HHS Public Access

Author manuscript

Immunol Lett. Author manuscript; available in PMC 2015 November 01.

Published in final edited form as:

Immunol Lett. 2014 November ; 162(1 0 0): 59–67. doi:10.1016/j.imlet.2014.07.004.

Targeting human dendritic cells *in situ* to improve vaccines

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Abstract

Dendritic cells (DCs) provide a critical link between innate and adaptive immunity. The potent antigen presenting properties of DCs makes them a valuable target for the delivery of immunogenic cargo. Recent clinical studies describing *in situ* DC targeting with antibodymediated targeting of DC receptor through DEC-205 provide new opportunities for the clinical application of DC-targeted vaccines. Further advances with nanoparticle vectors which can encapsulate antigens and adjuvants within the same compartment and be targeted against diverse DC subsets also represent an attractive strategy for targeting DCs. This review provides a brief summary of the rationale behind targeting dendritic cells in situ, the existing pre-clinical and clinical data on these vaccines and challenges faced by the next generation DC-targeted vaccines.

Keywords

Dendritic cell; targeted vaccines; nanoparticles

1. Introduction

Vaccines represent one of the major success stories of modern medicine [1]. However in spite of considerable effort, it has proven harder to develop effective vaccines against certain pathogens (such as human immune deficiency virus and tuberculosis), and chronic diseases (such as cancer) wherein strong cell-mediated immunity is desired [2-4]. The major goal of vaccination against these conditions is generation of high avidity antigen-specific CD8+ T cells capable of cytotoxic T lymphocyte (CTL) response and generation of long-lived memory cells [4,5].

Dendritic cells (DCs) are specialized antigen-presenting cells (APCs) that play a central role in initiating and regulating immunity [6]. DCs efficiently capture both foreign and self-antigens from the environment and process and present them to T cells [6]. They induce differential immune responses according to the accompanying stimulus and thus regulate development of immunity or tolerance [7,8]. Owing to their potent antigen presentation

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capacity and ability to generate distinct T cell responses, they have received particular attention in the field of immunotherapy.

2. Dendritic cells as potent antigen presenting cells

Dendritic cell regulate innate as well as acquired immunity and serve as a bridge between these two arms. They possess intrinsic specialized features which make them particularly efficient to capture, process and present antigens [9]. Firstly, DCs are present at the self-environment intersection (i.e. skin and mucosal surfaces) and hence strategically located to encounter pathogens and other foreign material. Secondly, they have specialized uptake receptors and downstream endocytic system for antigen processing and presentation (classical MHC molecules I and II for presentation of peptides, and CD1d system for presentation of lipid antigens). The specialized surface or intracellular receptors, called pattern recognition receptors (PRRs), include C-lectin type receptors (CLRs), Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-1 like receptors (RLRs) and helicases [7,10,11]. Thirdly, they undergo a process called maturation on exposure to a wide range of stimuli or ‘danger signals’ (bacterial lipopolysaccharide, viral nucleic acids etc.) which are recognized by TLRs, NLRs and RLRs. It is now well appreciated that vaccine adjuvants act by inducing DC maturation, which improves antigen processing and presentation [9]. Several TLR agonists [Poly I:C (TLR3 agonist), MPLA (TLR4 agonist), CpG ODN (TLR9 agonist) and Resiquimod/ R848 (TLR7/8 agonist)] have thus been administered along with vaccines to deliver concomitant DC activation signals. Lastly, they comprise of multiple subsets with distinct location, phenotype and function, and differential expression of specialized receptors [12,13]. These receptors can be used to target specific subsets through incorporation of monoclonal antibodies in the vaccines [14,15]. These subsets respond uniquely to different stimuli and thus contribute to the generation of a broad spectrum of immune responses.

3. Diversity and biology of human dendritic cell subsets

Human dendritic cells have been typically divided into blood and cutaneous subsets for classification purposes, largely because these compartments are easier to study in humans. Blood DCs are further sub-classified into three categories- BDCA2 (CD303)+ plasmacytoid, BDCA1 (CD1c)+ myeloid and BDCA3 (CD141)+ myeloid DCs [16-19]. Cutaneous DCs comprise of epidermal (Langerhans cells) and dermal (CD14+ DCs and CD1a+ myeloid) DCs [16]. Another distinct category, inflammatory DCs are putatively derived from monocytes unlike the above mentioned DC subsets which are derived from bone marrow precursors [16,20]. These inflammatory DCs have distinct functions, dependent upon the inflammatory environment [16,21]. The properties of different DC subsets have been succinctly described in reviews [3,16,22,23], with some key features described below and in Table 1.

