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Turbocharging Vaccines: Emerging Adjuvants for Dendritic Cell Based Therapeutic Cancer Vaccines

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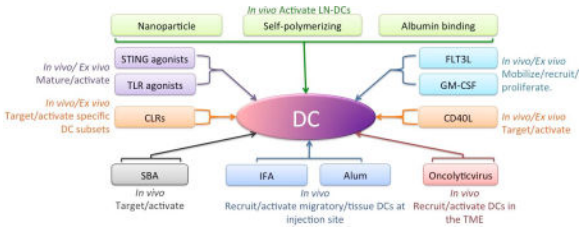
Abstract

Development of therapeutic cancer vaccines has been hindered by the many pro-tumorigenic mechanisms at play in cancer patients that serve to suppress both antigen presenting cells and T cells. In face of these obstacles, cancer vaccines are most likely to promote anti-tumorigenic immune responses only when formulated with strong adjuvants, and in combination with new immune interventions designed to reverse immune suppression and exhaustion of T cells in the tumor microenvironment. Dendritic cells (DCs) are often termed “nature’s adjuvant” due to their exceptional capacity for initiating both innate and adaptive immune responses. Hence, the past decade has witnessed a flurry of activity in testing DC based immunotherapies for cancer intervention. In this review we will discuss advances in conventional adjuvants and provide insight into new adjuvants as they pertain to DC cancer therapy.

Graphical Abstract: Role of Adjuvants in DC targeting



All adjuvants that play a role in carrying the vaccine cargo to the lymph nodes (LNs) such as nanoparticles, self-polymerizing platforms and albumin binding platforms are in essence targeting LN-DCs. On the other hand, oncolytic viruses recruit and activate intra-tumoral DCs. TLR and STING agonists serve to instigate and maintain DC activation *ex vivo* and *in vivo*. There are adjuvants that directly influence DC proliferation and recruitment such as, FLT3L and GM-CSF. C type lectin receptor (CLR) agonists, CD40Ls, and Saponin based adjuvants (SBAs) promote specific DC targeting and crosspresentation.

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Keywords

Cancer; Immunotherapy; Vaccine; Dendritic cells; Adjuvants

INTRODUCTION

Even armed with immunogenic tumor associated antigens (TAAs, BOX1), a successful cancer vaccine still requires a powerful adjuvant to meet the minimal criteria for engaging the immune system. Dendritic cells (DCs) are recognized for their unmatched capacity for activating both innate and adaptive immune pathways [1]. Moreover, DCs in general and $CD141^{+}$DCs in particular (Fig 1), are unique in their ability for processing exogenous tumor antigens through their cross-presentation pathway and facilitating activation of tumor specific $CD8^{+}$ cytotoxic T lymphocytes (CTLs) [2]. As a result, new cancer vaccine strategies (BOX2) consider adjuvants that activate cross-presenting DCs to yield maximum clinical success. In this review developments in conventional adjuvants and introduction of new adjuvant platforms over the past two years will be discussed with a special emphasis on how these adjuvants may be harnessed for maximizing DC activation towards cancer therapy.

NEW DEVELOPMENTS FOR CONVENTIONAL ADJUVANTS

A major obstacle in the way of vaccine induced anti-tumor immunity, is the plethora of immunosuppressive factors generated by the tumor [3]. Hence, choosing a suitable adjuvant is important as it can potentially override immunosuppression and allow the vaccine to maximize its therapeutic potential.

Aluminum Salts (Alum)

Alum, the first adjuvant to be used in human vaccines, is thought to function by adsorbing and then slowly releasing antigens *in vivo* to enhance the immune response. Moreover, alum has been reported to activate the inflammasome pathway particularly in DCs [4]. However, alum's predisposition for causing pro-tumorigenic Th2 differentiation makes it a questionable adjuvant for cancer immunotherapy. Interestingly, supplementing alum vaccines with other adjuvants and cytokines like Montanide (NCT00031733) and IL12 have been reported to elicit Th1 vaccine response [5]. Moreover, recently alum was demonstrated to improve vaccine efficacy through induction of IL17 secretion in mucosal innate lymphoid cells (ILCs) [6]. These studies suggest that alum plays a more complex role in shaping the immune response than previously believed and speak to the benefit of testing multiple adjuvants in combinations.

