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FOCUSSED RESEARCH REVIEW

Activation of dendritic cells by targeted DNA: a potential addition to the armamentarium for anti-cancer immunotherapy

Marlene Fyrstenberg Laursen[1] · **Emil Kofod-Olsen**[1] · **Ralf Agger**[1]

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Abstract

In the past decade, remarkable progress has been made in immunotherapy against cancer. Specifically, the introduction of immune checkpoint inhibitors has revolutionized the field. However, many patients are unable to benefit significantly from this treatment option. One of the major reasons for this is most likely the absence of an adequate tumor-specific T cell response in these patients. A way to circumvent this problem might be to combine immune checkpoint inhibitor treatment with new strategies to activate tumor-specific T cells. One such strategy could be to activate and mature dendritic cells in situ. Dendritic cells carry an array of external and internal pattern recognition receptors that induce cell activation and maturation when interacting with their corresponding damage-associated or pathogen-associated molecular patterns (DAMPs or PAMPs). Targeting such molecular patterns directly to dendritic cells might be a way to evoke stronger immune responses. Here, we review our recent findings using antibody-targeted DNA. We summarize the results from our experiments showing that dendritic cells can be actively targeted in vivo through the $\alpha_X\beta_2$ integrin subunit CD11c, and that DNA delivered through this receptor in vitro leads to maturation of dendritic cells via the cytosolic cGAS/STING DNA-sensing pathway.

Keywords Cancer · Cancer immunotherapy · Dendritic cells · STING · PIVAC 18

Abbreviations

cDC1	Classical type 1 dendritic cell
cDC2	Classical type 2 dendritic cell
CDN	Cyclic di-nucleotide
cGAMP	Cyclic GMP–AMP
cGAS	Cyclic GMP–AMP synthase
CR4	Complement receptor 4
DAMP	Damage-associated molecular pattern
ER	Endoplasmic reticulum
IRF3	Interferon regulatory factor 3
moDC	Monocyte-derived dendritic cell
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid dendritic cell
PRR	Pattern recognition receptor
STING	Stimulator of interferon genes
TBK1	TANK-binding kinase 1

Introduction

One of the crucial steps that allow mutated cells to develop into cancer is the development of resistance to attacks from the immune system [1]. Many innate and adaptive immune mechanisms participate in the defence against neoplasms, but the activation and appropriate function of tumor-specific cytotoxic T cells seem to be particularly important. The remarkable progress in immunotherapy against cancer, brought about by the introduction of immune checkpoint inhibitors, has revealed that many cancer patients have developed tumor-specific T cells that are able to kill cancer cells, once the restraint on the T cells is lifted by treatment with checkpoint-blocking agents [2]. However, clinical trials have also shown that a large fraction of patients do not benefit significantly from the treatment. One of the important reasons for this is most likely that these patients do not have the tumor-specific CD8+ and CD4+ T cells that are necessary for initiating a strong attack on the cancer cells, when the negative checkpoints become blocked [3]. Consequently, it is of great interest to explore approaches that could stimulate the generation of tumor-specific CD8+ and CD4+ T cell clones in these patients.

✉ Ralf Agger
agger@hst.aau.dk

[1] Laboratory of Immunology, Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 3B, 9220 Aalborg, Denmark

The activation of naïve T cells is predominantly executed by dendritic cells (DCs). These cells control whether an antigen is ignored, whether active tolerance develops or whether an adaptive immune response is initiated and if so, they potently shape the type and strength of the response [4]. Consequently, to obtain large numbers of tumor-specific T cells, it is crucial that the DCs are immunostimulatory rather than tolerogenic.

Dendritic cells in cancer

DCs infiltrate tumors, sample and process tumor-associated antigens (TAA), and then migrate to the draining lymph nodes and activate naïve TAA-specific T cells. TAA-specific cytotoxic effector T cells subsequently migrate to the tumor tissue and kill cancer cells that present the matching peptides on MHC class I. Some activation of T cells may also take place in the tumor tissue [5, 6].