Myeloid DCs (MDCs) are the major antigen-presenting cells. Out of the BDCA1+ and BDCA3+ MDCs, the latter constitutes a minor, yet significant subset with superior cross antigen-presentation capacity [24-27]. Plasmacytoid dendritic cells (PDCs), on the other hand, secrete large amounts of interferon-alpha on exposure to viruses [28,29] as well as

maintain tolerance against self-antigens [30,31]. This may explain why their dysfunction has been linked to the pathogenesis of autoimmune conditions such as systemic lupus erythematosus and immune thrombocytopenic purpura [32,33]. Langerhans cells (LCs) display a striking duality of function. They can prime T cell immunity as well as induce regulatory and IL-22 secreting T cells [34-36]. Therefore the role of LCs has evolved in recent years to include their tolerogenic function and broader roles in epithelial homeostasis [16]. Dermal CD14+ DCs, on the other hand, primarily stimulate humoral immunity [34,36-38].

4. Human versus mouse dendritic cell subsets

The human counterparts for the two most studied mouse DC subsets - CD8α+ and CD8α- DCs are BDCA3+ MDCs [26,27] and BDCA1+ MDCs respectively. Plasmacytoid DCs, on the other hand, are shared by both human and murine immune system. Although the majority of TLRs and CLRs on the major DC subsets are common in both human and mouse counterparts, clear differences exist. TLR9 which is found in all murine major DC subsets, is expressed only by PDCs in humans [12]. Other examples include CLRs DC-SIGN [39] and DC-ASPGR [40] whose biology differ between murine and human DCs.

The major murine DC subsets - CD8α+/DEC205+ and CD8α-/DCIR+ DCs show a remarkable division of labor in terms of their predominant response. While CD8α+ DCs efficiently cross-prime CD8+ T cell immunity through MHC class I antigen presentation [41], CD8α- DCs stimulate predominant CD4+ T cell response through MHC class II presentation [42]. This has been explained partly by some inherent characteristics of CD8α+ DCs- high endosomal pH, low antigen degradation, high antigen export to cytosol and more pre-synthesized stores of MHC class I molecules [22,43]. In humans, BDCA3+ MDCs were initially described to have superior cross-presentation capability than other DC subsets, however the cross-presentation capacity is not restricted to this subset [27]. Chatterjee et al found that cross-presentation capacity of human DCs was highly influenced by antigen delivery and whether antigens were delivered to early or late endosomes [44]. While late endosomal delivery through DEC205 maintained the superiority of BDCA3+ MDCs over BDCA1+ MDCs, this was eliminated on antigen delivery to early endosomes through CD40 or CD11c [45]. Another study showed all freshly isolated tonsilresident DC subsets – BDCA1+ MDCs, BDCA3+ MDCs and PDCs- possessed similar antigen cross-presentation capacity [46]. All three DC subsets could export proteins into cytosol efficiently and both BDCA1+ and BDCA3+ MDCs displayed similar phagosomal pH and production of reactive oxygen species. These findings are supported by numerous other studies where other DC subsets – PDCs [47-49], BDCA1+ MDCs [26,50], Langerhans cells [34,35] and CD1a+ DCs in skin-draining lymph nodes [51] cross-primed efficient CD8+ T cell immunity in culture.

