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# Chemically engineered glycan-modified cancer vaccines to mobilize skin dendritic cells

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**Abstract**

Dendritic cell (DC)–targeting vaccines show great promise in increasing antitumor immunity. Glycan-engineered vaccines facilitate both DC targeting and increased uptake by DCs for processing and presentation to $CD4^+$ and $CD8^+$ T cells to induce tumor-specific T-cell responses. However, the complexity of various DC subsets in skin tissues, expressing different glycan-binding receptors that can mediate vaccine uptake or drainage of vaccines via lymphatics directly to the lymph node–resident DCs, complicates the success of vaccines. Moreover, the influx of inflammatory immune cells to the site of vaccination, such as monocytes that differentiate to DCs and coexpress glycan-binding receptors, may contribute to the strength of DC-targeting glycovaccines for future clinical use.

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Cancer vaccines, Dendritic cells, Glycans, C-type lectins, DC-SIGN, Langerin, Immunity.

## Introduction

Over the past decade, cancer immunotherapy has become a popular treatment modality for patients with cancer. Its principle to stimulate antitumor immunity by removal of immunosuppressive circuits or by facilitating processing and presentation of tumor neoantigens by dendritic cells (DCs) is are thereby key elements [1]. Although multiple studies have shown promising results that lead to effective antitumor immunotherapy, whether it is by removing the break by immune checkpoint inhibition [2] or by stimulating anti-tumor immunity, there is still much room for improvement of vaccine formulations [3–5]. In this opinion review, we provide insights into the requirements of cancer vaccines that are aimed to instruct DCs to induce long-lasting immunity toward tumor-specific antigens.

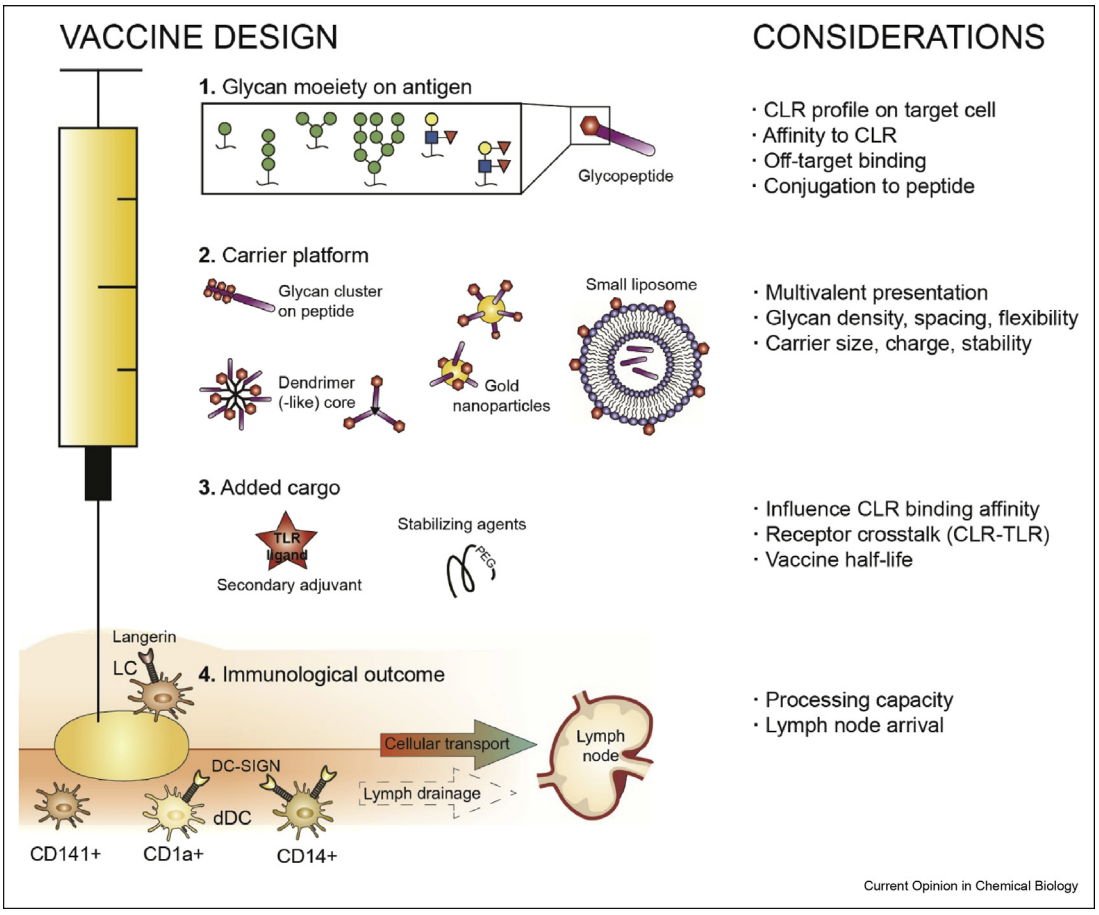
For cancer immunotherapy, *in vivo* DC-targeting strategies use the skin vaccination site to instruct antigen uptake by skin-resident DCs that present tumor antigens to $CD4^+$ and $CD8^+$ T cells [6–8]. As antigens, tumor-associated antigens or neoantigens [1], in the form of synthetic peptides, can be used to provide the 'tumor specificity' for $CD8^+$ and $CD4^+$ T-cell responses to attack or help attack the tumor. Additional innate stimuli are needed, often provided by adjuvants, that facilitate DC maturation and migration of the DCs to draining lymph nodes (LNs) to trigger local naive T cells [12].