Several types of DCs are present in the tumor tissue. Classical type 1 DCs (cDC1) [7], characterized by the expression of CD103 in the mouse, seem to be the most important cell type for transporting TAA from the tumor to the draining lymph nodes, where they prime tumor-specific CD8+ T cells [8]. Monocyte-derived DCs (moDCs), on the other hand, seem to be important for stimulating tumor-specific CD8+ T cells in the tumor tissue [9]. The role of classical type 2 DCs (cDC2) is less clear, but it is possible that activation of CD4+ T cells by this cell type is important for the development of an effective cytotoxic response against the cancer cells [10]. Plasmacytoid DCs (pDC) are also found in the tumor microenvironment and the capacity of this cell type for producing large amounts of type I interferons could potentially strengthen the immune response against the cancer cells [11].

Tumors avoid effective immune attacks by a multitude of mechanisms, including interference with the optimal function of DCs. Tumors can inhibit DC recruitment to the tumor tissue by tumor-intrinsic active β-catenin signaling, which in turn contributes to a lack of T cell infiltration in the tumor tissue, as demonstrated in melanoma by Spranger et al. [12]. Several cytokines produced by cancer cells have been shown to inhibit proper function of DCs. VEGF, important for neovascularization of the tumor tissue and produced by most human tumors, prevents the functional maturation of dendritic cells [13]. IL-6, that may be produced by cancer cells, as well as other cell types in the tumor, may prompt the DCs to decrease expression of MHC class II molecules and the chemokine receptor 7 (CCR7), important for DC homing to the lymph nodes [14, 15] IL-10, which may be produced by some cancer cells and several types of immune cells present in the tumor microenvironment, reduces the ability of DCs to induce T cell responses [16, 17]. These cytokines, together with many other biomolecules present in the tumor microenvironment (recently reviewed in [18]) create a milieu in which DCs assume tolerogenic or immunosuppressive characteristics instead of immunostimulatory properties.

Potentially, the DC-suppressive regime, induced by the tumor cells in cancer patients, could be lifted if the DCs could be suitably stimulated. One strategy could be to target a strong maturational stimulus to the DCs in situ.

Dendritic cell targeting

Many groups have shown that targeting antigens to DCs in situ is a potent way to induce both T- and B cell responses. The targeting element has typically been a monoclonal antibody (mAb), either as a full immunoglobulin molecule or a single chain variable fragment (scFv). A number of different surface molecules may function as receptors for antibody-mediated targeting of antigen, including CD11c, CD205, CD209 (DC-SIGN), CD369 (Clec7A) CD370 (Clec9A) (reviewed in [19]).

To directly compare potential DC surface molecules regarding their potential use as targets for antigen delivery, we screened ten different target molecules in parallel. In these experiments, splenocytes from mice immunized with non-targeting rat immunoglobulin isotype controls were isolated and cultured with rat mAbs specific for different surface molecules. T cell activation was evaluated by interferon-γ (IFN-γ) and interleukin-4 (IL-4) ELISPOT assays. Targeting the CD11c molecules induced a high number of IFN-γ-producing T cells. By flow cytometry and confocal microscopy, we could show that the mAb targeting CD11c was internalized by the DCs [20]. We also targeted eight different DC surface molecules in vivo. Mice were immunized twice with a rat mAb targeting a DC surface marker or an isotype-matched, non-targeting control mAb. In this way, the rat mAb served as both targeting unit and antigen. Blood samples were drawn at different time points and the presence of anti-rat IgG antibodies was determined by ELISA. CD11c proved to be among the best targets for obtaining a strong antibody response upon delivery of antigen in vivo, indirectly confirming the efficiency of CD11c as an effective antigen-delivery receptor for the activation of antigen-specific CD4+ T cells [21]. Other authors have also identified CD11c as an interesting structure for antigen targeting, showing that it is effective for the generation of both CD4- and CD8 T cell responses [22, 23].

