Minireview

Recombinant antigen delivery to dendritic cells as a way to improve vaccine design

Guilherme Antonio de Souza-Silva[1*], Fernando Bandeira Sulczewski[1*] and Silvia Beatriz Boscardin[1,2]

[1]Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 05508-000, Brazil; [2]Instituto de Investigação em Imunologia (iii), Instituto Nacional de Ciência e Tecnologia, São Paulo, 05401-350, Brazil
*These authors shared co-first authorship.
Corresponding author: Silvia Beatriz Boscardin. Email: sbboscardin@usp.br

Impact Statement

Dendritic cells are the most effective antigen-presenting cells described to date. Their biology, positioning in the body, and migratory capacity allow them to continuously sense the environment and quickly detect infection/inflammation. In this way, they acquire antigens and take them to the lymphoid organs to initiate adaptive immune responses. Their central role makes them excellent targets for manipulating immune responses. The use of monoclonal antibodies to direct antigens of interest to these cells has been used as a promising vaccination strategy, since a relatively small amount of antigen can induce strong immune responses.

Abstract

Dendritic cells are central to the development of immunity, as they are specialized in initiating antigen-specific immune responses. In this review, we briefly present the existing knowledge on dendritic cell biology and how their division in different dendritic cell subsets may impact the development of immune responses. In addition, we explore the use of chimeric monoclonal antibodies that bind to dendritic cell surface receptors, with an emphasis on the C-type lectin family of endocytic receptors, to deliver antigens directly to these cells. Promising preclinical studies have shown that it is possible to modulate the development of immune responses to different pathogens when monoclonal antibodies fused to pathogen-derived antigens are used to deliver the antigen to different subsets of dendritic cells. This approach can be used to improve the efficacy of vaccines against different pathogens.

Keywords: Dendritic cell, monoclonal antibody, antigen targeting, vaccination, T cell response, B cell response

Experimental Biology and Medicine **2023; 248: 1616–1623. DOI: 10.1177/15353702231191185**

Dendritic cells: function and classification

Dendritic cells (DCs) are professional antigen-presenting cells responsible for initiating and controlling adaptive immune responses. They are perfectly positioned to act as sentinels and capture antigens whenever and wherever they enter the body. After capture, DCs specialize in the processing and presentation of antigens through major histocompatibility complexes (MHCs). Therefore, DCs provide the main signals required for T cell activation, such as antigen recognition, costimulation, and cytokine production.[1] DCs are essential to promote adaptive responses mediated by CD4+ and CD8+ T cells to support immunity to a variety of pathogens.[2,3] However, DCs also promote tolerogenic immune responses in the absence of inflammation/infection either by deleting specific T cells or by expanding regulatory T cells.[4,5] This important role allows them to regulate autoimmune responses.

DCs were initially classified according to their location and expression of several membrane markers. Currently, they are divided according to their ontogeny based on the expression of different transcriptional factors.[6] In this way, DCs are classified in three main subsets: plasmacytoid dendritic cells (pDCs), conventional type 1 dendritic cells (cDC1s), and conventional type 2 dendritic cells (cDC2s). While pDCs are mainly found in lymphoid tissues and in the blood, cDC1s and cDC2s are found in lymphoid and non-lymphoid organs including lung, liver, skin, and gut. The main function of pDCs is the production of type 1 interferons (IFNs) during viral infections.[7] Whereas cDC1s are highly specialized in antigen cross-presentation to CD8+ T cells, as well as CD4+ T helper (Th)1 and T regulatory (Treg) cell polarization, cDC2s have been associated with the instruction of Th17 and T follicular helper cells (Tfh).[8–10]

cDCs in secondary lymphoid organs exist in an immature state, allowing them to sample their environment and capture antigens. Immature cDCs express lower levels of MHC

Copyright © 2023 by the Society for Experimental Biology and Medicine

molecules and co-stimulatory molecules, making them poor stimulators of T cells. However, upon encountering microbial products, inflammatory signals, or cytokines, maturation occurs with a series of molecular and morphological changes, transforming them into potent antigen-presenting cells. During maturation, cDCs upregulate the expression of MHC and co-stimulatory molecules such as CD80, CD86, and CD40, which are essential for T cell activation. The process of cDC maturation is regulated by different factors, including cytokines, chemokines, and danger signals. The interleukin-1 (IL-1) cytokine is known to promote cDC maturation by upregulating the expression of MHC class II (MHC II) molecules, co-stimulatory molecules, and cytokine production. Other cytokines, such as IFN-gamma (IFNγ) and tumor necrosis factor-alpha (TNF-α), also play a role in cDC maturation and activation. In addition to cytokines, the maturation of cDCs in the spleen is also influenced by chemokines, such as CXCL12 and CCL19, which promote cDCs migration to T cell rich areas of the secondary lymphoid organs. Once in these areas, cDCs encounter T cells and begin to present antigens and co-stimulatory molecules, initiating the activation of T cells.[11–14] In this way, DCs link innate and adaptive immune responses.

DC surface receptors: sensing the environment

The central role of DCs in the induction of adaptive immune responses relies on their incredible capacity to sense the environment and respond to infection/inflammation. In this way, DCs express a myriad of receptors, often also expressed by other immune cells, which are not only capable of recognizing different classes of pathogens and/or inflammatory mediators, but can also improve their capacity to process and present antigens, and travel from the periphery to lymphoid organs where antigen presentation and T cell priming actually take place.[15]

On the cell surface, CD11c, CD45, and MHCII are markers constitutively expressed by DCs. Upon stimulation, they also upregulate the costimulatory molecules CD40, CD80, and CD86. Other cell surface molecules also expressed on the surface of the DCs help to define the different subsets. For example, CD8α and CD103 are used to distinguish cDC1s from nonlymphoid and lymphoid tissues, respectively, while CD11b and CD172a (SIRPα) distinguish cDC2s. cDC1s also express the XC-chemokine receptor 1 (XCR1)[16] and distinct C-type lectin receptors compared to cDC2s. Differentially expressed C-type lectin receptors include DEC205 (CD205 or LY75), CLEC9A (or DNGR1), and CD207 (CLEC4K or langerin), which are expressed primarily by cDC1s, while DC inhibitory receptor 2 (DCIR2; or CLEC4A4) and DC-associated C-type lectin-1 (dectin-1; or CLEC7A) are expressed by cDC2s.[17–20]