## Designing intradermal vaccine formulations for DC-induced antitumor immunity

In human skin, four main subtypes of DCs have been characterized [8]. In the dermis, three DC subsets are distinguished based on the expression of the membrane markers CD1a, CD14, and CD141, whereas Langerhans cells (LCs) are found in the upper layer of the epidermis [8]. These four different DC subsets may respond differently to vaccines; they express different C-type lectin receptors (CLRs) for glycan uptake of antigens and different Toll-like receptors (TLRs) for maturation purposes. Thus, careful vaccine design is required (Figure 1) [9].

$CD141^+$ dermal DCs are considered the most potent to induce tumor-specific killer $CD8^+$ T cells via exogenous uptake of antigens (cross-presentation) and also promote $CD4^+$ T cells to provide help for induction of B-cell responses [10,11]. However, depending on the vaccines, the other skin DC subsets also cross-present antigens and induce tumor-specific killer $CD8^+$ T cells,

**Figure 1**




**Schematic overview of (considerations in) vaccine design for immunotherapy**. The design of a novel glycan-based immunotherapeutic vaccine consists of four steps. First, the carbohydrate moiety must be selected (1), taking the CLR to target into mind and its affinity to the receptor (or other receptors). Covalent binding to the peptide antigen or conjugation as separate moieties to the carrier is also dependent on the carrier platform (2). Compliant to the targeted receptor, multivalent presentation with optimal density, spacing, and flexibility of the carbohydrates must be sought out. The overall carrier size, charge, and stability on incorporation of the glycopeptides must additionally be considered. Supplemental cargo can be added to boost the immunization process or increase the half-life (3), provided there are no negative CLR-binding effects or alterations in processing via receptor cross talk. The overall formulation should also benefit the immunological outcome (4), by enhancing processing capacity of the dendritic cells or stable transport to the lymphoid tissues. DC-SIGN, dendritic cell specific ICAM grabbing non-integrin; CLR, C-type lectin receptor; DC, dendritic cell; LC, Langerhans cell; TLR, Toll-like receptor.

indicating that the type of vaccine matters [12]. Next to skin also, LN-resident cDC1 DCs may contribute to tumor-specific $CD8^+$ T-cell activation and the induction of immunological memory, indicating that even next to the local effects in the skin, the drainage of the vaccine via the lymphatics to the LN may contribute to the strength of a given vaccine.