5. Ex vivo as opposed to in situ dendritic cell targeting

Efficient and specific delivery of antigens to dendritic cells is the cornerstone for generating strong immune responses. Two major strategies have been utilized to engage dendritic cells [52]. The first approach involves ex vivo loading of autologous DCs with antigens/adjuvants and re-injecting them into patients while the second one targets DCs in situ through vaccine

conjugated to DC receptor-specific monoclonal antibodies. Most studies to date have focused on adoptive transfer of DCs and found it to be safe and immunogenic [52-54]. Two broad approaches have been tried, injection of naturally occurring DCs, or differentiation of DCs from progenitors ex vivo, before adoptive transfer. Adoptive transfer of naturally occurring DCs is best exemplified by Sipuleucel-T therapy, which involves isolation and ex-vivo culture of patient's APCs with prostatic acid phosphatase (PAP) and GM-CSF fusion protein. GM-CSF is added in addition to PAP antigen to promote activation of APCs, manifest as increased expression of HLA class II, co-stimulatory molecules and secretion of cytokines [55]. A large, randomized, double-blind, placebo-controlled phase III trial (IMPACT study) showed a median survival benefit of 4.1 months following Sipuleucel-T, leading to FDA approval for treatment of asymptomatic or minimally symptomatic patients with metastatic prostate cancer [56]. Ex-vivo vaccines were also tested in melanoma using patients' plasmacytoid dendritic cells loaded with tumor antigen-associated peptides. Specific CD4+ and CD8+ T cells were generated in addition to a much desirable interferon signature [57]. However, unlike the above two studies which employed naturally occurring DCs, majority of the work with ex-vivo DC vaccines utilized monocyte-derived dendritic cells (Mo-DCs), which are not physiological DCs. These studies included treatment of patients with melanoma [58], breast [59] and ovarian cancer [60] and HIV infection [61,62], as well generation of tolerogenic response in autoimmune conditions such as rheumatoid arthritis and multiple sclerosis [63,64]. Of note, decrease in HIV viral load was reported in two studies after injection of DCs loaded ex vivo with chemically inactivated autologous virus [61,62]. A summary of clinical trials using ex vivo DC vaccines is provided in a recent review [52]. Widespread application of adoptive DC transfer has been limited by cost, labor requirements and technical complexity of the procedure [13,65]. Targeting dendritic cells in situ will circumvent these problems and provide readily available off-the-shelf products. Moreover, after ex vivo injections, DCs need to migrate to lymph nodes, while in case of in situ targeting, vaccines can be directly targeted to desired DC subsets present in desired locations [12].

6. Antibody-based targeting – lessons learnt from preclinical and early clinical studies targeting DEC205

The pioneering studies in the field of in situ DC targeting by Steinman and Nussenzweig laboratories through anti-DEC205 antibody constructs laid the groundwork for the clinical development of these vaccines [15,66,67]. A critical finding was the generation of tolerance when antigens were targeted to steady state DCs [15]. Application of adjuvants along with targeted vaccines, however, led to the generation of protective antigen-specific cellular immunity. This led to further testing of vaccines targeting DCs via antibodies to generate protective and therapeutic immunity against chronic infections and cancer (Table 2) [9].

Both these principles have been evaluated in numerous preclinical studies. Targeting DCs through DEC205 in the presence of adjuvants (TLR3, TLR7/8 or CD40 ligands) led to protective immunity against pathogens (HIV [68-70], tuberculosis and dengue [71]) and cancer. On the other hand, DEC205 targeting in the absence of DC activators leads to tolerance in experimental models of type 1 diabetes mellitus and experimental allergic

encephalomyelitis [72-74]. Similarly, targeting through other DC surface receptors such as DC-SIGN [75], CLEC9A [69,76], DCIR [35,77], Dectin-1 [78] and Langerin [69,79] along with adjuvants stimulated integrated humoral and cellular immune responses.

It is important to note the plasticity of DC subsets which are capable of generating differential immune responses when targeted through different DC receptors [42,67]. In one study, targeting human DCs in the absence of adjuvant through DC-ASPGR led to the generation of IL-10 producing suppressive CD4+ T cells, while targeting through LOX-1 led to stimulation of IFN-γ producing CD4+ T cells [40]. In another study, where vaccines were targeted to both conventional and plasmacytoid murine DCs, Siglec-H targeting was found inferior to initiate either MHC-I or MHC-II antigen presentation, compared to BST-2 or DEC205 targeting [80].