Model antigens delivery to DCs: establishing the requirements for induction of specific immune responses

Antigen targeting to DCs consists of a strategy to deliver recombinant antigens directly to these cells. Generally, antigens are coupled to receptor ligands or genetically fused to mAbs directed to receptors expressed on the membrane of DCs (Figure 1).[21]

Initial studies involving DC targeting were conducted with mAbs specific for Fcγ receptors (FcγRs), MHC II, and CD40, and showed that antigen delivery through these receptors improves adaptive cellular and humoral immunity, the latter not requiring the administration of additional adjuvants.[22–25] Other DC surface receptors such as the CD11c and CD11b integrins were also targeted. The targeting of ovalbumin (OVA) to CD11c induced better proliferation of CD4+ and CD8+ transgenic T cells compared to targeting to MHC II,[26] and better humoral immune responses compared to targeting CD11b, MHC II, and CD40, after a single dose and without any adjuvant.[27] XCR1-expressing DCs were also targeted through constructs containing its ligand Xcl1 fused to OVA. Strong proliferation of CD4+ and CD8+ transgenic T cells was also observed.[28]

The last 20 years have experienced an exponential increase in research using mAbs directed at C-type lectin endocytic receptors such as DEC205, CLEC9A, CLEC12A, mannose receptor 1 (or CD206), DC-specific ICAM3-grabbing nonintegrin (DC-SIGN; or CD209), CD207, DC inhibitory receptor (DCIR; or CLEC4A), DCIR2, and dectin-1, among others (Figure 1).[29]

The first attempts to use mAbs to target antigens to DC C-type lectin endocytic receptors were made at Rockefeller University by Michel Nussenzweig and Ralph Steinman groups.[4,30] Hawiger *et al*.[4] and Bonifaz *et al*.[30] demonstrated that model antigens such as chicken egg lysozyme (HEL) or OVA could be selectively delivered to cDC1s *in vivo* through the DEC205 receptor, using a chimeric αDEC205 (clone NLDC-145) mAb coupled to the antigen (αDEC-HEL or αDEC-OVA). The antigen sent to cDC1s was effectively processed and presented to both transgenic CD4+ and CD8+ T cells. When the chimeric mAb was injected without a DC maturation stimulus, the result was the induction of peripheral tolerance (measured by the deletion of transgenic specific T cells). On the contrary, the combined administration of chimeric mAb together with a DC maturation stimulus (such as the agonist αCD40 mAb) led to prolonged CD4+ and CD8+ T cells activation. Furthermore, immunity induced by targeting DEC205 was long-lasting and more effective than administration of more potent adjuvants, such as complete Freund's adjuvant. Promising initial results also demonstrated that it was possible to use αDEC205 chimeric mAb in vaccination protocols. Mice vaccinated with αDEC-OVA together with αCD40 resisted challenge with a transgenic vaccinia virus and with a tumor line expressing OVA. This protective response was dependent on CD4+ and CD8+ T cells activation.[31] Furthermore, immunization with αDEC-OVA together with αCD40+Poly (I:C) triggered the activation of memory CD4+ T cells that were essential for the production of anti-OVA.[32] It is important to mention that Treg cells were also induced when αDEC-OVA was injected without any DC maturation stimulus.[5]

The first evidence that αDCIR2 (clone 33D1) mAb was able to target antigens to cDC2s was published in 2007. Immunization of animals with αDCIR2-OVA induced proliferation of transgenic CD4+ T cells much more efficiently than when the αDEC-OVA mAb was used.[18]

