



# Are Conventional Type 1 Dendritic Cells Critical for Protective Antitumor Immunity and How?

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Dendritic cells (DCs) are endowed with a unique potency to prime T cells, as well as to orchestrate their expansion, functional polarization and effector activity in non-lymphoid tissues or in their draining lymph nodes. The concept of harnessing DC immunogenicity to induce protective responses in cancer patients was put forward about 25 years ago and has led to a multitude of DC-based vaccine trials. However, until very recently, objective clinical responses were below expectations. Conventional type 1 DCs (cDC1) excel in the activation of cytotoxic lymphocytes including CD8<sup>+</sup> T cells (CTLs), natural killer (NK) cells, and NKT cells, which are all critical effector cell types in antitumor immunity. Efforts to investigate whether cDC1 might orchestrate immune defenses against cancer are ongoing, thanks to the recent blossoming of tools allowing their manipulation *in vivo*. Here we are reporting on these studies. We discuss the mouse models used to genetically deplete or manipulate cDC1, and their main caveats. We present current knowledge on the role of cDC1 in the spontaneous immune rejection of tumors engrafted in syngeneic mouse recipients, as a surrogate model to cancer immunosurveillance, and how this process is promoted by type I interferon (IFN-I) effects on cDC1. We also discuss cDC1 implication in promoting the protective effects of immunotherapies in mouse preclinical models, especially for adoptive cell transfer (ACT) and immune checkpoint blockers (ICB). We elaborate on how to improve this process by *in vivo* reprogramming of certain cDC1 functions with off-the-shelf compounds. We also summarize and discuss basic research and clinical data supporting the hypothesis that the protective antitumor functions of cDC1 inferred from mouse preclinical models are conserved in humans. This analysis supports potential applicability to cancer patients of the cDC1-targeting adjuvant immunotherapies showing promising results in mouse models. Nonetheless, further investigations on cDC1 and their implications in anti-cancer mechanisms are needed to determine whether they are the missing key that will ultimately help switching cold tumors into therapeutically responsive hot tumors, and how precisely they mediate their protective effects.

**Keywords:** conventional type 1 dendritic cells, tumor, type I IFN, CD8<sup>+</sup> T cells, NK cells, immunotherapy, cancer immunosurveillance, clinical trials

## INTRODUCTION

Immune responses against cancer are sculpted by the tumor microenvironment, including its composition in terms of cell types and their physiological states. Indeed, tumors escape host immune defenses not only through decreasing their intrinsic immunogenicity but also by shaping a specific immunosuppressive microenvironment (1, 2). Exogenous factors such as the microbiota and its metabolites also modulate the tumor microenvironment and hence antitumor immune responses (3). According to their degree of infiltration by immune cells and to their capacity to activate antitumor immune responses, tumors have been classified as immunologically “Hot” or “Cold.” “Hot” tumors are immunogenic, T cell-inflamed, and efficiently rejected by the immune system. They are characterized by the presence of activated CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs), by the expression of T cell-attracting chemokines, and by a type I interferon (IFN-I) transcriptional signature (4). “Cold” tumors lack T cell infiltration, which is correlated with an absence of IFN-I signature and with a poor chemokine production (4). They are ignored by the immune system due to their poor immunogenicity and are very poorly responsive to immunotherapies. We propose to refine this bimodal classification through the addition of two other tumor states, which we called “Icy” and “Warm.” We define as “Icy” the tumors that develop potent, active, mechanisms to prevent immune recognition and T cell activation, by inducing a highly immunosuppressive microenvironment very early on during their development. Hence, “Icy” tumors are even more refractory to immune control than “Cold” tumors. “Warm” tumors present an intermediate level of infiltration by “exhausted” CTLs, which have been functionally paralyzed by the local immunosuppressive environment that has been progressively shaped during tumor development. The exhaustion of CTLs is at least in part due to engagement of their immune checkpoint receptors by ligands expressed by the tumor cells themselves or by infiltrating antigen (Ag)-presenting cells. “Warm” tumors are more prone to be controlled by immune checkpoint blockade (ICB) treatments. These monoclonal antibody (mAb)-based immunotherapies have revolutionized cancer patient care, by significantly increasing not only overall survival rates but also very long-term remissions for tumor types previously difficult to treat. Despite this major advance, the majority of patients with difficult-to-treat cancers do not respond to ICB. To overcome this issue, it is critical to find additional means of manipulating the microenvironment of the “Cold” or “Warm” tumors unresponsive to ICB, in order to convert them into “Hot” tumors. It should be possible to achieve this by combining ICB with adjuvant immunotherapies able to counteract the other immune escape mechanisms established by these tumors, in order to (i) trigger *de novo* or enhance T cell infiltration, (ii) enhance cross-presentation of tumor-associated Ag, and (iii) promote a better induction or reactivation of CTL effector functions.

