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COMMENTARY

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Enhancing the immunogenicity of cancer vaccines by harnessing CLEC9A

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ABSTRACT

Dendritic cell (DC) vaccines are a safe and effective means of inducing tumor immune responses, however, a better understanding of DC biology is required in order to realize their full potential. Recent advances in DC biology have identified a crucial role for cDC1 in tumor immune responses, making this DC subset an attractive vaccine target. Human cDC1 exclusively express the C-type-lectin-like receptor, CLEC9A (DNGR-1) that plays an important role in cross-presentation, the process by which effective $CD8^+$ T cell responses are generated. CLEC9A antibodies deliver antigen specifically to cDC1 for the induction of humoral, $CD4^+$ and $CD8^+$ T cell responses and are therefore promising candidates to develop as vaccines for infectious diseases and cancer. The development of human CLEC9A antibodies now facilitates their application as vaccines for cancer immunotherapy. Here we discuss the recent advances in CLEC9A targeting antibodies as vaccines for cancer and their translation to the clinic.

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Introduction

Dendritic cells (DC) are the initiators and orchestrators of adaptive immune responses including the tumor-specific $CD8^+$ T cell responses required to eradicate cancer.[1,2] DC loaded with tumor antigens (Ag) and adjuvants have been administered as vaccines to thousands of cancer patients and these early clinical trials validate this as a safe and effective means of enhancing tumor immune responses. However, their limited efficacy in improving clinical outcomes as a monotherapy, and the emergence and success of checkpoint inhibitor and CAR T cell immunotherapies have shifted the focus of cancer immunotherapy away from DC vaccines over the past few years. With the realization that many cancer patients fail to respond to checkpoint inhibitor immunotherapy, DC vaccines are now experiencing a resurgence as an attractive means of increasing tumor immunogenicity. To achieve this there are now over 200 registered clinical trials evaluating DC vaccines in combination with therapies including chemotherapy, radiotherapy, or immune checkpoint inhibitors. In addition to combining with other treatments, novel vaccine formulations that take advantage of recent advances in human DC biology promise to not only overcome the logistical challenges of cell-based vaccine therapies but also more precisely target the key pathways required for optimal $CD8^+$ T cell priming with enhanced efficacy and reduced side effects.

DC are comprised of distinct subsets with specialized functions that are largely conserved across species and tissues.[3] These are the conventional (c) DC1 (human CD141/BDCA-3^+ DC) subset specialized at driving $CD8^+$ T cell responses, the cDC2 (human CD1c/BDCA-1^+ DC) subset that primarily orchestrates $CD4^+$ T cell responses and plasmacytoid (p) DC that are potent type I interferon (IFN-I) producers. There is compelling evidence from mouse models that the cDC1 DC subset plays a critical role in driving $CD8^+$ T cell-mediated tumor control.[1,2] They are more effective than other DC subtypes at cross-presentation of cellular Ag, the process by which tumor-specific $CD8^+$ T cell responses are generated. Moreover, mice lacking cDC1 fail to respond to treatments such as radiotherapy, checkpoint inhibitors, adoptive T cell therapies, and oncolytic viruses that rely on the generation of effective $CD8^+$ T cell responses. Human cDC1 mirror many of the phenotypic and functional characteristics of their mouse counterparts. Human cDC1 transcripts are associated with good prognosis in many solid human tumor types,[4–7] and their presence is associated with responsiveness to PD-1 checkpoint inhibition in melanoma,[6] highlighting the importance of this DC subset in human tumor immunity. As a result of these key developments, there is a very solid rationale and intensifying efforts to develop new strategies that harness cDC1 to maximize tumor immunogenicity and increase response rates to cancer immunotherapy.

While DC differentiated in vitro from monocytes (MoDC) have been the mainstay of cancer vaccines, and cDC2 and pDC vaccines are also under evaluation,[8,9] the rarity of cDC1 in human blood currently makes their use as a cellular therapeutic challenging. New methodologies that enable expansion of cDC1 from hematopoietic progenitors in vitro may permit personalized cDC1 cellular vaccines in the future.[10,11] However, a more efficient and practical approach to cancer vaccination is to target vaccine cargo to them directly in vivo using antibodies (Ab) specific for Ag uptake receptors and surface markers expressed by DC. The potential of DC targeting Ab as clinically relevant vaccines has been recently demonstrated with CDX-1401, a vaccine comprising the widely

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expressed immunogenic tumor Ag, New York esophageal squamous cell carcinoma 1 (NY-ESO-1), conjugated to an Ab specific for human DEC-205, that is expressed by all DC subsets.[12–14] CDX-1401 is well tolerated, induces NY-ESO -1-specific humoral, $CD4^+$ and $CD8^+$ T cell responses in patients with advanced solid malignancies and myelodysplastic syndrome (MDS) and can be combined with other therapies including chemotherapy and checkpoint inhibitor therapies. Although human cDC1 express DEC-205, taking advantage of Ag uptake receptors and surface markers uniquely expressed by cDC1, such as the C-type-lectin-like receptor, CLEC9A (also known as DNGR-1) offer a more precise means of specifically targeting Ag for cross-priming by this subset.