Recently, in situ DC targeting through soluble antigen-DC receptor antibody construct was tested in a phase I clinical trial using CDX-1401 [81]. This vaccine (CDX1401, Celldex Therapeutics, Hampton, NJ, USA) consisted of a human anti-DEC205 monoclonal antibody fused to full-length tumor antigen NY-ESO-1 and was administered along with TLR agonists resiquimod (TLR 7/8 agonist) and Hiltonol (polyICLC, TLR3 agonist). Intracutaneous injection (combination of intradermal and subcutaneous injection) along with topical or subcutaneous administration of adjuvants led to generation of robust humoral and cellular immunity against NY-ESO-1. This was observed even in patients where NY-ESO-1 expression was not present in the patient tumor. Thirteen out of forty-eight patients had stabilization of disease with a median duration of 6.7 months (2.4+ to 13.4 months). Additionally, two patients experienced tumor regression. The vaccine did not result in any Grade 3/4 or dose-limiting toxicities. This first in-human study of a protein- antibody construct vaccine targeting DCs demonstrated that these vaccines are immunogenic, safe and well-tolerated. Of note, 6 out of 8 patients (75%) who received immune-checkpoint inhibitors within 3 months of receiving CDX-1401 had objective clinical responses. Of these patients, clinical responses were observed in 4 of 6 melanoma patients who received Ipilumimab following CDX-1401. These findings are encouraging but preliminary and need to be confirmed in the context of formal clinical trial testing this combination. These data nonetheless provide support for clinical studies to test whether combining DC targeted vaccines with strategies such as immune check-point inhibitors will lead to improved efficacy compared to immune checkpoint inhibitors alone. This approach may be particularly relevant for patients lacking immunity to tumor antigens at baseline prior to checkpoint blockade[82].

7. Emerging approaches – Nanoparticles

Another approach for in situ targeting that is approaching the clinic is to encapsulate antigens and adjuvants within delivery vehicles [12]. This will also eliminate the requirement for systemic administration of adjuvants and the consequent untoward systemic effects. Co-delivering antigens and adjuvants within the same compartment will also ensure that only the APC exposed to antigen receives the activation signal. This would prevent the dual problems of T cell anergy in the absence of co-stimulation and non-specific activation

of APCs which have not seen the antigen. It would also allow delivery of high dose of immunogenic cargo, all within the same vector [65].

Nanoparticles (NP) are rapidly emerging as the new vehicles for delivering vaccines [83]. These include polymeric particles, liposomes, virus-like particles (VLPs), nanocrystals and immune-stimulating complexes (ISCOMs). These particles are efficiently taken up by DCs because of their size and particulate structure which resembles pathogens. They can induce long-lasting immune responses by delivering antigens in a slow and sustained manner [84]. Importantly, their release properties can be easily controlled by modulating their physic-chemical properties. Of these, poly-lactic-co-glycolic acid (PLGA) nanoparticles have received the most attention because of their production from a biodegradable, FDA approved polymer. Liposomes and virus-like particles have also been extensively studied, but their clinical application may be limited by the stability issues with liposomes and vector immunogenicity issues with VLPs [12]. While this review focuses on DC-targeted nanoparticle-based vaccines, recent reviews [83,85,86] provide excellent summaries of bioengineering issues with nanoparticles. Although DC-targeted NPs have not been tested in the clinic, the use of NPs as vaccine-delivery vehicles has already reached the clinic. For example, in a phase I/II clinical trial involving stage II-IV melanoma patients, VLPs loaded with Melan-A/Mart-1 peptide along with CpG led to tumorspecific CD8 T cell responses in 14 out of 22 patients [87].

8. Preclinical data with nanoparticle-based DC-targeted approaches

Nanoparticles can be decorated on their surface with antibodies or carbohydrate ligands that bind specifically to DC receptors. While polymer nanoparticles and liposomes can be coated with antibodies by PEGylation or avidin-biotin interactions, virus-like particles can also be engineered to express receptor ligands. In one of the earlier studies, Cruz et al demonstrated that DC-SIGN targeted PLGA nanoparticles, but not microparticles specifically delivered antigens to human dendritic cells in vitro [88]. Consequently, only targeted nanoparticles were able to improve antigen presentation and T cell response. Mannan bound PLGA nanoparticles were also found to improve antigen-specific CD4+ and CD8+ T cell responses in mouse in vitro and in vivo systems [89]. Interestingly, both of these studies were able to achieve these results in the absence of TLR agonists, which is consistent with the possibility that NPs themselves provide an activation signal to DCs.