CD11c is the alfa chain of a heterodimer with CD18, forming the integrin $\alpha_X\beta_2$, also known as complement receptor 4 (CR4), and is, thus, a member of the beta-2 integrin family. CD11c/CD18 binds the complement fragment iC3b [24] but over the years, CD11c/CD18 has been shown to

bind to many diverse ligands, including complement fragments, heparin sulfates and bacterial lipopolysaccharide [25]. Binding of a vast repertoire of ligands to CR4, has been proposed to be largely due to a positively charged ridge in the I-domain of CD11c, which binds to negatively charged moieties in the ligand [25]. The large number of possible ligands, including complement fragments, and the demonstrated subsequent internalization of bound ligand place CR4 as a possible antigen-sampling receptor.

In man, the CD11c/CD18 complex is expressed on several subsets of DCs [26] as well as on macrophages, monocytes, granulocytes [27], activated B cells [28], and some T cell populations [29]. In the mouse, CD11c/CD18 is markedly expressed by most DC subsets and less so on other myeloid and lymphoid cells [26]. Consequently, CD11c is widely used as a DC-specific surface marker in the murine system.

With its predominant expression on antigen-presenting cells, CD11c constitutes an interesting receptor for targeted delivery of antigens in both the murine and the human system.

Activation of dendritic cells by a targeted DNA

Presentation of antigen to T cells by immature DCs leads to T cell anergy, and antigenic tolerance [4]. In case of a vaccination, this is naturally undesirable, and therefore modern vaccines include a stimulatory signal, or adjuvant, that induces DC maturation.

Originally, it was proposed that the immune system discriminates between self and non-self. This paradigm was later challenged by Polly Matzinger, who proposed the so-called danger theory, according to which the immune system discriminates between dangerous vs. harmless stimuli, rather than between self and non-self [30]. Today, it is widely accepted that the immune system reacts strongly to signals indicating tissue damage. These signals, or damage-associated molecular patterns (DAMPs), are typically intracellular constituents that are not normally present in the extracellular compartment. They are recognized by pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and the more recently discovered group of cytosolic DNA sensors.

DCs are central players in detecting danger signals and conveying this information to the adaptive immune system, and therefore express a wide range of PRRs. DCs stimulated by various types of DAMPs and pathogen-associated molecular patterns (PAMPs) ultimately display different cytokine profiles, and thereby influence the differentiation of antigen-specific T cells into different effector cell subsets [31]. If a DAMP or PAMP is employed as an adjuvant in a vaccine, it is, therefore, important to choose one that will induce the desired type of response.

In the case of solid tumors, the most likely DAMPs are substances originating from dying cells within the tumor [32]. They can be proteins like the high mobility group box 1 (HMGB1) protein or the heat shock proteins (HSP), which has been shown to signal through TLR4; or they may be small cytoplasmic molecules, like ATP, which binds to purinergic receptors, including the P2X family [33–36]. Of this family, the P2X7 receptor appears to play an important role during inflammation and cancer immune responses [37, 38]. Another important DAMP is DNA, and it was recently demonstrated by Woo et al. that tumor-infiltrating DCs are able to take up tumor cell-derived DNA in the B16 melanoma model [39]. Using lipofectamine transfection with double-stranded DNA and flow cytometric analysis of the surface markers CD83, CD86 and HLA-DR, we have shown that human moDCs mature after cytoplasmic exposure to DNA [40].

The pathway involved in the recognition of DNA in the cytosol has been under intense investigation in the past decade. In 2008, Ishikawa et al., discovered an endoplasmic reticulum (ER)-associated transmembrane adaptor protein, named stimulator of interferon genes (STING) that was involved in the induction of type I interferon production after cytosolic DNA exposure [41]. After activation, STING translocates from the ER to the Golgi, and recruits TANK-binding kinase 1 (TBK1) [42]. TBK1 phosphorylates interferon regulatory factor 3 (IRF3) and initiates its translocation to the cell nucleus, where it functions as a transcription factor for type I interferons [43–45].