## Pathogen recognition receptors to target human skin DCs for vaccine uptake and cross-presentation

Targeting the vaccine to specific receptors on DCs that facilitate uptake of the vaccine and presentation of tumor antigens to T cells improves the mode of action of vaccines. Glycan-binding receptors such as CLRs have been intensively studied for this application [13]. CLRs such as dendritic cell specific ICAM grabbing non-integrin (DC-SIGN) and Langerin recognize (among others) fucose-containing glycans of the Lewis-type oligosaccharides. The carbohydrate epitopes Lewis Y Fucα1-2Galβ1-4[Fucα1-3]GlcNAc and Lewis B Fucα1-2Galβ1-3[Fucα1-4]GlcNAc were shown to bind both DC-SIGN and Langerin, whereas Lewis X Galβ1-4[Fucα1-3]GlcNAc and Lewis A Galβ1-3[Fucα1-4]GlcNAc were mostly recognized by DC-SIGN, showing unique glycan specificity for CLRs, but also overlapping glycan specificity [14]. Glycan structures can be conjugated to synthetic peptides that encode tumor-associated antigens or neoantigens, or included as glycolipids in liposomes, or particles thereby successfully targeting DCs and LCs, as shown in multiple studies [15,16]. Glycan modification of antigens shows

improved tumor-specific $CD8^+$ T-cell responses via targeting of $CD14^+$ dermal DCs and LCs via DC-SIGN and Langerin, respectively [17]. Moreover, specific glycans such as Lewis Y can be used for targeting both Langerin and DC-SIGN simultaneously, and therefore, they are the preferred glycan structures to target multiple DCs in the skin [18].

Certain adjuvants in the vaccine can trigger TLRs to induce DC maturation and migration to the LN, but they also alter the intracellular fate of antigens in DCs (cross-presentation), thereby favoring the induction of tumor-specific $CD8^+$ T cells when combined with CLR targeting, as described for TLR4 and DC-SIGN [19]. Similarly, TLR3 triggering combined with Langerin targeting enhanced $CD8^+$ T-cell activation by LCs [17]. In addition, non-pattern recognition receptor (PRR)—targeting ligands as adjuvants, such as granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4, were shown to enhance the immunogenicity of peptide-based vaccines or tumor vaccines in the clinic [19].

## Considerations for vaccine formulation to target human skin DCs

The most straightforward manner of introducing a CLR-targeting moiety is the covalent attachment of a specific carbohydrate onto a specific amino acid in an antigenic peptide [12]. However, effective CLR receptor—glycan interactions require both affinity and avidity and multimerizations of the receptors. Carbohydrate-mediated interactions are generally very weak, and this low affinity is mostly compensated by the multivalent presentation of glycans that induce CLR multimerization (Figure 1) [15].

A recent bio-orthogonal approach has been described to synthesize glycopeptides with a multivalent glycosylation pattern [20]. Azido-lysines were specifically added via solid-phase peptide synthesis into the antigenic peptide sequence. Synthetic oligomannosides were equipped with an alkyne spacer at the reducing end followed by a copper(I)-catalyzed azide alkyne cycloaddition (click chemistry). In this way, well-defined multiple oligomannosides copies can be prepared, carrying up to six glycans per peptide [20].

The (multi)valency of a glycopeptide can be further increased by incorporation into carrier systems, such as dendrimers or dendrimer-like structures. These defined nanostructures are repetitively and uniformly branched molecules, available in a large array of sizes, generations, and terminal groups. Glycopeptide multimerization via a dendrimer core has been applied successfully for DC targeting and immunization. Gold nanoparticles are additionally widely used as a carrier system owing to their ease of changeability in size, shape, surface properties, and other chemical properties. The simultaneous functionalization of gold nanoparticles with thiol-containing antigenic peptides and with thiol-functionalized carbohydrates has been described with successful immunizing applications in vitro and *in vivo* [22,23]. Another carrier system in the nanorange is the naturally occurring outer membrane vesicles from pathogens, or small liposomes including carbohydrates in the lipid bilayer, with the antigen entrapped at the core [24].

Inclusion of TLR-targeting ligands as a parallel adjuvant is additionally explored. These pathogen-associated molecular patterns can be conjugated to the glycopeptide itself or added to the carrier systems.

Next to the carbohydrate moiety, the density, the spacing, and the flexibility of the carrier system can contribute to CLR targeting. We recently demonstrated that the preferential size restriction for DC-SIGN and Langerin could be met in a one-size-fits-all Lewis-sized dendrimer that showed both the requirement for DC-SIGN targeting and Langerin targeting [18]. The preference of DC-SIGN for larger particles, in the nanometer range, can be retraced to its spatial organization on the cell surface influencing ligand avidity [25,26]. However, high-affinity CLR binding cannot be directly translated to enhanced antigen presentation and T-cell responses [21].