The role of TLR agonists was evaluated by Tacken et al by targeting through DC-SIGN in human and DEC205 in mouse studies [90]. Co-encapsulating TLR3 and TLR7/8 ligands (poly IC and resiquimod/ R848 respectively) with the antigen in PLGA nanoparticles in this study did improve the generation of CTL responses. Of note, targeted delivery of TLR agonists reduced their dose requirement by 100 fold and was associated with decreased serum cytokine storm and related toxicities in vivo, compared to administration of soluble adjuvants. Similar results were achieved with mannose-targeted liposomes which showed higher anti-tumor therapeutic efficacy in vivo compared to non-targeted liposomes, thereby allowing use of lesser quantities of both TLR ligands and peptide epitopes [91]. On comparison between PLGA NP coated with either DC receptor-specific antibodies or carbohydrate ligands, targeting through former was shown to be more efficient to target

dendritic cells and induce immune responses [92]. Targeting to specific human dendritic cell subsets has also been evaluated. BDCA3+ MDCs targeted via PLGA NP through CLEC9A efficiently presented melanoma-associated antigens to CD4+ T cells as well as cross-presented them to CD8+ T cells [93]. Human plasmacytoid dendritic cells also cross-presented antigens delivered via PLGA NP co-encapsulating R848 and targeted through DEC205, DCIR, BDCA-2 or FcyR CD32. Notably, the presence of TLR agonist led to robust type I interferon secretion, a desirable effect in immune activation [94]. A summary of selected studies where nanoparticle-based vaccines were actively targeted to dendritic cells is provided in Table 3.

9. Unmet needs for nanoparticle-based strategies

As discussed earlier, an important aspect of DC biology is the presence of distinct subsets specialized for distinct effects on the immune system. However in terms of in situ DC targeting in humans, questions regarding the optimal DC subset, target receptor and adjuvant still remain unanswered [12]. It is notable that recent studies have challenged the superiority of BDCA3+ MDCs over other DC subsets to cross-present antigens in humans [45,46]. It is also increasingly appreciated that generation of optimal T helper-1 (Th-1) immunity may require the engagement of multiple DC subsets [95-97]. This is supported by the finding that the yellow fever vaccine 17D, one of the most effective vaccines in recent history, activates multiple TLRs in DC subsets [98]. In a recent study, we have shown that combinatorial targeting of BDCA3 and DC-SIGN+ DCs via NPs was superior to targeting either subset alone. The mechanism underlying this synergy involved IL15-dependent DC-DC crosstalk [99]. Therefore, active targeting of nanoparticle-based vaccines to a single DC subset, though effective in the pre-clinical studies, may deprive the resultant immune response of the benefit of cross-talk between different DC subsets. One possible strategy to target multiple DC subsets in situ is to target receptors such as CD40 or CD32, which are expressed by multiple DC subsets and also mediate DC maturation [100-102].

The ideal DC activation signal or TLR ligand for these vaccines also remains to be defined. This may depend on the specific DC subset being targeted, as different subsets express different TLRs. Importantly, prior studies have shown that co-encapsulating more than one TLR agonist within NP significantly improved CTL responses, when compared to single agonist vaccines [103,104]. The ideal approach would be to generate a nanoparticle-based platform targeting combination of DC subsets which yield synergistic effects.

One potential advantage of NP platform is the potential flexibility in terms of antigen loading. This is potentially very valuable in the setting of cancer as antigenic peptides specific for mutations could be loaded onto NPs. Ultimately, this should pave the way for development of truly personalized cancer vaccines.

10. Conclusions

To conclude, active targeting of dendritic cells in situ is emerging as an attractive approach to generate strong protective cellular immunity against chronic infectious diseases and cancer. The recently reported phase I trial of human in-situ CDX-1401 has laid the foundation for clinical application of these vaccines. The observed clinical responses in

patients receiving immune checkpoint blockade following the vaccine suggest that combining these vaccines with immune-checkpoint blockade (such as anti-CTLA4, anti-PD1) may be of therapeutic benefit in human cancer. Nanoparticles are also emerging as attractive vehicles to target antigens to DCs and recent data suggest that combinatorial targeting of multiple DC subsets may significantly enhance the efficacy of DC targeting. The development of such combinatorial approaches would allow us to harness the full potential of the human immune system in the fight against cancer and chronic infections [9].