STING is activated by small cyclic di-nucleotides (CDN) of bacterial or host cellular origin [46]. In mammalian cells, the CDN cyclic GMP–AMP (cGAMP) is generated by the cytoplasmic DNA sensor cyclic GMP–AMP synthase (cGAS) after binding of double-stranded DNA [47, 48].

The cGAS/STING pathway was first recognized as an important pathway in the immune response against viral infections and for its role in different autoimmune diseases [49]. However, in recent years it has become clear that this pathway is also an important part of the anti-tumor immune response, specifically in the generation of tumor-specific T cell responses. Therefore, it is of interest to investigate means of activating this pathway for cancer immunotherapy [39, 49].

Recent studies have shown that targeting STING is an efficient strategy to enhance cancer immunotherapy. Corrales et al. demonstrated that the STING agonist DMXAA (5,6-dimethylxanthenone-4-acetic acid), on its own, could induce anti-tumor immune reactions in mice after intratumoral injections [50]. Nakamura et al. tested a system where another STING agonist, the cyclic dinucleotide, c-di-GMP, was delivered to the cytosol of cells via liposomes. They found

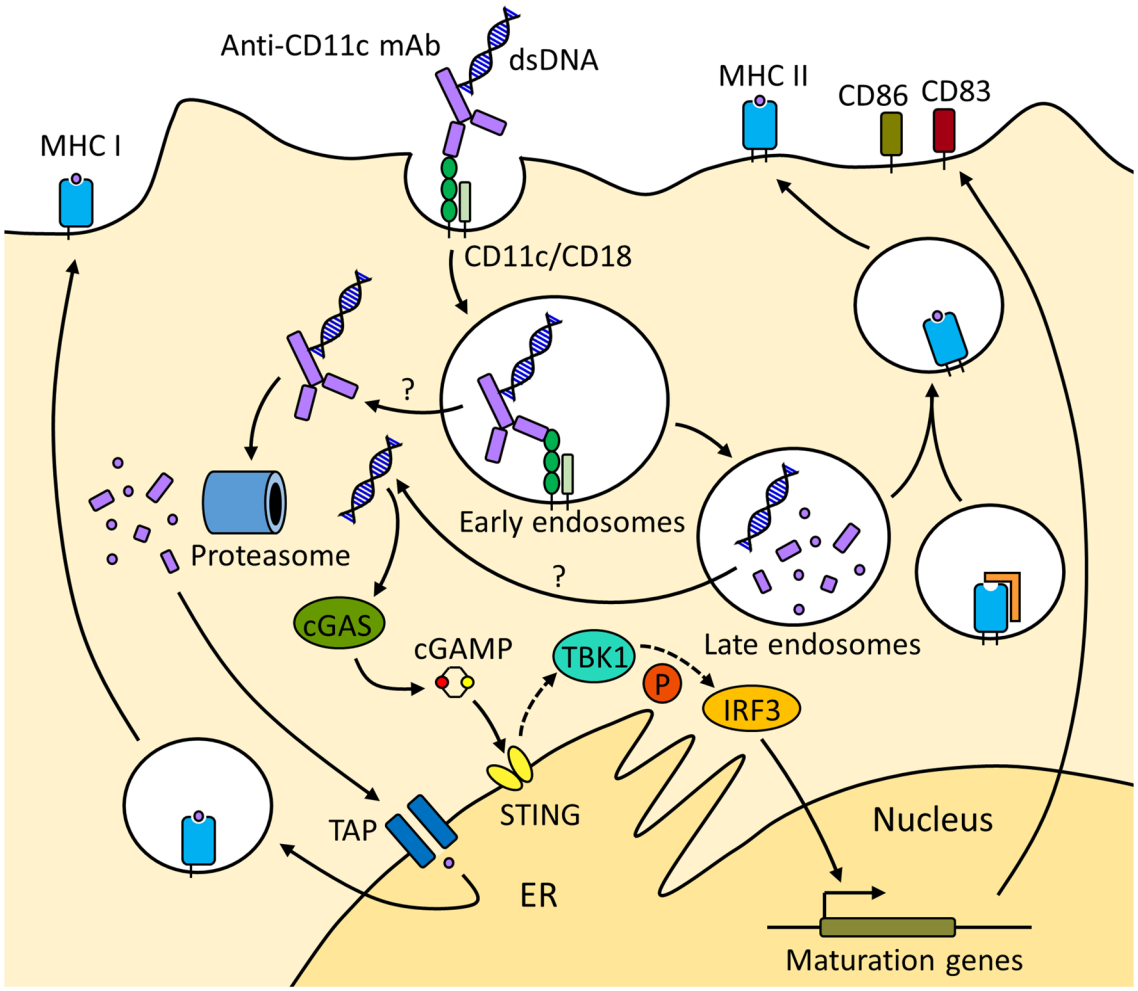

Fig. 1 Targeting DNA to DCs via CD11c leads to internalization and subsequent maturation. Upon binding, the mAb-DNA construct is internalized into early endosomes. From here, the DNA escapes the endosome by a so far unknown mechanism and is released into the cell cytoplasm, where it is free to interact with the cytosolic DNA sensor cGAS. cGAS activates STING via the second messenger, cGAMP, and recruits TBK1. TBK1 phosphorylates IRF3 and this induces transcription of genes involved in DC maturation and expression of CD83 and CD86. The targeting Ab will be degraded to facilitate MHC presentation, and activation of T cells. *dsDNA* double-stranded DNA, *MHC I* MHC class I, *P* phosphoryl group, *TAP* transporter associated with antigen processing