## Lymphatic drainage of vaccines: implication for DC targeting

For intradermal vaccination, targeting of numerous DC populations at their site of residence is important [10,11]; however, lymph drainage of the vaccine may reach LN-resident $CD8^+$ DCs. In addition, LN-resident DCs are capable of antigen cross-presentation on activation and induce strong $CD8^+$ T-cell responses [27]. Various vaccine delivery systems can promote antigen transport to lymphoid organs and enhance retention time within the lymphoid tissues. Particles of (up to) 200 nm size are preferentially trafficked into the lymphatic vessels [28], and multiple carrier systems comply with the size requirement. The surface chemistry, stability, and antigen release can also be adjusted by small modifications of the vaccine delivery system, such as PEGylation [29]. Nonetheless, each adjustment to the carrier system can again affect the DC CLR targeting and its biological outcome.

Multiple DC subsets are present in the skin-draining LN, including LN-resident DCs, DCs originated from the blood, and DCs that have migrated from the skin via the afferent lymph vessels [30]. In the steady state, DC-$SIGN^+$ DCs are present in the paracortex of the LN, the site where DCs and T cells interact [31]. In addition, $Langerin^+$ LCs can be found in the skin-draining LN [30]. Currently, it is not known which glycan-modified DC-targeting vaccines drain into the LN and instruct

also LN-resident DCs that express glycan-binding receptors.

## Intradermal vaccination follow-up: influx of innate immune cells

With the design of *in vivo* vaccination strategies, the main focus lies on the effect of the formulation on skin DCs because these are the cells able to initiate adaptive antitumor immune responses. However, with intradermal injections, the vaccine comes in contact with more than just DCs because the tissue constitutes different resident cell types, including stromal cell types such as keratinocytes, melanocytes, and fibroblasts. The vaccine is also able to accumulate various immune cells that extravasate from the blood vessel upon the inflammatory signals provided by the vaccine (Figure 2).

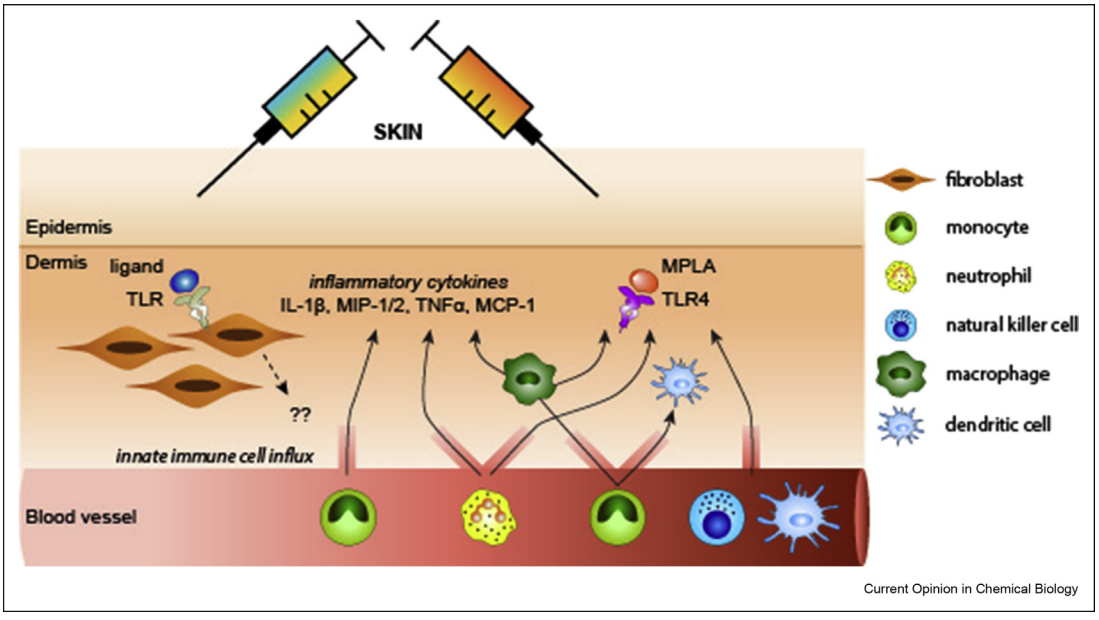
Skin-resident macrophages can activate and promote a proinflammatory environment to which $CCR2^{+}$ monocytes and neutrophils are recruited [32]. Intradermal vaccination of influenza virus using microneedle patches can increase multiple inflammatory cytokines in the skin, such as IL-1β, macrophage inflammatory protein 1 and 2, tumor necrosis factor (TNF-α), and monocyte chemoattractant protein 1, thereby attracting monocytes and neutrophils [33].