Incomplete Freund's Adjuvant (IFA)

Montanide adjuvants are an iteration of IFA, that function by forming depots to concentrate vaccines at the injection site and facilitate slow release of antigens to enhance antigenic uptake by antigen presenting cells (APCs) [7]. However, it is argued that vaccine depots may induce T cell depletion, especially when administered with small peptides [8]. Formulating Montanide vaccines with additional activating adjuvants and synthetic long peptides (SLPs)

avoids the loss of T cells and leads to generation of exceptional, clinically relevant Th1 polarized anti-tumor immune responses [8–10]. Multiple studies are ongoing to assess the benefit of using Montanide in combination with TLR agonists and standard chemotherapy (NCT02425306, NCT01079741, NCT02126579, NCT02293707, NCT02193347, NCT02795988) [11]. In addition, our group is conducting a trial to compare the efficacy of vaccinating with SLPs versus matured DCs loaded with TAAs, both in combination with Montanide and polyinosine-polycytidylic polylysine acid (PolyIC:LC) in melanoma patients. IFA may yet emerge as a strong and safe adjuvant specially when used along with Toll like receptor (TLR) ligands.

TLR agonists

TLR agonists activate and mature DCs and have the potential to reverse T cell anergy, thereby overcoming immune suppression [12,13]. Over the years TLR agonists have seen substantial improvements as discussed below.

Derivates and analogues of synthetic TLR3 ligand, PolyI:C, PolyIC:LC (Hiltonol) and Poly (I:C12C) (Ampligen), respectively, are widely used in clinical trials as adjuvants due to their capacity for DC maturation, interferon secretion, Th1 polarization and tumor suppression [14]. PolyI:C LC has also been used as an “autovaccination” platform when administered intra-tumorally to mimic a live viral infection yielding remarkable tumor regression [15].

TLR2 and 4 agonists play multiple roles in tumor suppression such as DC maturation and CTL activation. Indeed, TLR2/4 agonist, Picibanil (OK432) [16] and a non-toxic TLR4 agonist monophosphoryl lipid A [17] has been licensed for cancer therapy in Japan and the USA, respectively. Synthetic TLR4 ligand, glucopyranosyl lipid A, has displayed success in early clinical trials in eliciting Th1 polarized anti-tumor immunity (NCT02501473). Other mixed TLR2/4 ligands being evaluated for cancer therapy include OM-174 (NCT01800812) and IMM-101 (NCT01559818, NCT01303172).

TLR7 ligand Imiquimod expresses anti-tumor and anti-metastatic properties against multiple cancers [18] and is FDA approved for treating pre-cancerous skin lesions. TLR7/8 agonist Resiquimod is being (NCT02126579) tested in several trials alone and in combination with DC vaccines and chemotherapy, and has so far yielded a good safety and immunogenic profile [19].

TLR9 agonist, unmethylated CpG oligodinucleotide (CpG-ODN) used in peptide vaccines with other agonists such as Montanide [20] has been shown to boost anti-tumorigenic T cell responses. TLR9 agonists IMO-2055, CpG-28 and MGN1703 have been deemed safe and useful in phase I and II clinical trials in cancer patients [21] while DUK-CPG-001 (NCT02452697) and SD-101 are being studied in open trials in combination with chemotherapy and radiation therapy (NCT02927964 and NCT02521870) and anti-IL10 antibody MK-1966 (NCT02731742).

Recently tumor derived long non-coding RNAs have been shown to activate DCs, though the exact receptor remains unidentified [22]. Different DC subsets express different array of TLRs (Fig 1). Hence, choice of TLR ligand adjuvants is predicated on the subset of DCs

targeted. For instance, TLR9 is primarily expressed on B cells and plasmacytoid DCs (pDCs), while TLRs3 and 8 are expressed on all DCs. Thus, TLR3 and 8 agonists are more suitable adjuvants for activating a greater variety of DCs. However, if pDCs were the vaccine target then TLR9 agonists would be the preferred choice. Overall, inclusion of more than one TLR agonists, together or sequentially, may yield maximum DC engagement by mimicking the therapeutic effect of Coley's toxin [23] while avoiding any toxicity [24].