an increased activation of NK cells and a reduction in tumor load in a B16 lung melanoma mouse model [51]. In another study, Fu et al. used irradiated GM-CSF-secreting tumor cells together with synthetic CDNs (STINGVAX), which introduced anti-tumor responses in several mouse models. When combining STINGVAX and anti-PD-1 therapy, complete tumor regression was seen in the CT26 carcinoma mouse model, with no tumor growth seen after second inoculation of the tumor [52]. In an ongoing clinical study, the human STING agonist CDN MK-1454 has been shown to be safe to use as combinational therapy together with PD-1 inhibitor pembrolizumab on solid tumors or lymphomas. Preliminary data from this trial indicate that monotherapy with MK-1454 had no effect, whereas combinational therapy led to tumor regression in 24% (6 out of 25) of the treated patients [53].

We generated a targeted DNA by conjugating a murine anti-human-CD11c mAb to a 60 base pair, non-coding, string of double-stranded DNA (CD11c Ab-DNA). CD11c-targeted delivery of DNA to moDCs led to internalization of both antibody and DNA, and induced DC maturation, measured by upregulation of the surface markers CD86 and HLA-DR, and production of IL-12 mRNA [40]. Using mixed leukocyte reactions (MLR), we demonstrated that moDCs targeted with CD11c Ab-DNA induced increased proliferation of allogeneic T cells. Further, we used the human monocytic cell line THP1 to generate mature DC-like cells, and demonstrated that these cells upregulated the surface expression of CD86 and HLA-DR, and produced elevated levels of IL-12 in response to lipofectamine-delivered DNA as well as in response to CD11c Ab-DNA. Finally, using CRISPR/Cas9-generated cGAS and

STING knockout THP1 cells, we showed that knockout of either cGAS or STING prevented maturation, thus demonstrating that DNA delivered through the CD11c receptor is exposed in the cytosol and activates STING [40].

In conclusion, our data on targeting antigen to CD11c support and expand the finding by other authors that CD11c is a useful target structure for antigen delivery in vivo, leading to the induction of antigen-specific immune responses. Further, we have demonstrated that CD11c is also an interesting target for the delivery of double-stranded DNA, leading to DC maturation in vitro dependent on the cGAS/STING pathway (Fig. 1).

In the mouse, CD11c is expressed by not only cDC1, but also cDC2, pDC and monocyte-derived DCs [54, 55]. We speculate whether targeting DNA to CD11c on these cells in situ might break the tumor-induced immune suppression in tumor-bearing mice and lead to effective CD4+ and CD8+ T cell responses against the cancer cells.