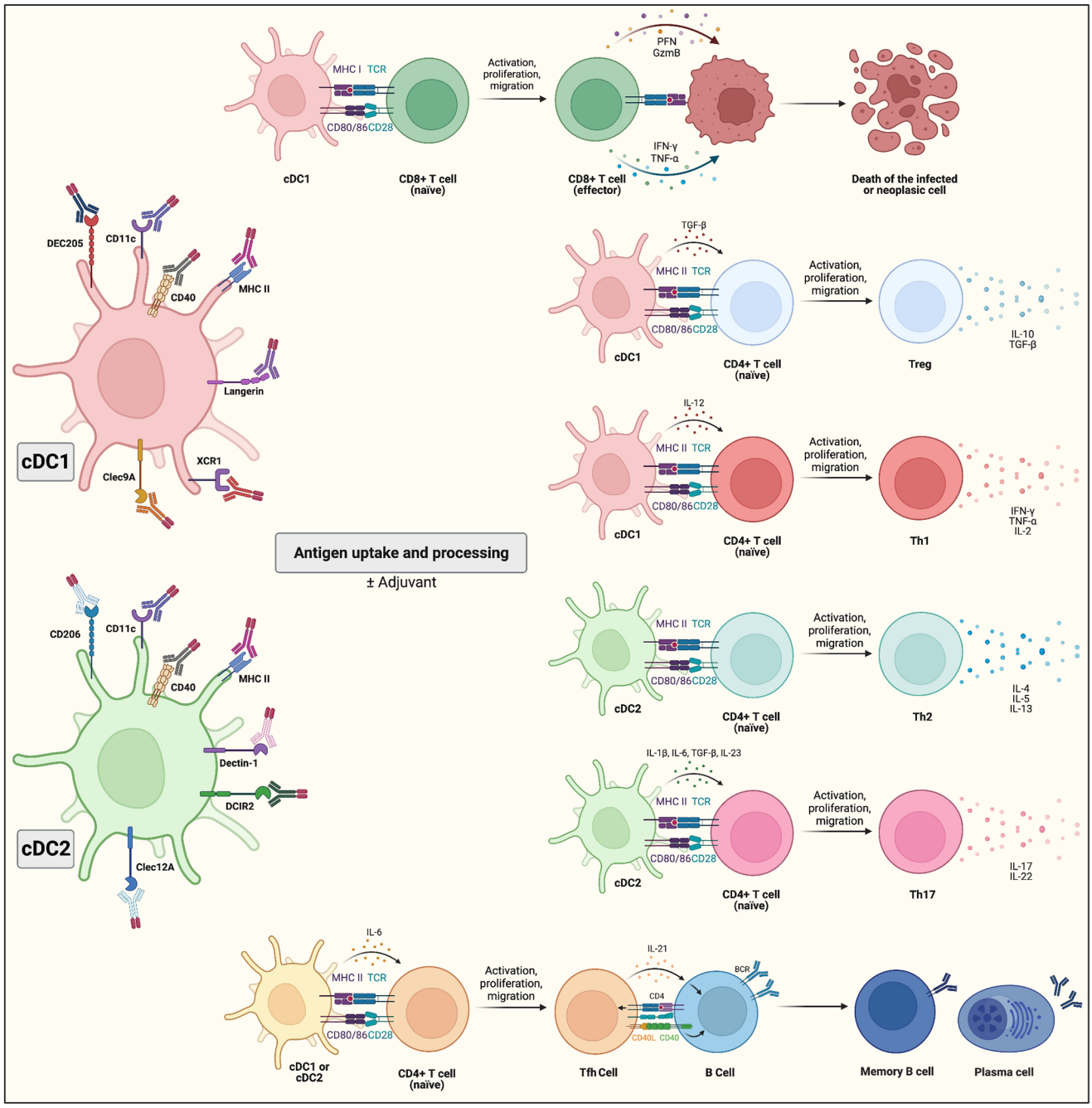

Figure 1. Receptors used for antigen delivery to conventional dendritic cell subsets, and the resulting T and B cell responses. Created with BioRender.com.

Complementary studies showed that cDC2s expressing the DCIR2 receptor were more efficient than cDC1s in inducing Tfh cells, leading to efficient humoral immunity.[9,10] These experiments with chimeric mAbs fused to model antigens were important because they established a functional difference between the cDC subsets that later became named cDC1s and cDC2s.[33]

The ability of CLEC9A to induce immune responses was studied using an αCLEC9A mAb fused to OVA. The targeting of OVA to CLEC9A induced the proliferation of CD4+- and CD8+-specific transgenic T cells, as well as antibody responses even in the absence of any adjuvant.[34,35] Furthermore, OVA peptide targeting to CLEC9A in the presence of αCD40 was also able to induce cytotoxic T lymphocytes (CTLs) and protect mice from a tumor cell line expressing OVA.[36] A strong Th1 cell response was also elicited after an OVA-derived CD4+ T cell epitope was delivered to CLEC9A together with Poly (I:C) as adjuvant. When curdlan (an agonist of Dectin-1) was administered together with the αCLEC9A mAb, a Th17 response was elicited. The absence of a DC maturation stimuli led to the induction of Treg,[37] as previously observed for DEC205 receptor.[5] OVA was also used to study antigen delivery to CLEC12A through an αCLEC12A-OVA mAb. The results showed that antigen delivery to CLEC12A induced specific cellular and humoral immune responses only in the presence of an adjuvant.[38,39]

Mannose receptor 1 and DC-SIGN were targeted using a highly glycosylated (o-glycan oligomannoses) immunoglobulin protein fused to OVA. Mouse immunization with this protein in the presence of the AbISCO®-100 adjuvant led to high and broad anti-OVA antibody responses together with the induction of Th1, Th2, and CTL responses.[40]

Delivery of clinically relevant antigens to DCs

The findings described above opened the possibility of using αDEC205 and αDCIR2 fused to clinically relevant antigens for the induction of protective immunity against different pathogens or against tumors.

The αDEC205 mAb was fused to the circumsporozoite protein (CSP) expressed by the sporozoite forms of *Plasmodium yoelii* (a species that causes malaria in rodents). Administration of a single dose of αDEC-CSP in the presence of αCD40+poly (I:C) induced specific IFNγ-producing CD4+ and CD8+ T cells in different mouse strains. In addition, the induction of an antibody response, measured by the assessment of anti-CSP antibody titers, was also observed after administration of an additional dose, in the absence of any other adjuvant.[32] Immunization of rhesus monkeys with three doses of αDEC205 fused to *Plasmodium falciparum* (a species that causes human malaria) CSP together with Poly (I:C) induced polyfunctional CD4+ T cells, as well as anti-CSP antibodies that blocked 43% of the parasite invasion *in vitro*.[41] Another protein derived from *Plasmodium vivax* (another species that causes human malaria) merozoite surface protein 1 (MSP1), named $MSP1_{42}$, was also targeted at cDC1s by fusion with αDEC205. The results showed that high antibody titers were obtained, especially against a portion of this molecule known as $MSP1_{19}$.[42] Furthermore, the immunogenicity of the $MSP1_{19}$ protein itself was increased after its fusion with a synthetic CD4+ T cell epitope (Pan-allelic DR epitope; PADRE) designed to enhance humoral immune responses.[43]

Our group has extensively used the fusion protein $MSP1_{19}$-PADRE not only to study the possibility of developing an effective vaccine against *Plasmodium vivax* malaria but also to explore, in a little more detail, the biology of cDCs. To this end, we compared the T cell and antibody responses induced after $MSP1_{19}$-PADRE delivery to cDC1s via DEC205 and to cDC2s via DCIR2 shortly after (on days 2, 3, 4, 5, and 6) the administration of αDEC205-$MSP1_{19}$-PADRE and αDCIR2-$MSP1_{19}$-PADRE together with Poly (I: C). Our results confirmed and extended previously published data, since we showed that DCIR2 antigen targeting increased Tfh cell frequencies on day 5 after immunization, suggesting that cDC2s are particularly good to prime these cells. In addition, we detected an increased frequency of germinal center B cells and plasma cells, suggesting that Tfh cells support the formation of germinal centers and also plasma cell differentiation. Besides the activation of Th1 cells induced by DEC205 targeting to cDC1s, our results also showed that, after a booster dose, αDEC205-$MSP1_{19}$-PADRE mAb induced Th1-like Tfh cells, which probably contributed to the observed IgG class switch and to the increase of anti-$MSP1_{19}$ antibody titers. More importantly, a subset of Treg capable of producing IL-10 was also detected.[44] These results are relevant to show that, despite their different functions, DCs from different subsets are plastic and can lead to different immune outcomes depending on the context in which they present the antigen. More recently, we have used antigen targeting to DCs to study in more detail their signaling pathways. We have shown that the canonical signal transducer and activator of transcription 3 (STAT3) pathway regulates the capacity of cDC1s to support CD4+ T cell responses after antigen delivery to the DEC205 receptor,[45] while the STAT6 pathway controls B cell positioning in the germinal centers after antigen delivery to the DCIR2 receptor.[46]