Acknowledgments

MVD and KMD are supported by funds from NIH (CA135110, CA106802, AI079222), Dana Foundation, and Multiple Myeloma Research Foundation.

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Highlights

- The specialized antigen-presentation capability of dendritic cells and the plasticity of different DC subsets make them valuable targets for immunotherapy.
- The success of recently reported phase I trial of NY-ESO1-anti-DEC205 antibody vaccine has set the stage for further clinical testing of in-situ DC-targeted vaccines.
- Nanoparticles also represent an attractive strategy for targeting DCs in situ.

Table 1

Major human dendritic cell subsets

	Blood DCs			Cutaneous DCs		Inflammatory DCs
	Myeloid DCs Type I	**Myeloid DCs Type II**	**Plasmacytoid DCs**	**Langerhans Cells**	**CD14+ DCs**	
Phenotype	**BDCA1 (CD1c)+** CD11c+ CD13+ CD33+ CD11b+	**BDCA3 (CD141)+** CD11c+ CD13+ CD33+ CD11b+	**BDCA2 (CD303)+ BDCA4 (CD304)+** CD123 (IL-3R α)+ ILT7+	**Langerin+**, CD1a+	**CD14+**, CD11c+	**CD14+,** CD11c+
Location	Blood, tissue & lymphoid organs	Blood, tissue & lymphoid organs	Blood, tissue & lymphoid organs	Epidermis & stratified squamous epithelia	Dermis & non-lymphoid organs	Site of inflammation
TLRs	TLR 1-8, 10	TLR 1, 2, 3, 6, 8, 10	TLR 7, 9	TLR 1, 2, 3, 6, 10	TLR 2, 4, 5, 6, 8, 10	
Other receptors	**Dectin-1**, Dectin-2, DCIR, Mannose receptor	**CLEC9A, XCR1, NECL2**	DCIR, CD32, Siglec-H, BST-2	DCIR, Dectin-1, Dectin-2	DC-SIGN, LOX-1, Dectin-1, DCIR	SIRPα, FcεR
Major cytokines produced on activation	TNF-α, IL-8, IL-10,IL-23	**IFN-λ**, TNF-α, CXCL10	**IFN-α**, IL-6, IL12 p40	IL-15	IL-1β, IL-6, IL-8, IL-10, IL-12, GM-CSF, MCP, TGF- β	IL-1β, TNF-α, IL-6, IL-23
Major function	Antigen-presentation	Antigen-presentation/cross-presentation	Anti-viral response, Maintenance of tolerance	Tolerance/immune-regulation; epithelial homeostasis	Formation of follicular helper T cells, B cell help	Depends upon inflammatory environment
Predominant immune response	Th2, Th1, Th17	Th1	Type I IFN, Tregs	Th22, Th1	Tfh	