Since a requirement for a good clinical response to immune checkpoint inhibition in cancer patients is the ability to mount an adequate T cell response, we hypothesize that combining immune checkpoint inhibition with this novel targeted DNA alone, or together with an anti-cancer vaccine, may be a path leading to a more effective immunological clearing of tumor cells. As shown both in pre-clinical and clinical trials [52, 53], activation of STING enhances the anti-cancer effects of immune check point inhibitors. We hope that our way of stimulating the cGAS/STING pathway may eventually improve the effect of the treatment with immune checkpoint inhibitors for cancer patients.

Author contributions Marlene Fyrstenberg Laursen, Emil Kofod-Olsen and Ralf Agger all contributed equally to the writing of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674. https://doi.org/10.1016/j.cell.2011.02.013
2. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12:252–264. https://doi.org/10.1038/nrc3239
3. Wei SC, Levine JH, Cogdill AP et al (2017) Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. Cell 170:1120–1133. https://doi.org/10.1016/j.cell.2017.07.024.Distinct
4. Mellman I (2013) Dendritic cells: master regulators of the immune response. Cancer Immunol Res 1:145–149. https://doi.org/10.1158/2326-6066.CIR-13-0102
5. Böttcher JP, Reis e Sousa C (2018) The role of type 1 conventional dendritic cells in cancer immunity. Trends Cancer 4:784–792. https://doi.org/10.1016/j.trecan.2018.09.001
6. Thompson ED, Enriquez HL, Fu Y-X, Engelhard VH (2010) Tumor masses support naive T cell infiltration, activation, and differentiation into effectors. J Exp Med 207:1791–1804. https://doi.org/10.1084/jem.20092454
7. Guilliams M, Ginhoux F, Jakubzick C et al (2014) Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol 14:571–578. https://doi.org/10.1038/nri3712
8. Roberts EW, Broz ML, Binnewies M et al (2016) Critical Role for CD103+/CD141+ dendritic cells bearing CCR8 for tumor antigen trafficking and priming of T cell immunity in melanoma. Cancer Cell 30:324–336. https://doi.org/10.1016/j.ccell.2016.06.003
9. Diao J, Gu H, Tang M et al (2018) Tumor dendritic cells (DCs) derived from precursors of conventional DCs are dispensable for intratumor CTL responses. J Immunol 201:1306–1314. https://doi.org/10.4049/jimmunol.1701514
10. Gardner A, Ruffell B (2016) Dendritic cells and cancer immunity. Trends Immunol 37:855–865. https://doi.org/10.1016/j.it.2016.09.006
11. Mitchell D, Chintala S, Dey M (2018) Plasmacytoid dendritic cell in immunity and cancer. J Neuroimmunol 322:63–73. https://doi.org/10.1016/j.jneuroim.2018.06.012
12. Spranger S, Bao R, Gajewski TF (2015) Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. Nature 523:231–235. https://doi.org/10.1038/nature14404
13. Gabrilovich DI, Ishida T, Nadaf S et al (1999) Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. Clin Cancer Res 5:2963–2970. https://doi.org/10.1158/1078-0432.ccr-06-2197
14. Park S-J, Nakagawa T, Kitamura H et al (2004) IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation. J Immunol 173:3844–3854. https://doi.org/10.4049/jimmunol.173.6.3844
15. Hegde S, Pahne J, Smola-Hess S (2004) Novel immunosuppressive properties of interleukin-6 in dendritic cells: inhibition of NF-kappaB binding activity and CCR15 expression. FASEB J 18:1439–1441. https://doi.org/10.1096/fj.03-0969fje
16. Sato T, Terai M, Tamura Y et al (2011) Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. Immunol Res 51:170–182. https://doi.org/10.1007/s12026-011-8262-6
17. Mittal SK, Roche PA (2015) Suppression of antigen presentation by IL-10. Curr Opin Immunol 34:22–27. https://doi.org/10.1016/j.coi.2014.12.009
18. Bandola-Simon J, Roche PA (2018) Dysfunction of antigen processing and presentation by dendritic cells in cancer. Mol Immunol. https://doi.org/10.1016/j.molimm.2018.03.025
19. Pugholm LH, Varming K, Agger R (2016) Antibody-mediated delivery of antigen to dendritic cells. Immunother Open Access 02:1–10. https://doi.org/10.4172/2471-9552.1000119
20. Pugholm LH, Varming K, Agger R (2015) In vitro assay for screening of optimal targets for antigen-delivery to murine dendritic cells. Scand J Immunol 82:498–505. https://doi.org/10.1111/sji.12365
21. Pugholm LH, Petersen LR, Søndergaard EKL et al (2015) Enhanced humoral responses induced by targeting of antigen to