EMERGING VACCINE ADJUVANTS

Nanoparticles (NPs)

NPs help vaccines in accessing the lymph node (LN) in two ways. First, by enabling direct deposition of vaccines to the LNs (intra-nodal injections) and provoking antigen specific CTL responses and sustained DC activation [25]. Second, NP vaccines may arrive at the LN through lymphatic drainage. NPs with diameters ranging to 25–100nm such as interbilayer-crosslinked multilamellar vesicles (ICMVs) can convect to LNs from the injection site. As a result, ICMVs loaded with adjuvants and antigens are able to activate LN-DCs, promote cross-presentation and induce potent $CD8^+$T cell and humoral immunity [26,27]. Newer platforms incorporating biologics like; a) immune activating cytokines and growth factors (like IL12, IL2 and Granulocyte-macrophage colony-stimulating factor (GM-CSF)), b) neutralizing antibodies against immunosuppressive cytokines (like Transforming growth factor beta), c) factors that selectively target specific DC subset (anti CD141 and Clec9a antibodies or CD40 ligands (CD40L)) and stimulatory ligands (like TLR agonists), along with antigens will be able to utilize the remarkable properties of NPs towards cancer therapy.

Self-polymerizing scaffolds

TLR agonists are widely used to induce DC activation but their potency is prematurely lost through rapid dissolution and limited LN retention. Dr. Seder's group introduced a temperature sensitive biodegradable platform consisting of TLR agonists and peptide antigens linked to a polymer scaffold. These molecules remain water soluble at room temperature but upon *in vivo* administration, undergo temperature-dependent self-polymerization forming immunogenic particles that drain to the local LNs and successfully activate DCs [28]. Thermo-sensitive hydrogel loaded with LPS and TAAs (BOX1) have also been demonstrated to instill anti-tumor immunity in mice with tumors [29]. These platforms may achieve the "vaccine depot" effect while avoiding the long-lasting side effects observed with traditional emulsion adjuvants [8].

Albumin binding

Albumin is an abundantly expressed migratory protein in blood and lymph and an ideal carrier for delivering vaccines to the LN [30]. "Amph-vaccines" consist of adjuvants and TAAs engineered to display lipid tails with high affinity for serum albumin. Such amph-vaccines have been reported to safely enhance vaccine sequestering within the LNs thereby improving $CD8^+$T cell activation and impeding melanoma and cervical cancer progression in mice [31]. Moreover, amph-vaccine administered in combination with peptide antigen targeting antibodies, IL-2 and checkpoint inhibitor was shown to further enhance tumor regression and establish memory against tumor challenges in mice [32]. Further studies

extending these results to clinical trials will determine the benefits of amph-vaccines over other vaccine delivery platforms.

Oncolytic Viruses

Oncolytic virus therapy (virotherapy) activates DCs and T cells to induce an inflammatory milieu favorable for tumor regression [33]. Oncoviruses (BOX3) are naturally drawn to tumor cells or genetically engineered to enable enhanced tumor tropism, TAA expression and cytokine secretion [34]. However, a major problem with virotherapy is the risk of developing virus specific immunity that diminishes the anti-tumor immune response. A “prime boost” immunization strategy, sequential immunization with different strains of oncoviruses expressing the same TAA, has been demonstrated to negate the risk of generating “distracting” anti-viral immunity in mice [35] and is now being evaluated in clinical trials (NCT02285816). Attenuated herpes simplex virus (HSV) engineered to express human GM-CSF, Talimogene laherparepvec (TVec), is the first FDA approved virotherapy used in humans. TVec is administered intratumoraly and is found to be maximally effective in early stages of melanoma (NCT00769704) [36]. Currently, TVec is being evaluated in combination with checkpoint blockade drugs (NCT01740297). Overall, with data incoming from all the open trials (BOX3) it will be exciting to see how virotherapy may be leveraged for provoking DC mediated anti-tumor immunity.