Proteins derived from other protozoans have also been targeted to cDCs *in vivo*. The protective capacity of a vaccine against *Leishmania major* was investigated when the αDEC205 sequence was fused to stress-inducible protein 1 (LmSTI1a). Targeting of this protein to mature cDC1s increased the number of antigen-specific CD4+ T cells that produced IFNγ, IL-2, and TNF-α in two mouse strains. Furthermore, using an LmSTI1a protein peptide library, it was possible to map at least two distinct MHC II epitopes in this protein in each mouse strain. Protection against a *Leishmania major* challenge was also obtained in BALB/c mice (a highly susceptible strain) after challenge.[47] *Trypanosoma cruzi* amastigote surface protein 2 (ASP-2) was also fused to αDEC205 and used to immunize animals together with Poly (I:C) as adjuvant. As previously demonstrated with proteins from other pathogens, it was possible to map a new CD4+ T cell epitope present in this protein.[48] *Toxoplasma gondii* tachyzoites main surface antigen, SAG1, was fused to a variable chain fragment of the αDEC205 mAb (scDEC). Intranasal and subcutaneous immunizations together with Poly (I:C) induced protective responses against chronic infection that were probably mediated by polyfunctional Th1 cells.[49]

The αDEC205 mAb was also coupled to the human immunodeficiency virus (HIV) gag p24 protein, and the administration of a single dose of αDEC-gag p24, together with αCD40 agonist mAb and Poly (I:C), was able to induce a strong immune response mediated mainly by IFNγ-producing CD4+ T cells. Protection was also observed when immunized animals were challenged with a transgenic vaccinia virus expressing the gag protein. The interesting results obtained with these experiments showed that relatively low concentrations of chimeric mAbs (between 3 and 10 μg per animal) were able to induce strong immune responses.[50] The αDEC-gag p24 mAb was also used to test different adjuvants that could induce DC maturation. The most potent adjuvant for this type of immunization was Poly (I:C), along with other more stable and less toxic analogues such as Poly ICLC[51] or Poly ($I:C_{12}U$).[52] At the same time, the number of doses was also tested, and it was noticed that a homologous prime-boost strategy was more efficient than the administration of only one dose.[51,52] Another strategy using the p24 gag protein involved the administration of αDEC-gag p24 as a single chain (sc). Immunization of mice with two doses of scDEC-gag p24 in the presence of Poly ICLC was able to induce protection after an intranasal challenge with a vaccinia virus expressing the gag protein.[53] This type of strategy is also very promising because it is easier to scale up for mass administration, since a single-chain antibody is easier

to produce than a whole antibody containing two chains. Antigen delivery to the DEC205 receptor was also used in an attempt to increase the immunogenicity of DNA vaccines. In this case, a plasmid encoding scDEC205 fused to the HIV p41 gag protein was used to immunize mice. The results showed that, after electroporation with only one dose of the plasmid encoding the scDEC-gag p41, a specific and polyfunctional CD4+ and CD8+ T cell response was detected and provided protection against challenge with a vaccinia virus expressing the gag protein.[54] These results showed that the effectiveness of a DNA vaccine could be improved by including sequences, such as single-chain antibodies, to deliver the antigen to DCs.

The protein gap p24 was also fused to αDEC205, αDCIR2, αCLEC9A and αLangerin to more systematically compare the immune responses elicited in the presence of αCD40+ Poly (I:C) as adjuvant. Comparable levels of Th1 and CD8+ T cells producing IFNγ were observed when the antigen was delivered to DEC205, CLEC9A, and Langerin, corroborating data showing that cDC1s are indeed well specialized in antigen cross-presentation to CD8+ T cells, as well as Th1 polarization.[55]

In addition to the p24 gag protein, the αDEC205 mAb was also conjugated to a sequence containing eight conserved epitopes derived from different HIV proteins that were recognized by T lymphocytes from 90% of HIV-1-infected individuals (clade B) in different clinical stages of the disease.[56] This chimeric protein was created in an attempt to present HIV immunogenic epitopes outside the context of their flanking proteins, in order to suppress the escape mechanisms developed by this virus throughout its evolution in the human host. The results demonstrated that the injection of two doses of αDEC205 fused to the eight epitopes (αDEC-HIVBr8), together with Poly (I:C), was capable of inducing a broad and polyfunctional response of both CD4+ and CD8+ T lymphocytes, especially when this strategy was compared with immunization with two doses of a DNA vaccine encoding identical epitopes.[57]

In addition to HIV, proteins from other viruses were also targeted using both αDEC205 and αDCIR2 mAbs. In the case of dengue 2 virus, immunization with two doses of chimeric mAbs fused to the nonstructural protein 1 (NS1) together with Poly (I:C) induced high anti-NS1 antibody titers. However, partial protection was only observed in animals immunized with αDEC205-NS1 and was dependent both on CD4+ and CD8+ T cells.[58] In another strategy, the animals were immunized with three doses of a plasmid encoding domain III of the viral envelope protein (EDIII) fused to scDEC205. As a control without targeting, a single chain encoding a mAb unable to bind to any murine receptor was used. In this case, higher anti-EDIII antibody titers and a more robust polyfunctional CD4+ T cell response were obtained in animals administered with the plasmid that encoded the scDEC-EDIII. However, sera obtained from immunized animals in both groups were able to block invasion by the dengue 2 virus in eukaryotic cells.[59]

The αDEC205 and αDCIR2 mAbs were further coupled to the LcrV antigen of *Yersinia pestis*, the bacteria that causes bubonic plague, and used to immunize mice. A homologous prime-boost strategy, in which two doses of each mAb were injected together with Poly (I:C), showed that protective antibody titers against this pathogen were higher in αDCIR2-LcrV immunized mice.[60]

In addition to studies conducted with *Yersinia pestis*, the sequence of the C-terminal portion of the botulinum neurotoxin heavy chain (serotype A) derived from the bacterium *Clostridium botulinum* was cloned in phase with the sequence of scDEC205, the plasmid being used to immunize animals. After administration of only two doses of the targeted DNA vaccine, it was possible to observe DC maturation at the inoculation site, proliferation of T lymphocytes, and the production of high levels of antibodies capable of protecting animals against a lethal challenge with botulinum toxin.[61]