murine dendritic cells. Scand J Immunol 82:515–522. https://doi.org/10.1111/sji.12387
22. Castro FVV, Tutt AL, White AL et al (2008) CD11c provides an effective immunotarget for the generation of both CD4 and CD8 T cell responses. Eur J Immunol 38:2263–2273. https://doi.org/10.1002/eji.200838302
23. Wei H, Wang S, Zhang D et al (2009) Targeted delivery of tumor antigens to activated dendritic cells via CD11c molecules induces potent antitumor immunity in mice. Clin Cancer Res 15:4612–4621. https://doi.org/10.1158/1078-0432.CCR-08-3321
24. Bilsland CA, Diamond MS, Springer TA (1994) The leukocyte integrin p150,95 (CD11c/CD18) as a receptor for iC3b. Activation by a heterologous beta subunit and localization of a ligand recognition site to the I domain. J Immunol 152:4582–4589
25. Vorup-Jensen T, Jensen RK (2018) Structural immunology of complement receptors 3 and 4. Front Immunol 9:2716. https://doi.org/10.3389/fimmu.2018.02716
26. Ganguly D, Haak S, Sisirak V, Reizis B (2013) The role of dendritic cells in autoimmunity. Nat Rev Immunol 13:566–577. https://doi.org/10.1038/nri3477
27. Hogg N, Takacs L, Palmer DG et al (1986) The p150,95 molecule is a marker of human mononuclear phagocytes: comparison with expression of class II molecules. Eur J Immunol 16:240–248. https://doi.org/10.1002/eji.1830160306
28. Postigo AA, Corbí AL, Sánchez-Madrid F, de Landázuri MO (1991) Regulated expression and function of CD11c/CD18 integrin on human B lymphocytes. Relation between attachment to fibrinogen and triggering of proliferation through CD11c/CD18. J Exp Med 174:1313–1322
29. Qualai J, Li L-X, Cantero J et al (2016) Expression of CD11c is associated with unconventional activated T cell subsets with high migratory potential. PLoS One 11:e0154253. https://doi.org/10.1371/journal.pone.0154253
30. Matzinger P (1994) Tolerance, danger, and the extended family. Annu Rev Immunol 12:991–1045. https://doi.org/10.1146/annurev.iy.12.040194.005015
31. Gornati L, Zanoni I, Granucci F (2018) Dendritic cells in the cross hair for the generation of tailored vaccines. Front Immunol 9:1484. https://doi.org/10.3389/fimmu.2018.01484
32. Galluzzi L, Buqué A, Kepp O et al (2017) Immunogenic cell death in cancer and infectious disease. Nat Rev Immunol 17:97–111. https://doi.org/10.1038/nri.2016.107
33. Di Virgilio F, Dal Ben D, Sarti AC et al (2017) The P2X7 receptor in Infection and Inflammation. Immunity 47:15–31. https://doi.org/10.1016/j.immuni.2017.06.020
34. El-Moatassim C, Dubyak GR (1992) A novel pathway for the activation of phospholipase D by P2z purinergic receptors in BAC1.2F5 macrophages. J Biol Chem 267:23664–23673
35. Kim S, Kim SY, Pribis JP et al (2013) Signaling of high mobility group box 1 (HMGB1) through toll-like receptor 4 in macrophages requires CD14. Mol Med 19:88–98. https://doi.org/10.2119/molmed.2012.00306
36. Vabulas RM, Ahmad-Nejad P, Ghose S et al (2002) HSP70 as Endogenous stimulus of the toll/interleukin-1 receptor signal pathway. J Biol Chem 277:15107–15112. https://doi.org/10.1074/jbc.M111204200
37. Ferrari D, Chiozzi P, Falzoni S et al (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J Immunol 159:1451–1458