STING agonists

Stimulator of interferon genes (STING) is a pattern recognition receptor that potently induces an interferon response upon activation. STING ligands are cytosolic double stranded DNA, host signaling second messenger molecule cGAMP and pathogen derived cyclic dinucleotide (CDNs) [37]. STING agonists, can effectively induce tumoricidal effects in mice [38,39]. The STINGVAX vaccine platform using GM-CSF secreting cells along with modified STING agonists was found to induce tumor specific immunity and cause regression of established tumors in mice formerly resistant to PD1 inhibition [40]. Methods to improve STING agonist adjuvancy such as generation of rationally designed synthetic agonists and encapsulation within NPs [41] are likely to make these stimuli more appealing for use in humans. Indeed, synthetic STING agonists such as ADU-S100 are being tested in patients with advanced solid tumors (NCT02675439). Moreover, DNA vaccines, such as VGX-3100 (BOX2), may have the inherent potential for activating STING as a means of immune activation [42].

Listeria monocytogenes (Lm) is a STING activator [43]. Attenuated Lm strains (att-Lms) promote antigen cross-presentation and anti-tumoral immune responses especially in combination with checkpoint inhibitors [44]. Furthermore, our results demonstrate that DCs primed with att-Lms regain their potential for Th1 differentiation [45]. Overall, att-Lms have the capacity to deliver TAAs and also act as adjuvants for boosting DC vaccines by neutralizing immunosuppressive factors (NCT02575807), recruiting DCs by expressing GM-CSF (NCT01417000) or inducing DC activation through TLR and STING signaling [45]. Moving forward it will be interesting to see how att-Lms compare with STINGVAX or STING agonists.

FLT3L

Fms related tyrosine kinase 3 ligand (FLT3L) is a pleiotropic cytokine required for DC proliferation. Recombinant human FLT3L (CDX-301) safely expands pDCs, $CD1c^+$ and $CD141^+$ DCs *in vivo* in healthy humans [46]. Furthermore, combination of checkpoint blockade with systemic FLT3L administration was shown to enhance proliferation of $CD103^+$DCs and induce tumor regression in mice [47]. A recently completed clinical trial studied the efficacy of combined vaccination with DC targeting vaccine (CDX-1401: DEC205-NY-ESO-1) and CDX-301 along with PolyIC:LC in patients with resected melanoma (NCT02129075). Results from the study show a higher tumor specific immunogenic response in patients receiving FLT3L (unpublished data). Other trials will parse the usefulness of FLT3L administration in combination with radiotherapy (NCT02839265) and adenoviral TAA expression (NCT01811992). So far, FLT3L therapy has not been clinically beneficial against advanced tumors alone or in combination with vaccines. However, when injected intratumorally with polyIC:LC (NCT01976585) it has shown clinical efficacy together with radiation in low grade B cell lymphomas (personal communication with Dr. Brody).

GM-CSF

GM-CSF is a cytokine that supports DCs differentiation and aids in tumor rejection [48]. Multiple vaccine platforms include GM-CSF in their formulations. GVAX is a vaccine platform comprising of irradiated allogeneic or autologous tumor cells engineered to secrete GM-CSF which can illicit T cell immunity against TAAs to improve median survival in patients [49]. The concept of leveraging cancer stem cells for the formulation of GVAX platform has been validated in murine models of breast cancer [50]. GM-CSF secreting, STINGVAX, has proven to be a promising anti-tumorigenic vaccine in murine studies [40]. Furthermore, GM-CSF inclusion in vaccines can enhance melanoma homing markers on $CD8^+$T cells [51]. Altogether these studies underscore the promising nature of optimized GM-CSF platforms for mobilizing not just DCs but also other immune cells to enhance anti-tumor immunity.

C type Lectin Receptors (CLRs)

Different DC subsets can be distinguished by expression of specific CLRs (e.g. Clec9a is expressed by $CD141^+$DCs (Fig 1)). Endocytic antigen uptake through CLRs like DEC-205, has been shown to induce cross-presentation. Thus, CLR ligands can be exploited for delivering antigens to desirable DC subsets and promoting cross-presentation of these antigens to induce $CD8^+$ T cell immunity. CDX-1401, a vaccine comprising of DEC-205 fused with tumor antigen NY-ESO-1, has been proven therapeutic and safe against advanced malignancies [52] and is being evaluated for use in patients (NCT02166905, NCT01834248 and NCT02129075). A vaccine by Celldex targeting mannose receptors (MRs) on DCs called, CD1307, in combination with TLR agonists has yielded promising results in a phase I clinical trial with patients afflicted with advanced solid tumors [53]. In murine models, Clec9a antibodies have been shown to induce robust T cell, antibody response and tumor regression in mice [54]. Thus, strategies targeting CLRs and MRs on DCs are emerging as a

promising approach for *in vivo* DC vaccination and T cell activation especially in combination with checkpoint inhibitors.