CLEC9A as a target for vaccine enhancement

CLEC9A is a type II transmembrane protein that is selectively expressed by cDC1.[15–17] CLEC9A is a damage recognition receptor that recognizes cytoskeletal actin filaments or complexes revealed by dead or damaged cells and facilitates cross-presentation of dead cell-associated Ag.[18–21] While CLEC9A does not appear to be critical for the uptake of dead cells, it plays an important role in the trafficking of dead cell-associated antigenic material to early and recycling endosomes that are favorable for cross-presentation and hence effective $CD8^+$ T cell responses.[22]

In line with the role of CLEC9A as a specialized receptor for Ag handling, delivery of Ag to cDC1 using Ab specific for CLEC9A has demonstrated exceptional potential as a vaccine platform for enhancing immune responses in mice and non-human primates. Consistent with its role in facilitating MHCI processing and presentation, targeting Ag to mouse Clec9A enhances Ag-specific $CD8^+$ T cell responses.[15,16] Furthermore, targeting Ag using Clec9A induces potent Ab responses, even in the absence of adjuvant, in mice and non-human primates.[15,23–25] Targeting Ag to mouse Clec9A induces protective immune responses for infectious diseases including influenza[25] and malaria[26] as well as cancer.[16] Several factors are proposed to contribute to this efficacy, including the selective expression of Clec9A on DC1, enabling the persistence of Clec9A targeted Ag,[23] its delivery of Ag to low degradative compartments,[22] and display of Ag on the surface of cDC1 that facilitates induction of T follicular helper T cells.[23,27]

Translating Clec9A-targeting vaccines from mouse to man

Extending the promise of Clec9A in mouse and primate models, human CLEC9A Ab have recently been developed that are highly effective at delivering Ag exclusively to human cDC1 and induce potent $CD4^+$ and $CD8^+$ T cell responses specific to a variety of human viral and tumor antigens in preclinical models.[28–31] Two vaccines have recently been developed comprised of anti-human CLEC9A Ab genetically fused to the well-characterized, highly immunogenic human tumor Ags, NY-ESO-1, and Wilms' tumor 1 (WT1).[30,31] CLEC9A Ab vaccines were highly efficient at promoting cross-presentation by human cDC1 and inducing naïve and memory $CD8^+$ T cell responses specific for several NY-ESO-1 and WT1 epitopes, and importantly, were more effective than vaccines comprising the same Ag delivered by human DEC-205 Ab or untargeted protein. Although there is some debate as to the contribution of other DC subsets in anti-tumor immune responses,[8] these human preclinical studies, as well as several mouse models[23,32,33] demonstrate that CLEC9A targeting is at least equivalent to, and in some cases more efficient than targeting Ag to receptors expressed more broadly by many DC subsets. WT1 MoDC, peptide and protein vaccines have already demonstrated potential in an extensive range of solid and hematological malignancies including acute myeloid leukemia (AML), glioblastoma, breast, lung, renal, ovarian, and pancreatic cancers.[34–38] Likewise, NY-ESO-1 is widely expressed by many cancer types including melanoma, sarcoma, neuroblastoma, bladder, head and neck, ovarian, prostate, and breast cancers, and vaccine formulations have been shown to induce potent NY-ESO-1-specific humoral and cellular immune responses.[14,39] Thus, specific targeting of cDC1 via CLEC9A-WT1 and CLEC9A-NY-ESO-1 vaccines offers enormous potential to further improve immunogenicity and outcomes for the wide range of cancer types where NY-ESO-1 or WT1 vaccines have already shown potential. Moreover, with rapid advances in Ab engineering and production technologies, vaccines comprising CLEC9A Ab fused to other tumor Ag, including shared and neoAg can be rapidly developed.

The requirement for DC activation for optimal tumor immune responses is well established from both mouse models and early clinical trials. As binding of ligands or Ab to CLEC9A does not directly induce cDC1 activation in mice or humans, co-administration of an appropriate adjuvant will be required for CLEC9A-targeting vaccines. For clinical translation, stabilized poly I:C derivatives such as poly-ICLC (Hiltonol) are the current adjuvants of choice, firstly because of their established safety profile,[40] and secondly because they activate TLR3 that is highly expressed by cDC1.[41] Poly-ICLC has been safely combined with DEC-205 Ab vaccines in cancer patients[12,14] and with CLEC9A Ab in non-human primates.[24] cDC1 is a major source of Type III interferon (IFN) in response to poly I:C, and although Type III IFN and cDC1 have recently been associated with good prognosis in breast cancer,[42] the role of this pathway in tumor adjuvanticity remains to be investigated.[43] By contrast, Type I IFN signaling by cDC1 is a known requirement for optimal tumor immunity in mouse models[44,45] and is likely provided indirectly to cDC1 via Type I production by other cell types.[46] An alternative approach to activating cDC1 is therefore more targeted Type I IFN delivery. In this regard, CLEC9A Ab has also demonstrated potential in mouse and human preclinical models as delivery vehicles of modified Type I IFN for more localized direct activation of cDC1.[47]