*Th= T helper response, Tfh= T follicular helper response

Table 2

DC targeted antibody-based vaccines

Antigen (Ag)	Target	Adjuvant	Key findings	Ref.
			Animal in vitro and in vivo	
Model Ag:				
• OVA	DEC-205	CD40 Ab	Enhanced resistance to rapidly growing tumor and mucosal viral infection.	[105,106]
		CpG	Ab-Ag-adjuvant conjugates more effective than Ab-Ag conjugates + soluble adjuvant or Ab-free Ag-adjuvant conjugates.	[107]
	Clec9A/DNGR-1	CD40 Ab /Poly IC	Promoted tumor immunity. No adjuvant led to Treg differentiation, addition of Poly I:C led to Th1 & curdlan to Th17 polarization	[76] [108]
	DC-SIGN	CD40 Ab	Protective against infection with OVA expressing L. monocytogenes	[109]
	Dectin-1	Poly IC	Preferential induction of CD4 T cell and Ab response on targeting to CD8a- DCs.	[106]
	Mannose R	CpG	CpG enhanced effector T cell immunity and Ag-specific protective tumor immunity	[110]
	BST-2	Poly IC / R848	Targeted to PDCs. Protective immunity against subsequent viral infection & tumor growth	[80,111,112]
	Siglec-H	- / CpG	Targeted to PDCs or cDCs. Inferior to BST-2 & DEC-205 targeting	[111,113]
• TT & KLH	DC-SIGN	-	Inhibited tumor growth in NOD/SCID mice	[39]
Infectious Ag:				
• HIV gag p24	DEC-205	Poly IC Poly dA:dT	Most effective adjuvant and matured DCs by inducing Type I IFN response Type I IFN production & improved IFN-y+ CD8 & CD4 T cell response	[68,70,114-116]
	Clec9A/ DNGR-1	Poly IC / CD40 Ab	Comparable stimulation of Th1 & CD8 immunity by CD8a+ DC targeting by Clec9A, Langerin & DEC205. Greater than targeting to CD8a- DCs through DCIR.	[69]
• Dengue NS1	DEC-205	Poly IC	Protection from lethal intracranial challenge with DENV2 NGC strain. In comparison, DCIR targeting led to Ab response only	[71]
• Leishmania LmSTI1a	DEC-205	PolyICLC & CD40 Ab	Multi-epitope Th1 CD4+ T cell immunity, protective against L. major challenge.	[117]
• Mycobacterial ESX Ag	DEC-205	Poly IC	Targeted to airway CD205+ DCs. Significant protection against virulent M.tuberculosis	[118]
• EBNA1	DEC-205	PolyICLC	Targeted to CD141+ cDCs. Protective against autologous EBV-transformed B cells	[119]
• Influenza HA1	LOX-1	-	Ag-specific IFN-y producing CD4+ T cells in non-human primates	[40]
Tumor Ag:				
• HER2/neu	DEC-205	PolyICLC	Protective against neu-expressing mammary tumor challenge	[120]
• PSA fusion protein	LOX-1	-	Ag-specific IFN-y producing CD4+ T cells in non-human primates	[40]
			Human in vitro	
Model Ag:				

Antigen (Ag)	Target	Adjuvant	Key findings	Ref.
• TT	DC-SIGN	-	Targeted delivery induced durable T cell responses in vaccinated donors	[75]
• KLH	Clec9A	Poly IC & R848	Induced recall CD4 T cell response	[93]
	DCIR	CpG	Targeting to PDCs caused proliferation of PBLs	[121]
Infectious Ag:				
• HIV gag p24	DEC-205	-	Superior to DC-SIGN/ CD209 targeting	[122]
• 5 HIV peptide regions	CD40	CD40 Ab	Broad repertoire of multifunctional CD8 T cells generated with cytotoxic effects, ability to kill autologous target cells & suppress viral replication in vitro	[123]
Tumor Ag:				
•gp100/ pmel17	DC-SIGN	Poly IC & R848	Equally efficient as cell-penetrating peptide PolyR at cross-presenting antigens	[124]
	Mannose R	CD40L	Cytotoxicity towards gp100 (+) HLA-matched melanoma targets	[125]
• MART-1	Dectin-1	-	Targeted to IFNDCs efficiently cross-presented Ag to stimulate functionally active $CD8^+$ T cell responses.	[78]
	DCIR	CL075, poly IC, LPS or CD40L	Targeted to ex-vivo generated DCs, skin Langerhans cells, and blood MDCs & PDCs. CL075 (TLR7/8) agonist found to be the most potent adjuvant, especially when combined with CD40L	[35]
• hCG beta	Mannose R	CD40L or poly IC & R848	CTLs capable of killing human cancer cell lines.	[126,127]
		Clinical trials		
Tumor Ag:				
• NY-ESO1	DEC-205	Resiquimod + Poly-ICLC	Phase I trial reported robust humoral and cellular immunity against NY-ESO1. Stabilization of disease in 13 out of 48 patients, with tumor regression in 2 patients	[81]
• hCG beta	Mannose R	Poly-ICLC + Resiquimod	Phase I studies in patients with advanced epithelial malignancies. Humoral and T-cell responses seen.	[128]