CD40L

CD40 engagement is a requirement for DC activation. Pre-clinical data indicates the feasibility of using CD40L or antibodies to CD40 for eliciting DC and macrophage mediated cytokine secretion, antigen processing, tumor stroma destruction and T cell activation against established tumors [55]. Studies in non-human primates have also indicated the efficacy of systemically administered CD40 antibody with PolyIC:LC in inducing DC activation and LN homing accompanied by T cell activation [56]. Several vaccine strategies have been designed to leverage CD40-mediated DC activation in humans, including vaccinating with; tumor cell lines modulated to over-express CD40L (NCT00458679, NCT02719015, NCT02466568), anti-CD40 antibody (NCT02376699, NCT02482168, NCT01103635) and recombinant CD40L (NCT00001145). Along with co-stimulation, CD40L is also being evaluated for maturing and activating DCs *ex vivo* (NCT00053391). Overall, it may be too early to predict how CD40Ls or antibodies will fare as adjuvants in humans, but the promising results from murine and early human studies [57] suggest that these may improve DC activation and over all tumor immunity of cancer vaccines.

Saponin Based Adjuvants (SBAs)

Saponins are plant-derived glycosides that form stable Immune stimulating complexes (ISCOMs) along with cholesterol and phospholipids. The ISCOM Matrix Adjuvants (ISCOMATRIX) directly deliver their cargo to the DCs enabling antigen cross-presentation, particularly in $CD11c^+$ DCs [58]. Moreover, the SBA QS-21, was demonstrated to activate inflammasome-signaling [59]. SBAs formulated with NY-ESO-1 administered prior to immunization with fowlpox virus expressing NY-ESO-1, in a prime boost strategy, induced antibody and T cell responses in melanoma patients [60], but failed to show clinical efficacy. Co-administration of regulatory T cell (Treg) depleting agent, cyclophosphamide, with NY-ESO-1-ISCOMATRIX vaccine significantly improved the induction of $CD4^+$T cell responses indicating that multiple treatment modalities are necessary for SBAs to achieve clinically relevant results [61]. In essence, SBAs are a safe candidate for targeting DCs *in vivo* for tumor intervention and are being explored for their anti-tumorigenic potency in combination with novel platforms such as NPs, oncoviruses and chemotherapy agents.

The best vaccine approach would involve mobilizing DCs with FLT3L followed by vaccinating with peptide/RNA/DNA/DC vaccine using a platform that includes DC activating factors, facilitates LN homing and enables slow antigen release. Alternatively, following FLT3 mobilization, virotherapy or att-Lms maybe administered to potentiate tumor cell targeting. Moreover, prime boost vaccine regimens using oncolytic viruses [35] or DNA vaccines followed by viral vectors [62] are yet another promising example of how multiple vaccine modalities and adjuvants could be used in synchrony to promote anti-tumor immunogenicity. Either way, the end goal would be to enhance antigen presentation on DCs in the LN to promote tumor specific CD8 and $CD4^+$ T cell activation and expansion.

CONCLUSION

"DC targeting tumor vaccine" is an oxymoron as all tumor vaccines either directly or indirectly target DCs (Graphical abstract). An ideal cancer vaccine should selectively mount an immune response against tumor cells, achieve complete regression and generate memory cells to prevent any relapses. However, vaccine trials to date have failed to match these standards. Several factors might account for this. First, most trials have been conducted in patients with advanced tumors hampered by high tumor burden and associated immune suppression. Second, the right combinations of adjuvants and immune modulators that complement each vaccination platform have yet to be optimized. Third, immunosuppressive cells such as Tregs and myeloid derived suppressive cells (MDSCs) tend to accumulate with tumor progression facilitating tumor escape and T cell exhaustion. Hence, it seems that there is a loosely defined, narrow window of opportunity, after tumor onset but before significant progression, where therapeutic tumor vaccines may yield maximum benefit [36]. Chemotherapy and immunotherapeutics that actively neutralize specific immune-suppressive factions such as Tregs (cyclophosphamide), T cell anergy (checkpoint inhibitors), suppressive cytokines (neutralizing antibodies) and MDSCs (signaling inhibitors) [63,64] need to be co-administered with cancer vaccines to unleash the immune components and allow the vaccines to engage the entire arsenal of immune cells to overcome the tumor [32].