The αDEC205 mAb was also fused to antigens expressed in tumors and used in immunization protocols. When the survivin antigen was directed at cDC1s, a robust CD4+ T cell response was observed, mainly after depletion of Treg. However, in this particular model, activation of CD8+ T cells was not observed.[62] On the contrary, when the soluble form of the human epidermal growth factor receptor, an antigen highly expressed in breast cancer, was sent to the cDC1s, both CD4+ and CD8+ T lymphocytes were activated, with significant protection was observed in a breast tumor model.[63] A single study showed protection against melanoma in animals immunized with either αDCIR2-OVA or αDEC-OVA challenged with an OVA expressing melanoma cell line. In this case, immunization was performed with the mAbs administered together with of αCD40+Poly (I:C), and protection was evaluated both in prophylactic and therapeutic schemes.[64] Protection against tumors associated with human papilloma virus (HPV) was also evaluated after immunization with αDEC205 genetically fused to HPV16 oncoprotein E7. Mice challenged with E7+ tumors were vaccinated with αDEC-E7 mAb together with Poly (I:C) and protection with prevention of tumor recurrence, mediated by the induction of CD8+-specific T lymphocytes, was observed.[65]

Taken together, these data show that it is possible to experimentally treat some types of cancer with this targeting strategy. Results like these paved the way for clinical trials. In fact, two phase 1 clinical trials were carried out using a human αDEC205 mAb (clone 3G9) fused to the NY-ESO-1 antigen.[66] In preclinical studies, targeting the DEC205 receptor through 3G9 mAb efficiently cross-presented NY-ESO-1 to CD8+ T cells when compared to the NY-ESO-1 protein administered alone.[67] In the first clinical phase study, 3G9-NY-ESO-1 mAb was injected in conjunction with the adjuvants resiquimod (a 7/8 TLR agonist), Poly ICLC, or both, in increasing doses. Combinations of this mAb with adjuvants were administered to 45 patients with advanced-stage tumors expressing the NY-ESO-1 antigen. Treatment induced T and B cell responses to NY-ESO-1 at various doses and combinations of adjuvants, and high toxicity was not reported. Thirteen patients had the disease stabilized at intervals ranging from 2.4 to 13.4 months, and in two patients, the tumor regressed.[68] 3G9-NY-ESO-1 with Poly ICLC was also administered to patients with acute myeloid leukemia who received the drug decitabine (which induces increased expression of NY-ESO-1). In several patients, induction of specific T cell responses was also observed.[69] An αDEC205-NY-ESO-1 was also used in a phase II clinical

trial after the administration of the fms-like tyrosine kinase 3 ligand (FLT3L) to patients with melanoma. FLT3L expanded DCs and led to increased proliferation of cDC1s and cDC2s subsets. The subsequent administration of αDEC205-NY-ESO-1 resulted in an improved immune response against NY-ESO-1+ tumors, as well as an increased duration of the response compared to the phase 1 clinical trial.[70]

The results gathered in this section clearly show that antigen delivery to DCs is an efficient way to induce immune responses not only in mice, but also in humans. The different immune outcomes elicited by targeting different receptors can be exploited to customize the required immune response for a specific pathogen and cancer type.

Antigen targeting to DCs: what is next?

Antigen targeting is a strategy that increases the immunogenicity of recombinant proteins based on DCs ability to support cellular immune responses. The use of chimeric mAbs fused with antigens has also been shown to be an efficient approach to studying the biology and function of different DC subsets *in vivo*. This approach provides for a better understanding of how DCs control their function and regulate adaptive immune responses. In addition, the knowledge generated is helping in the development of new vaccines and therapies based on specific functions of each DC subset.

AUTHORS' CONTRIBUTIONS

GASS and FBS, and SBB contributed to the literature review. GASS and FBS contributed to writing original draft. GASS contributed to image production. SBB contributed to review, editing and supervision.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the São Paulo Research Foundation (FAPESP, grant no. 2018/07142-9), and the Coordination for the Improvement of Higher Level Personnel (CAPES, Finance Code 001). FBS. and GASS. received fellowships from FAPESP (2018/00145-2 and 2022/00204-4, respectively). SBB received a fellowship from the Brazilian National Research Council (CNPq).

ORCID IDS

Fernando Bandeira Sulczewski https://orcid.org/0000-0001-5611-6967
Silvia Beatriz Boscardin https://orcid.org/0000-0002-7845-7110