Adjuvants, such as the TLR4 agonist monophosphoryl lipid A, that stimulate DC maturation can recruit macrophages, whereas the imidazoquinoline (Resiquimod), R848, a TLR7/8 agonist, affects the relative percentages of macrophages and inflammatory monocytes in the skin [34]. Importantly, fibroblasts can play an important role in induction of the innate immune cell influx because they can respond to TLR triggers by secretion of IL-6 and IL-8, thereby inducing either proinflammatory or anti-inflammatory responses and neutrophil recruitment, respectively [35] (Figure 2). Moreover, the influx of monocytes upon intradermal vaccination may also result in transport of the vaccine by monocytes to the LN.

## Bottleneck of studying intradermal vaccination strategies

There is a significant gap between our knowledge on how vaccines work and the efforts to design tailored vaccines aimed at forcing a complex immune system into clinical efficacy. Because immune responses are compartmentalized both in space and time, the complete complexity can only be modeled in live organisms such as mice; however, they show many differences between men [36]. Central to the efficacy of clinically approved adjuvants is the DCs [37]. Many vaccine adjuvants have shown to depend on local activation and recruitment of neutrophils or monocytes that may differentiate into DCs [38]. Indeed, squalene-based adjuvants MF59 or AddaVax (oil-in-water nanoemulsion formulations), which are regularly used in seasonal influenza vaccines [39], have been shown to induce monocyte recruitment via the CCR2/CCL2 axis

**Figure 2**




**Influx of immune cells in the skin after vaccination**. Upon vaccination, inflammatory cytokine (IL-1β, MIP-1/2, TNF-α, and MCP-1) levels are increased, to which monocytes, neutrophils, and macrophages are attracted. Because fibroblasts express TLRs, they might react to TLR ligands after which fibroblasts can change the local environment of the skin, thereby inducing the innate immune cell influx. After injection of a MPLA-containing vaccine NK cells, neutrophils and monocytes/macrophages are recruited from blood to the skin. NK, natural killer cells; TNF-α, tumor necrosis factor; IL, interleukin; MIP-1/2, macrophage inflammatory protein 1 and 2; MCP-1, monocyte chemoattractant protein 1; TLR, Toll-like receptor; MPLA, monophosphoryl lipid A.

in mice [40]. Monocytes may take up antigens at the vaccination site and differentiate into monocyte-derived DCs, capable of inducing T- and B-cell activation in the draining LN [41]. It has previously been shown that the addition of small amounts could increase the migration capacity of intramuscular differentiated monocyte-derived DCs [42]. In fact, MF59 may have similar effects in the skin, recruiting monocyte-derived DCs expressing the mouse ortholog of DC-SIGN [43].

## Future perspectives

Cancer immunity has shown their potential in both murine models and intradermal vaccination strategies using human skin explants by targeting vaccines to DCs. However, the human model systems lack dynamics of adjuvant-induced immunocompetence owing to lack of the innate immune influx and vaccine drainage to the LN, indicating that there is an urgent need for humanized models [44]. These can be mouse models that include microbial experience [45], expression of human receptors (for example, DC-SIGN [46]), humanized immune cell compositions [47,48], and the inclusion of both sexes [49]. Although a mouse model with human DC-SIGN (hSIGN) under the control of the CD11c promotor is generated [50], this does not reflect the DC-restricted DC-SIGN expression we see in humans.

On the other hand, current skin-on-chip technology [51], which mimics the physiology of the human skin, does not include all immune cells that are naturally present. Besides, the contributing effect of skin-resident cells, also the influx of other immune cells after intradermal injection of the vaccine, should be investigated, as has been demonstrated to play a role in mouse studies [52]. Future directions should be aimed to develop these complete ex vivo human immune networks that allow us to study the dynamics of adjuvant-induced immunocompetence of DC-targeted vaccines.

## Conflict of interest statement

Nothing declared.

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