The goal of cancer vaccines is to foster mass immunity against any and all tumorigenisis. The only widely available preventive vaccines protect against cancers caused by infectious agents and rely on the microbial nature of the pathogens to instill protection. Vaccinating against non-infectious tumors is entirely reliant on the availability of early tumor biomarkers. Several such antigens have been suggested but have yet to fare through clinical trials [65]. In the future, powered by new technologies, early and unique markers of tumorigenisis will be discovered and together with existing therapies shall enable preventive cancer vaccination.

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BOX1

Tumor Associated Antigens (TAAs)

TAAs are antigens expressed largely, if not solely, by tumorigenic cells. Such antigens are the bedrock of cancer immunotherapy and are broadly classified into different categories based on their origin and distribution.

Cancer testis (CT) antigens

CT antigens are normally restricted to germ line cells (in testis and placenta) but are aberrantly over expressed in several tumors. In fact it has been proposed that epigenetic dysregulation in CT antigen expression program may be an underlying factor for tumorigenisis. CT antigens like NY-ESO-1 are being fervently pursued as candidates for peptide, cell based, RNA and DNA vaccines alone and in combination with virotherapy and standard chemotherapy [68].

Oncoviral Antigens

These are antigens expressed by viruses, like human papilloma virus (HPV) and Merkel cell polyomavirus that cause tumorigenic transformation in cells. As these antigens are only found on the infected cells they are recognized by the immune system as “non-self” [9].

Neoantigens

These are unique MHC restricted antigens created by mutations in tumor cells. Vaccines designed to target these antigens should theoretically be able to specifically target tumor cells while avoiding general autoimmunity or tolerance. However, not all tumors express immunogenic neoantigens. Moreover, tumors and patients have unique neoantigen repertoires necessitating personalized neoantigen discovery platforms that enable personalized vaccines against predicted neoantigen epitopes [69].

Overexpressed Antigens

Overexpression antigens is an umbrella term that covers antigens that are present in both normal and tumor cells but are substantially over expressed in tumor cells. These include antigens such as Her2/Neu [70], mesothelin [71], lineage and tissue restricted differentiation antigens such as melanoma differentiation antigens (Tyrosinase Related Protein-2 and Melan-A (MART-1)) and Oncofetal antigens (Carcinoembryonic antigen) [72].

BOX2

Current vaccine platforms

DC based vaccines

Multiple platforms are being developed to harness DC vaccines for cancer immunotherapy. These platforms include the DCVax-Direct and DCVaxL wherein, *ex vivo* activated DCs are administered with or without antigen loading, respectively. Similarly, Individualized Vaccines Against Cancer (IVAC) platform aims at using autologous DCs loaded with individually sequenced neo-antigens (NCT02035956, NCT02316457). Moreover, multiple efforts are being made to optimize DC yield for use in vaccines. These efforts include, use of natural DCs (blood isolated DCs) (NCT01690377) and generating cross presenting $XCR1^{+}Clec9a^{+}$ DCs [73] directly from stem cells. Novel approaches such as mRNA transfection [74] and lentiviral transduction to promote favorable DC phenotype, function and antigen presentation [75] are currently underway.

RNA vaccines

A novel approach pioneered by Sahin group uses lipid based positively charged nanoparticles to deliver RNA, encoding TAA, to target DCs *in vivo* and simulate an anti-viral response concomitantly. This is currently being tested in a phase 1 trial, in melanoma patients (NCT02410733). Two component RNA vaccine platforms launched by Curevac have also yielded promising results in early clinical trials in cancer patients (NCT00923312) [76].