REFERENCES

1. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. *Cell* 2001;**106**:255–8
2. Guermonprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 2002;**20**:621–67
3. MacLennan I, Vinuesa C. Dendritic cells, BAFF, and APRIL: innate players in adaptive antibody responses. *Immunity* 2002;**17**:235–8
4. Hawiger D, Inaba K, Dorsett Y, Guo M, Mahnke K, Rivera M, Ravetch JV, Steinman RM, Nussenzweig MC. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J Exp Med* 2001;**194**:769–79
5. Yamazaki S, Dudziak D, Heidkamp GF, Fiorese C, Bonito AJ, Inaba K, Nussenzweig MC, Steinman RM. CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3+ regulatory T cells. *J Immunol* 2008;**181**:6923–33
6. Yin X, Chen S, Eisenbarth SC. Dendritic cell regulation of T helper cells. *Annu Rev Immunol* 2021;**39**:759–90
7. Swiecki M, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. *Nat Rev Immunol* 2015;**15**:471–85
8. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, Ho AW, See P, Shin A, Wasan PS, Hoeffel G, Malleret B, Heiseke A, Chew S, Jardine L, Purvis HA, Hilkens CM, Tam J, Poidinger M, Stanley ER, Krug AB, Renia L, Sivasankar B, Ng LG, Collin M, Ricciardi-Castagnoli P, Honda K, Haniffa M, Ginhoux F. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. *Immunity* 2013;**38**:970–83
9. Shin C, Han JA, Choi B, Cho YK, Do Y, Ryu S. Intrinsic features of the CD8α(-) dendritic cell subset in inducing functional T follicular helper cells. *Immunol Lett* 2016;**172**:21–8
10. Shin C, Han JA, Koh H, Choi B, Cho Y, Jeong H, Ra JS, Sung PS, Shin EC, Ryu S, Do Y. CD8α(-) dendritic cells induce antigen-specific T follicular helper cells generating efficient humoral immune responses. *Cell Rep* 2015;**11**:1929–40
11. Capucha T, Mizraji G, Segev H, Blecher-Gonen R, Winter D, Khalaileh A, Tabib Y, Attal T, Nassar M, Zelentsova K, Kisos H, Zenke M, Sere K, Hieronymus T, Burstyn-Cohen T, Amit I, Wilensky A, Hovav AH. Distinct murine mucosal langerhans cell subsets develop from pre-dendritic cells and monocytes. *Immunity* 2015;**43**:369–81
12. Collin M, McGovern N, Haniffa M. Human dendritic cell subsets. *Immunology* 2013;**140**:22–30
13. Hovav AH. Dendritic cells of the oral mucosa. *Mucosal Immunol* 2014;**7**:27–37
14. Platt CD, Ma JK, Chalouni C, Ebersold M, Bou-Reslan H, Carano RA, Mellman I, Delamarre L. Mature dendritic cells use endocytic receptors to capture and present antigens. *Proc Natl Acad Sci U S A* 2010;**107**:4287–92
15. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu Rev Immunol* 2013;**31**:563–604
16. Dorner BG, Dorner MB, Zhou X, Opitz C, Mora A, Guttler S, Hutloff A, Mages HW, Ranke K, Schaefer M, Jack RS, Henn V, Kroczek RA. Selective expression of the chemokine receptor XCR1 on cross-presenting dendritic cells determines cooperation with CD8+ T cells. *Immunity* 2009;**31**:823–33
17. Crowley M, Inaba K, Witmer-Pack M, Steinman RM. The cell surface of mouse dendritic cells: FACS analyses of dendritic cells from different tissues including thymus. *Cell Immunol* 1989;**118**:108–25
18. Dudziak D, Kamphorst AO, Heidkamp GF, Buchholz VR, Trumpfheller C, Yamazaki S, Cheong C, Liu K, Lee HW, Park CG, Steinman RM, Nussenzweig MC. Differential antigen processing by dendritic cell subsets in vivo. *Science* 2007;**315**:107–11
19. Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM, Nussenzweig MC. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 1995;**375**:151–5
20. Carter RW, Thompson C, Reid DM, Wong SY, Tough DF. Preferential induction of CD4+ T cell responses through in vivo targeting of antigen to dendritic cell-associated C-type lectin-1. *J Immunol* 2006;**177**:2276–84
21. Macri C, Dumont C, Johnston AP, Mintern JD. Targeting dendritic cells: a promising strategy to improve vaccine effectiveness. *Clin Transl Immunology* 2016;**5**:e66

