib, on other hand, is a tyrosine kinase inhibitor against c-kit and Abl kinases reported to down-regulate expression of indole-pyrrole 2,3-dioxygenase in myeloid cells and increasing intra-tumoral CTLs (Larmonier *et al.* 2008). Concomitant use of CTLA-4 antibody and Imatinib shows increased IFNγ production. Interestingly, monocyte-derived DCs when differentiated in presence of Imatinib results show dampened capacity to activate T-cells (Taieb *et al.* 2004; Appel *et al.* 2005). DCs are shown to secrete functionally active CTLA-4 that interferes with T-cell activation by binding to B7 co-stimulatory molecules; hence, DC vaccines can be used concomitantly with Ipilimumab for improved clinical results (Halpert *et al.* 2016).

Thus, with the availability of vast anti-cancer therapies, it becomes essential to optimally select the combinations with appropriate biomarker-based patient selection, dosage of administration of individual treatments along with management of adverse events.

9. Discussion

The most desirable therapeutic vaccine for cancer would be a personalized weapon targeting tumour cells specifically with no toxicity. DC-based vaccines offer positive clinical results in both personalized antigen loads like whole tumour lysate, whole tumour RNA as well as non-patient-specific antigen sources like recombinant proteins and lysate from tumour cell lines. Studies across the globe have shown variable benefits in clinical settings owing to different methods used for generation of DCs, maturation stimuli, antigen source and route of vaccine administration. DCs can be generated from either CD14+ monocytes supplemented with GM-CSF and IL-4/IL-13 or from CD34+ monocytes incubated with GM-CSF, Flt3-ligand and TNF-α. Loading of antigens *ex vivo* is followed by DC maturation that allows better migration of DCs to the lymph nodes. Intradermal/intranodal routes of administration are shown to be superior to the intravenous route (Mullins *et al.* 2003). Alternatively, DCs can be targeted and activated *in vivo* to avoid the labour-intensive protocol for *ex vivo* generation of DCs for all patients.

DCs expanded *in vivo* by Flt3-ligand are inactivated, leading to enhanced antigen uptake (Maraskovsky *et al.* 2000). An important consideration while evaluating efficacy of DC vaccine is the concept of pseudo-progression of the tumour. Activated DCs enhance the immune system's potential to fight tumours, which also leads to the infiltration of activated immune cells to the tumour site which is seen as increased tumour mass and categorized as disease progression by the response evaluation criteria in solid tumours (RECIST) criteria. Thus, immune0related response criteria (irRC) define better conditions to characterize the disease status after administration of activated DCs. DC vaccines must be assayed for induction of immune response and stability before administration and the patients must be followed-up for sufficient long time perioda to validate the development of memory T-cell response. Much needs to be standardized for improved efficacy and consistency of the DC-based vaccine products. Since cancer cells have the potential to induce suppressor immune cells and escape immune detection, immunological conditioning prior to or parallel to DC-based vaccines can increase the success rate. Conventional cancer treatment like few anticancer drugs and radiation therapy can enhance recognition of the tumour cells by the activated immune cells. Small molecules immunomodulators like tyrosine kinase inhibitors can aid in T-cell activation, memory response and down-regulating immune suppressive molecules. Thus, DCs in conjunction to other anti-cancer therapies needs to be explored for patients' improved clinical and symptomatic management.

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