The integrity of cDC1 in advanced cancer patients is another important consideration for the success of CLEC9A-targeting Ab vaccines. Circulating and lymphoid resident cDC1 can be deficient in advanced cancer patients[1] so combining CLEC9A-targeting vaccines with strategies to increase cDC1 numbers could be advantageous. Fms-like tyrosine kinase 3 ligand (Flt3L), a growth factor required for cDC1 development, is an obvious candidate for this given its safety profile and ability to expand human cDC1 in vivo.[48] Furthermore, Flt3L

administration was recently demonstrated to enhance the immunogenicity of a DEC-205 Ab vaccine combined with poly-ICLC.[14] The combination of Flt3L with CLEC9A Ab targeting and poly-IC was also effective at priming tumor-specific T cell responses in a humanized mouse model,[31] providing a strong rationale for translating this approach to the clinic. Another promising approach for enhancing cDC1 numbers is the epigenetic modulation of transcriptional repressors that control their development. One such candidate is decitabine, a demethylating agent already used in the clinic for the treatment of AML, MDS, and triple-negative breast cancer. In addition to upregulating expression of tumor Ag and Ag presentation machinery in tumors, decitabine also enhances expression of IRF8, a master regulator of DC development in humans.[49,50] Notably, decitabine has been safely combined with a DEC-205 Ab vaccine in MDS patients, and vaccine-induced immune responses were associated with increased cDC1.[13] This demonstrates the feasibility and a strong rationale to trial CLEC9A-targeting Abs in malignancies where decitabine is standard of care.

The ability of Clec9A Ab to deliver Ag to cDC1 for efficient activation of $CD4^+$ T cells as well as $CD8^+$ T cells in both mice and humans[15,29] was initially surprising in the context of the emerging DC subset specialization paradigm whereby cDC1 preferentially prime $CD8^+$ T cells whilst cDC2 mediate $CD4^+$ T cell responses. Although this paradigm conflicts with the decades-old premise that Ag must be presented by the same DC to both $CD4^+$ and $CD8^+$ T cells for optimal priming,[51,52] several recent studies have identified lymphoid resident cDC1 as the platform on which simultaneous interactions with $CD4^+$ and $CD8^+$ T cells mediate optimal priming of anti-viral and anti-tumor immunity.[53–55] Supporting this, DC vaccines delivered directly to lymph nodes are known to induce better immune responses than other administration routes[9] and there is some evidence to suggest that transfer of Ag from the DC vaccine to lymph node resident cDC1 is required for their efficacy.[56] These observations imply that delivery of vaccines directly to lymphoid cDC1 could be a more efficient and effective strategy for enhancing tumor immunogenicity. Intranodal or intravenous administration of Clec9A Ab is hypothetically feasible; however, subcutaneous or intradermal vaccination will be a more practical and safer approach for clinical translation, as already demonstrated with DEC-205 Ab vaccines.[12,14] Subcutaneously administered CLEC9A Ab induces potent immune responses in mice and in non-human primates,[24,25] despite lower expression of CLEC9A on skin DC,[57,58] suggesting this route will be the most practical, efficient, and possibly even more efficacious approach to deliver human CLEC9A Ab to lymphoid cDC1.

Concluding remarks

There is a compelling argument for harnessing cDC1 to enhance tumor immunogenicity and outcomes for cancer patients. Accumulating evidence from mouse, non-human primates and human preclinical models highlights the potential of CLEC9A Ab as a highly efficient and practical means of achieving this. DC vaccines are safe and can induce tumor immune responses in many cancer types, including tumors with low mutational burden and those that respond poorly to checkpoint inhibitors.[9,59] Ongoing clinical trials evaluating DC vaccines in combination with standard treatments including surgery, chemotherapy, targeted therapies, and checkpoint inhibitors will offer new insights into where DC vaccines are likely to be most effective. Harnessing Ab specific to DC receptors as a vaccine offers key advantages to cell-based therapies with encouraging results in early clinical outcomes that warrant further development. We predict that CLEC9A-targeting vaccines will have utility as next-generation cancer vaccines with high potential to further enhance response rates in a range of tumor settings where existing DC vaccines have already demonstrated safety and some evidence of efficacy.

Disclosure of potential conflicts of interest

MHL is listed as an inventor on patents relating to Clec9A antibodies.

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