22. Carayanniotis G, Barber BH. Adjuvant-free IgG responses induced with antigen coupled to antibodies against class II MHC. *Nature* 1987;**327**:59–61
23. Frleta D, Demian D, Wade WF. Class II-targeted antigen is superior to CD40-targeted antigen at stimulating humoral responses in vivo. *Int Immunopharmacol* 2001;**1**:265–75
24. Serre K, Machy P, Grivel JC, Jolly G, Brun N, Barbet J, Leserman L. Efficient presentation of multivalent antigens targeted to various cell surface molecules of dendritic cells and surface Ig of antigen-specific B cells. *J Immunol* 1998;**161**:6059–67
25. Snider DP, Segal DM. Targeted antigen presentation using crosslinked antibody heteroaggregates. *J Immunol* 1987;**139**:1609–16
26. Castro FV, Tutt AL, White AL, Teeling JL, James S, French RR, Glennie MJ. CD11c provides an effective immunotarget for the generation of both CD4 and CD8 T cell responses. *Eur J Immunol* 2008;**38**:2263–73
27. White AL, Tutt AL, James S, Wilkinson KA, Castro FV, Dixon SV, Hitchcock J, Khan M, Al-Shamkhani A, Cunningham AF, Glennie MJ. Ligation of CD11c during vaccination promotes germinal centre induction and robust humoral responses without adjuvant. *Immunology* 2010;**131**:141–51
28. Fossum E, Grødeland G, Terhorst D, Tveita AA, Vikse E, Mjaaland S, Henri S, Malissen B, Bogen B. Vaccine molecules targeting Xcr1 on cross-presenting DCs induce protective CD8+ T-cell responses against influenza virus. *Eur J Immunol* 2015;**45**:624–35
29. Kastenmuller W, Kastenmuller K, Kurts C, Seder RA. Dendritic cell-targeted vaccines—hope or hype? *Nat Rev Immunol* 2014;**14**:705–11
30. Bonifaz L, Bonnyay D, Mahnke K, Rivera M, Nussenzweig MC, Steinman RM. Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral CD8+ T cell tolerance. *J Exp Med* 2002;**196**:1627–38
31. Bonifaz LC, Bonnyay DP, Charalambous A, Darguste DI, Fujii S, Soares H, Brimnes MK, Moltedo B, Moran TM, Steinman RM. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* 2004;**199**:815–24
32. Boscardin SB, Hafalla JC, Masilamani RF, Kamphorst AO, Zebroski HA, Rai U, Morrot A, Zavala F, Steinman RM, Nussenzweig RS, Nussenzweig MC. Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. *J Exp Med* 2006;**203**:599–606
33. Ginhoux F, Guilliams M, Naik SH. Editorial: dendritic cell and macrophage nomenclature and classification. *Front Immunol* 2016;**7**:168
34. Caminschi I, Proietto AI, Ahmet F, Kitsoulis S, Shin Teh J, Lo JC, Rizzitelli A, Wu L, Vremec D, van Dommelen SL, Campbell IK, Maraskovsky E, Braley H, Davey GM, Mottram P, van de Velde N, Jensen K, Lew AM, Wright MD, Heath WR, Shortman K, Lahoud MH. The dendritic cell subtype-restricted C-type lectin Clec9A is a target for vaccine enhancement. *Blood* 2008;**112**:3264–73
35. Li J, Ahmet F, Sullivan LC, Brooks AG, Kent SJ, De Rose R, Salazar AM, Reis e, Sousa C, Shortman K, Lahoud MH, Heath WR, Caminschi I. Antibodies targeting Clec9A promote strong humoral immunity without adjuvant in mice and non-human primates. *Eur J Immunol* 2015;**45**:854–64
36. Sancho D, Mourão-Sá D, Joffre OP, Schulz O, Rogers NC, Pennington DJ, Carlyle JR, Reis e Sousa C. Tumor therapy in mice via antigen targeting to a novel, DC-restricted C-type lectin. *J Clin Invest* 2008;**118**:2098–110
37. Joffre OP, Sancho D, Zelenay S, Keller AM, Reis e, Sousa C. Efficient and versatile manipulation of the peripheral CD4+ T-cell compartment by antigen targeting to DNGR-1/CLEC9A. *Eur J Immunol* 2010;**40**:1255–65
38. Lahoud MH, Ahmet F, Kitsoulis S, Wan SS, Vremec D, Lee CN, Phipson B, Shi W, Smyth GK, Lew AM, Kato Y, Mueller SN, Davey GM, Heath WR, Shortman K, Caminschi I. Targeting antigen to mouse dendritic cells via Clec9A induces potent CD4 T cell responses biased toward a follicular helper phenotype. *J Immunol* 2011;**187**:842–50
39. Lahoud MH, Proietto AI, Ahmet F, Kitsoulis S, Eidsmo L, Wu L, Sathe P, Pietersz S, Chang HW, Walker ID, Maraskovsky E, Braley H, Lew AM, Wright MD, Heath WR, Shortman K, Caminschi I. The C-type lectin Clec12A present on mouse and human dendritic cells can serve as a target for antigen delivery and enhancement of antibody responses. *J Immunol* 2009;**182**:7587–94
40. Ahlén G, Strindelius L, Johansson T, Nilsson A, Chatzissavidou N, Sjöblom M, Rova U, Holgersson J. Mannosylated mucin-type immunoglobulin fusion proteins enhance antigen-specific antibody and T lymphocyte responses. *PLoS ONE* 2012;**7**:e46959
41. Tewari K, Flynn BJ, Boscardin SB, Kastenmueller K, Salazar AM, Anderson CA, Soundarapandian V, Ahumada A, Keler T, Hoffman SL, Nussenzweig MC, Steinman RM, Seder RA. Poly(I:C) is an effective adjuvant for antibody and multi-functional CD4+ T cell responses to plasmodium falciparum circumsporozoite protein (CSP) and αDEC-CSP in non human primates. *Vaccine* 2010;**28**:7256–66
42. Amorim KN, Rampazo EV, Antonialli R, Yamamoto MM, Rodrigues MM, Soares IS, Boscardin SB. The presence of T cell epitopes is important for induction of antibody responses against antigens directed to DEC205(+) dendritic cells. *Sci Rep* 2016;**6**:39250
43. Antonialli R, Sulczewski FB, Amorim K, Almeida BDS, Ferreira NS, Yamamoto MM, Soares IS, Ferreira LCS, Rosa DS, Boscardin SB. CpG oligodeoxinucleotides and flagellin modulate the immune response to antigens targeted to CD8α(+) and CD8α(-) conventional dendritic cell subsets. *Front Immunol* 2017;**8**:1727
44. Sulczewski FB, Martino LA, Almeida BDS, Zaneti AB, Ferreira NS, Amorim KNDS, Yamamoto MM, Apostolico JS, Rosa DS, Boscardin SB. Conventional type 1 dendritic cells induce TH 1, TH 1-like follicular helper T cells and regulatory T cells after antigen boost via DEC205 receptor. *Eur J Immunol* 2020;**50**:1895–911
45. Sulczewski FB, Martino LA, Salles D, Yamamoto MM, Rosa DS, Boscardin SB. STAT3 signaling modulates the immune response induced after antigen targeting to conventional type 1 dendritic cells through the DEC205 receptor. *Front Immunol* 2022;**13**:1006996
46. Sulczewski FB, Martino LA, da Silva Almeida B, Yamamoto MM, Rosa DS, Boscardin SB. STAT6 signaling pathway controls germinal center responses promoted after antigen targeting to conventional type 2 dendritic cells. *Curr Res Immunol* 2021;**2**:120–31
47. Matos I, Mizenina O, Lubkin A, Steinman RM, Idoyaga J. Targeting antigens to dendritic cells in vivo induces protective immunity. *PLoS ONE* 2013;**8**:e67453
48. Rampazo EV, Amorim KN, Yamamoto MM, Panatieri RH, Rodrigues MM, Boscardin SB. Antigen targeting to dendritic cells allows the identification of a CD4 T-cell epitope within an immunodominant trypanosoma cruzi antigen. *PLoS ONE* 2015;**10**:e0117778
49. Lakhrif Z, Moreau A, Hérault B, Di-Tommaso A, Juste M, Moiré N, Dimier-Poisson I, Mévélec MN, Aubrey N. Targeted delivery of toxoplasma gondii antigens to dendritic cells promote immunogenicity and protective efficiency against toxoplasmosis. *Front Immunol* 2018;**9**:317
50. Trumpfheller C, Finke JS, Lopez CB, Moran TM, Moltedo B, Soares H, Huang Y, Schlesinger SJ, Park CG, Nussenzweig MC, Granelli-Piperno A, Steinman RM. Intensified and protective CD4+ T cell immunity in mice with anti-dendritic cell HIV gag fusion antibody vaccine. *J Exp Med* 2006;**203**:607–17
51. Longhi MP, Trumpfheller C, Idoyaga J, Caskey M, Matos I, Kluger C, Salazar AM, Colonna M, Steinman RM. Dendritic cells require a systemic type I interferon response to mature and induce CD4+ Th1 immunity with poly IC as adjuvant. *J Exp Med* 2009;**206**:1589–602
52. Trumpfheller C, Caskey M, Nchinda G, Longhi MP, Mizenina O, Huang Y, Schlesinger SJ, Colonna M, Steinman RM. The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. *Proc Natl Acad Sci U S A* 2008;**105**:2574–9
53. Ngu LN, Nji NN, Ambada G, Ngoh AA, Njambe Priso GD, Tchadji JC, Lissom A, Magagoum SH, Sake CN, Tchouangueu TF, Chukwuma GO, Okoli AS, Sagnia B, Chukwuanukwu R, Tebit DM, Esimone CO, Waffo AB, Park CG, Überla K, Nchinda GW. Dendritic cell targeted HIV-1 gag protein vaccine provides help to a recombinant Newcastle disease virus vectored vaccine including mobilization of protective CD8(+) T cells. *Immun Inflamm Dis* 2018;**6**:163–75
54. Nchinda G, Kuroiwa J, Oks M, Trumpfheller C, Park CG, Huang Y, Hannaman D, Schlesinger SJ, Mizenina O, Nussenzweig MC, Uberla K, Steinman RM. The efficacy of DNA vaccination is enhanced in mice by targeting the encoded protein to dendritic cells. *J Clin Invest* 2008;**118**:1427–36