* Ab = Antibody, Mannose R = Mannose receptor, CD40L = CD40 Ligand

Table 3

DC targeted nanoparticle-based vaccines

Vector	Antigen (Ag)	Target	Targeting moiety	Key Findings	Ref.
			Animal in vitro and in vivo		
Polymer Particles	***Model Ag:***				
	• OVA	DEC205	Monoclonal Ab	100 fold less adjuvant required, as compared to soluble forms. Reduced serum cytokine storm and related toxicity	[90]
		DC-SIGN	PMAM dendrimers	Robust CD4+ & CD8+ T cell responses when loaded to BMDCs derived from DC-SIGN-transgenic mice	[129]
		Mannose R	Mannan	Enhanced CD4+ and CD8+ T cell responses in vitro and in vivo	[89]
			Mannosylated-alginate	Targeted nanogel delivery induced more efficient Th1 response in vitro	[130]
Liposomes	***Model Ag:***				
	• OVA	DEC205	Monoclonal Ab	Strong Ag-specific CTL response in splenic T cells and marked protection against tumor growth. LPS or IFN-y used as adjuvant.	[131]
		DC-SIGN	Glycans	Only glycan-modified non-PEGylated liposomes could bind to DC-SIGN	[132]
		Mannose R	Glycan ligands	Enhanced cross-presentation and Th1 skewing. Found to be independent of TLR-mediated signaling	[133]
		TLR5	Flagellin-related peptides	Induced DC maturation and Ag-specific CD8 & humoral immunity, which significantly inhibited tumor growth/metastasis &induced complete tumor regression in majority of mice tumor models	[134]
	Infectious Ag:				
	• HIV gp120 18IIIB	Mannose R	Oligomannose – neoglycolipid	These coated liposomes were proposed as adjuvants. Induced CD8+ CTL response.	[135]
	• Neisseria PorA	Mannose R	Ligands	On subcutaneous immunization, Increased localization in draining lymph nodes and improved bactericidal Ab response	[136]
	Tumor Ag:				
	• ErbB2 p63-71	Mannose R	Mannosylated ligands	Mannose-targeted liposomes had higher anti-tumor therapeutic efficiency allowing use of lesser quantities of both TLR ligands & peptide epitopes. TLR2/6 agonists found to be more efficient than TLR1/2 agonist for tumor eradication	[91]
	• HPV16 E7	Mannose R	Mannan	Coating of DOTAP liposomes with mannan significantly enhanced both preventive & therapeutic anti-tumor effects in vivo	[137]
Viruses /Virus-likeparticles	***Model Ag:***				

Vector	Antigen (Ag)	Target	Targeting moiety	Key Findings	Ref.
	• OVA	DC-SIGN	Engineered Sindbis viral glycoprotein	Specific transduction and maturation of DCs by lentivector. Generated Ag-specific CD8+T cells and significant Ab response. Protected against tumor growth and induced regression of established tumors	[138]
	Tumor Ag:				
	• $TRP2_{180-188}$	CD40	CD40L extracellular domain	Targeted adenovector enhanced both transduction & maturation of DCs. Improved CD8+ T cell immunity and therapeutic efficacy in a melanoma model	[139]
			Human in vitro		
Polymer Particles	***Model Ag:***				
	• TT	DC-SIGN	Monoclonal Ab	Nanoparticles, but not microparticles improved antigen presentation. No adjuvant used	[88]
	Tumor Ag:				
	• gp100	DC-SIGN	Monoclonal Ab / carbohydrate ligands	Targeting antigens and adjuvants within the same particles enhanced CD8+ T cell stimulation potential. Receptor-specific antibodies more effective than carbohydrates	[90,92]
		Clec9A	Monoclonal Ab	Cross-presented by BDCA3+ DCs. Adjuvants R848 & poly IC	[93]
		DEC205, DCIR, BDCA2, FcR CD32	Monoclonal Ab	Delivered to plasmacytoid dendritic cells. Adjuvant R848. Triggered robust Type I IFN response	[94]
Liposomes	***Tumor Ag:***				
	• NY-ESO-1	Fcy-R	Fc fragment from IgG	Co-encapsulated with adjuvants Palm-IL-1 & MAP-IFN-y, generated potent immunological responses	[140]
	•LHRH	Fcy-R	Fc fragment from IgG	Enhanced immune response compared to non-targeted NP & soluble peptides	[141]
Viruses /Virus-like particles	***Infectious Ag:***				
	• Haem-influenza M1	CD40	Monoclonal Ab Fv fragment	Superior ability to activate antigen-specific cytotoxic T lymphocyte response, compared to non-targeted adenoviral vectors	[142]

* Fcy= constant fragment of IgG, Fv= variable fragment.