38. Ghiringhelli F, Apetoh L, Tesniere A et al (2009) Activation of the NLRP3 inflammasome in dendritic cells induces IL-1β–dependent adaptive immunity against tumors. Nat Med 15:1170–1178. https://doi.org/10.1038/nm.2028
39. Woo SR, Fuertes MB, Corrales L et al (2014) STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity 41:830–842. https://doi.org/10.1016/j.immuni.2014.10.017
40. Laursen MF, Christensen E, Degn LLT et al (2018) CD11c-targeted Delivery of DNA to dendritic cells leads to cGAS- A nd STING-dependent maturation. J Immunother 41:9–18. https://doi.org/10.1097/CJI.0000000000000195
41. Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455:674–678. https://doi.org/10.1038/nature07317
42. Ishikawa H, Ma Z, Barber GN (2009) STING regulates intracellular DNA-mediated, type i interferon-dependent innate immunity. Nature 461:788–792. https://doi.org/10.1038/nature08476
43. Tanaka Y, Chen ZJ (2013) STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal. https://doi.org/10.1126/scisignal.2002521.STING
44. Fitzgerald KA, McWhirter SM, Faia KL et al (2003) IKKE and TBKI are essential components of the IRF3 signalling pathway. Nat Immunol 4:491–496. https://doi.org/10.1038/ni921
45. Chen Q, Sun L, Chen ZJ (2016) Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. Nat Immunol 17:1142–1149. https://doi.org/10.1038/ni.3558
46. Barber GN (2014) STING-dependent cytosolic DNA sensing pathways. Trends Immunol 35:88–93. https://doi.org/10.1016/j.it.2013.10.010
47. Wu J, Sun L, Chen X et al (2013) Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339:826–830. https://doi.org/10.1126/science.1232033
48. Sun L, Wu J, Du F et al (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339:786–791. https://doi.org/10.1126/science.1229963
49. Corrales L, Gajewski TF (2015) Molecular pathways: targeting the stimulator of interferon genes (STING) in the immunotherapy of cancer. Clin Cancer Res 21:4774–4779. https://doi.org/10.1158/1078-0432.CCR-15-1362
50. Corrales L, Glickman LH, McWhirter SM et al (2015) Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell Rep 11:1018–1030. https://doi.org/10.1016/j.celrep.2015.04.031
51. Nakamura T, Miyabe H, Hyodo M et al (2015) Liposomes loaded with a STING pathway ligand, cyclic di-GMP, enhance cancer immunotherapy against metastatic melanoma. J Control Release 216:149–157. https://doi.org/10.1016/j.jconrel.2015.08.026
52. Fu J, Kanne DB, Leong M et al (2015) STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. Sci Transl Med 7:283ra52. https://doi.org/10.1126/scitranslmed.aaa4306
53. Harrington KJJ, Brody J, Ingham M et al (2018) Preliminary results of the first-in-human (FIH) study of MK-1454, an agonist of stimulator of interferon genes (STING), as monotherapy or in combination with pembrolizumab (pembro) in patients with advanced solid tumors or lymphomas. Ann Oncol 29:viii712. https://doi.org/10.1093/annonc/mdy424.015
54. Merad M, Sathe P, Helft J et al (2013) The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol 31:563–604. https://doi.org/10.1146/annurev-immunol-020711-074950
55. Kuhn S, Yang J, Ronchese F (2015) Monocyte-derived dendritic cells are essential for CD8+ T cell activation and antitumor responses after local immunotherapy. Front Immunol 6:1–14. https://doi.org/10.3389/fimmu.2015.00584