55. Idoyaga J, Lubkin A, Fiorese C, Lahoud MH, Caminschi I, Huang Y, Rodriguez A, Clausen BE, Park CG, Trumpfheller C, Steinman RM. Comparable T helper 1 (Th1) and CD8 T-cell immunity by targeting HIV gag p24 to CD8 dendritic cells within antibodies to langerin, DEC205, and Clec9A. *Proc Natl Acad Sci U S A* 2011;**108**:2384–9
56. Fonseca SG, Coutinho-Silva A, Fonseca LA, Segurado AC, Moraes SL, Rodrigues H, Hammer J, Kallas EG, Sidney J, Sette A, Kalil J, Cunha-Neto E. Identification of novel consensus CD4 T-cell epitopes from clade B HIV-1 whole genome that are frequently recognized by HIV-1 infected patients. *AIDS* 2006;**20**:2263–73
57. Apostólico JS, Lunardelli VA, Yamamoto MM, Souza HF, Cunha-Neto E, Boscardin SB, Rosa DS. Dendritic cell targeting effectively boosts T cell responses elicited by an HIV multiepitope DNA vaccine. *Front Immunol* 2017;**8**:101
58. Henriques HR, Rampazo EV, Gonçalves AJ, Vicentin EC, Amorim JH, Panatieri RH, Amorim KN, Yamamoto MM, Ferreira LC, Alves AM, Boscardin SB. Targeting the non-structural protein 1 from dengue virus to a dendritic cell population confers protective immunity to lethal virus challenge. PLoS Negl Trop Dis 2013;**7**:e2330
59. Zaneti AB, Yamamoto MM, Sulczewski FB, Almeida BDS, Souza HFS, Ferreira NS, Maeda DLNF, Sales NS, Rosa DS, Ferreira LCS, Boscardin SB. Dendritic cell targeting using a DNA vaccine induces specific antibodies and CD4(+) T cells to the dengue virus envelope protein domain III. *Front Immunol* 2019;**10**:59
60. Do Y, Koh H, Park CG, Dudziak D, Seo P, Mehandru S, Choi JH, Cheong C, Park S, Perlin DS, Powell BS, Steinman RM. Targeting of LcrV virulence protein from Yersinia pestis to dendritic cells protects mice against pneumonic plague. *Eur J Immunol* 2010;**40**:2791–6
61. Chen BY, Zhou G, Li QL, Lu JS, Shi DY, Pang XB, Zhou XW, Yu YZ, Huang PT. Enhanced effects of DNA vaccine against botulinum neurotoxin serotype A by targeting antigen to dendritic cells. *Immunol Lett* 2017;**190**:118–24
62. Charalambous A, Oks M, Nchinda G, Yamazaki S, Steinman RM. Dendritic cell targeting of survivin protein in a xenogeneic form elicits strong CD4+ T cell immunity to mouse survivin. *J Immunol* 2006;**177**:8410–21
63. Wang B, Zaidi N, He LZ, Zhang L, Kuroiwa JM, Keler T, Steinman RM. Targeting of the non-mutated tumor antigen HER2/neu to mature dendritic cells induces an integrated immune response that protects against breast cancer in mice. *Breast Cancer Res* 2012;**14**:R39
64. Neubert K, Lehmann CH, Heger L, Baranska A, Staedtler AM, Buchholz VR, Yamazaki S, Heidkamp GF, Eissing N, Zebroski H, Nussenzweig MC, Nimmerjahn F, Dudziak D. Antigen delivery to CD11c+CD8- dendritic cells induces protective immune responses against experimental melanoma in mice in vivo. *J Immunol* 2014;**192**:5830–8
65. Silva MO, Almeida BS, Sales NS, Diniz MO, Aps LRMM, Rodrigues KB, Silva JR, Moreno ACR, Porchia BFMM, Sulczewski FB, Boscardin SB, Ferreira LCS. Antigen delivery to DEC205(+) dendritic cells induces immunological memory and protective therapeutic effects against HPV-associated tumors at different anatomical sites. *Int J Biol Sci* 2021;**17**:2944–56
66. Jungbluth AA, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer* 2001;**92**:856–60
67. Tsuji T, Matsuzaki J, Kelly MP, Ramakrishna V, Vitale L, He LZ, Keler T, Odunsi K, Old LJ, Ritter G, Gnjatic S. Antibody-targeted NY-ESO-1 to mannose receptor or DEC-205 in vitro elicits dual human CD8+ and CD4+ T cell responses with broad antigen specificity. *J Immunol* 2011;**186**:1218–27
68. Dhodapkar MV, Sznol M, Zhao B, Wang D, Carvajal RD, Keohan ML, Chuang E, Sanborn RE, Lutzky J, Powderly J, Kluger H, Tejwani S, Green J, Ramakrishna V, Crocker A, Vitale L, Yellin M, Davis T, Keler T. Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. *Sci Transl Med* 2014;**6**:232ra51
69. Griffiths EA, Srivastava P, Matsuzaki J, Brumberger Z, Wang ES, Kocent J, Miller A, Roloff GW, Wong HY, Paluch BE, Lutgen-Dunckley LG, Martens BL, Odunsi K, Karpf AR, Hourigan CS, Nemeth MJ. NY-ESO-1 vaccination in combination with decitabine induces antigen-specific T-lymphocyte responses in patients with myelodysplastic syndrome. *Clin Cancer Res* 2018;**24**:1019–29
70. Bhardwaj N, Friedlander PA, Pavlick AC, Ernstoff MS, Gastman BR, Hanks BA, Curti BD, Albertini MR, Luke JJ, Blazquez AB, Balan S, Bedognetti D, Beechem JM, Crocker AS, D'Amico L, Danaher P, Davis TA, Hawthorne T, Hess BW, Keler T, Lundgren L, Morishima C, Ramchurren N, Rinchai D, Salazar AM, Salim BA, Sharon E, Vitale LA, Wang E, Warren S, Yellin MJ, Disis ML, Cheever MA, Fling SP. Flt3 ligand augments immune responses to anti-DEC-205-NY-ESO-1 vaccine through expansion of dendritic cell subsets. *Nat Cancer* 2020;**1**:1204–17