DNA vaccines

Developed by Vaccibody, VB10.16 is a DNA based vaccine targeting HPV16. The clinical trial (NCT02529930) is set to launch and if successful stands to provide a novel and much needed non-invasive option for treating HPV caused cervical cancers. Trials have been scheduled to determine efficacy of combination vaccine designed by Inovio Pharmaceuticals called INO-3112, It comprises of synthetic plasmids encoding E6 and E7 (VGX-3100) [77] in combination with DNA based IL12 delivery (INO-9012), against cervical and head and neck cancers (NCT02172911, NCT02163057). Inovio's preventive anti-HIV DNA vaccine, PENNVAX-G, used for "priming" followed by "boosting" with modified pox viral vector has yielded a good safety and immunogenic profile [62]. This study has laid the groundwork for exploring the prime-boost regimen using DNA vaccines and viral boosts for designing cancer therapy vaccines.

BOX3

Oncolytic virus: Clinical and pre-clinical studies

Several onco-viruses have been engineered to express factors that activate and recruit DCs to the tumor microenvironment (TME). These viruses include GM-CSF expressing adenovirus ONCOS-102 (NCT01598129), IL12 secreting attenuated vaccinia virus JX-594 (NCT00554372); IL12 expressing Maraba Virus [78] and adenovirus expressing CD40L [79].

In addition, attenuated oncolytic Edmonston strain of measles virus, encoding the human thyroidal sodium iodide symporter, (MV-NIS) is being investigated as a therapy against multiple cancers (NCT02919449, NCT01846091, NCT02962167, NCT02364713, NCT02700230, NCT01503177, NCT02192775, NCT00450814, NCT02068794) [80].

Oncolytic Coxsackie virus strain A21, Cavatak, made by Viralytics is now being evaluated in conjuction with checkpoint inhibitor, Pembrolizumab, in patients with head and neck cancer (NCT028249650) and melanoma (NCT02565992).

Modified oncolytic New Castle Disease Virus (NDV) has been demonstrated to successfully generate immunity against tumor antigens, lift resistance to checkpoint inhibitors and achieve tumor regression in preclinical models of cancer [81]. Although there are no open trials for NDV available presently, multiple clinical trials in the past have evaluated the use of NDV based therapies and over all concluded that NDV is a safe and promising option for cancer therapy.

The Bergmann group recently demonstrated the therapeutic potential of oncolytic influenza virus that expresses IL15 in retarding the growth of established tumors in mice [82]. Vacthera is developing a modified oncolytic strain of influenza, OncoFluVec, as tumor virotherapy. Future clinical trials are awaited to determine the viability of using flu virus for treating cancers.

HIGHLIGHTS

- Antigen presentation by Dendritic cells to both CD4 and $CD8^+$ T cells is the cornerstone of successful vaccines
- Vaccine adjuvants are critical for overcoming cancer related immunosuppression
- All adjuvants target DCs directly or indirectly
- Advances in conventional adjuvants and advent of new adjuvants for cancer therapy
- Formulating multiple adjuvants in one vaccine platform and combination with checkpoint blockade immunotherapy

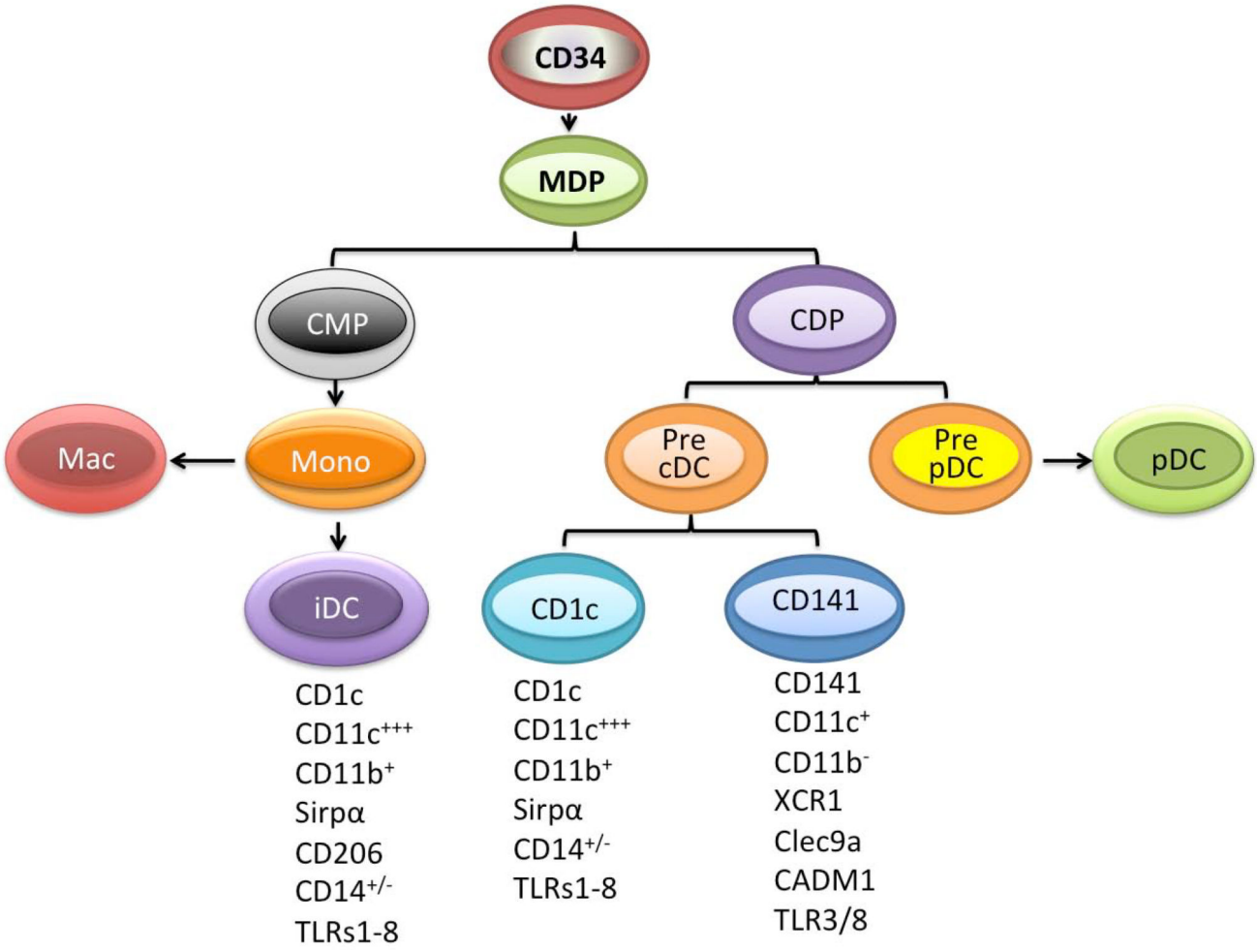

Fig 1. Current markers for selecting human DC subsets for research

Both lymph node resident DCs (LN-DCs) and migratory DCs stem from $CD34^+$ stem cells. $CD34^+$ cells differentiate into monocyte and dendritic cell progenitors (MDPs), which in turn bifurcate into committed monocyte or DC progenitors CMPs and CDPs, respectively. CDPs eventually differentiate into two broadly classified DC populations, namely conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Human cDCs are divided into $CD1c^+$ ($BDCA1^+$) and $CD141^+$ ($BDCA3^+$) DCs. The CD1c DCs express high CD11c and CD172α (Sirpα) where as the $CD141^+$ DCs express low CD11c, but high XCR1 and Clec9a. Apart from phenotypic distinctions, functional differences between $CD1c^+$ and $CD141^+$ DCs have been described. For instance, $CD1c^+$ DCs represent the majority of migrating DC proportion and express a wide range of TLRs (TLR1–8). On the other hand $CD141^+$ DCs comprise of a much smaller proportion of migrating DCs and only express TLR3 and TLR8. In addition, an inflammatory DCs (iDC) subset is reported that arises from monocytes in blood. The iDCs are close in phenotype to CD1c DCs and share most phenotypic markers with the exception of CD206. While it is recognized that all DC subsets may perform cross-presentation the lymph node resident $CD8^+$ DCs in mice and $CD141^+$

cDCs in humans are remarkably more efficient in this process and consequently capable of generating superior anti-tumor immunity [1,66,67].