Dendritic cells (DCs) are the most potent Ag-presenting cells, with a unique efficacy for priming naïve T cells and

inducing their functional polarization. They are more generally in charge of orchestrating the expansion and functions of T and natural killer (NK) cells in lymphoid and non-lymphoid tissues. Many clinical trials have been performed over the last 25 years to attempt harnessing DC functions for boosting protective antitumor CTL responses in cancer patients (5). Up to now, the results have been disappointingly far below expectations. These failures occurred at least in part because of the almost exclusive use of monocyte-derived DCs (MoDCs) for ACT in cancer patients. Indeed, later advancement of our basic understanding of the heterogeneity and functional plasticity of DCs suggested that other types than MoDCs should be better suited for this purpose (6–8). A relatively recent consensus has emerged on a universal and simplified classification of DC types both in mice and in humans, based on their ontogeny, gene expression programs, phenotype, functions and localization (9, 10). Five major types of DCs can be distinguished: plasmacytoid DC (pDCs), type 1 conventional DCs (cDC1), type 2 cDCs (cDC2), Langerhans cells and MoDCs. In mice, cDC1 encompass both the lymphoid tissue-resident CD8 $\alpha$ <sup>+</sup> cDCs as well as the CD103<sup>+</sup>CD11b<sup>-</sup> cDCs that reside in the parenchyma of non-lymphoid tissues and, once matured upon activation, can migrate to the draining lymph nodes. In humans, cDC1 correspond to the CD141 (BDCA3)<sup>high</sup>CD11b<sup>-/low</sup> cDCs. Both mouse and human cDC1 express specifically the chemokine receptor XCR1 and selectively the C-type lectin endocytic receptor CLEC9A (11). cDC1 can directly enter tissues from the blood, or differentiate locally from a dedicated progenitor, the pre-cDC1 that has been characterized both in the mouse and the human (12, 13). Mouse cDC2 correspond to the CD11b<sup>+</sup> cDCs, and human cDC2 to the CD1c (BDCA1)<sup>high</sup>CD11b<sup>+/high</sup> cDCs. For a very long time, MoDCs were the only DC type that could be produced *in vitro*, in high numbers and under clinical-grade conditions (5, 6, 8). They were therefore used for most immunotherapeutic clinical trials based on adoptive cell transfer (ACT) of *in vitro* derived autologous DCs. However, MoDCs strikingly differ from cDC1 and cDC2 that are the major types of DCs residing in secondary lymphoid organs and orchestrating immune responses *in vivo* (14–16). For example, MoDCs do not migrate efficiently to lymph nodes and are particularly prone to develop immunosuppressive functions, whereas cDC1 excel in the activation of CTLs, which are critical effector cell types for antitumor immunity (17). Thus, major efforts have been conducted in the last 10 years to investigate whether cDC1 might be critical for defense against cancer, and how. Here, we are reporting on studies addressing this issue in mice, under experimental conditions of spontaneous immune rejection of tumor grafts in syngeneic recipients, or in preclinical models of immunotherapies. We also summarize human studies that mined large datasets of tumor gene expression profiles to investigate correlations between clinical outcome and digital deconvolution of the tumor immune infiltrate. We discuss how the knowledge generated by these studies can instruct innovative immunotherapeutic strategies to harness cDC1 functions for the benefits of cancer patients.

## NO CURRENTLY AVAILABLE MUTANT MOUSE MODEL IS SPECIFICALLY TARGETING ONLY cDC1 *IN VIVO*

To determine whether and how a given type of immune cells plays a non-redundant role in antitumor immunity *in vivo*, it should be specifically and efficiently manipulated in mice. Different mutant mouse models have been generated to either deplete DCs, or inactivate candidate genes in DCs, as recently reviewed (18, 19). Here, we will specifically discuss the use of mutant mouse models to investigate the functions of cDC1 or their molecular regulation (**Table 1**) (14, 20–47). Mouse models expressing the Cre DNA recombinase under the control of the promoter of a gene selectively expressed in DCs have been generated to enable conditional deletion of candidate floxed genes in the targeted cells (e.g., *Itgax-Cre* targeting CD11c<sup>+</sup> cells and *Xcr1-Cre* targeting cDC1). Constitutive depletion models have been generated using two types of strategies. The first corresponds to the knock-out of a transcription factor shown to be crucial selectively for the development/homeostasis of cDCs (*Zbtb46*) or cDC1 (*Batf3*) (**Table 1**). The second consists in ectopic expression of the active subunit of the diphtheria toxin (*DTA*) selectively in DCs (e.g., *Xcr1-Cre;Rosa26-LSL-DTA* mice for cDC1, **Table 1**). Conditional depletion can be achieved upon diphtheria toxin administration in mutant animals engineered for ectopic expression of the gene encoding the human diphtheria toxin receptor (*hDTR*) selectively in DCs (e.g., *Karma-hDTR* or *Xcr1-hDTR* mice for cDC1).

One major caveat of using CD11c for targeting DCs is that the gene encoding this molecule, *Itgax*, is expressed by other immune cell types, including some that play critical roles in anti-tumor immunity, such as NK cells, effector memory CTLs, intraepithelial lymphocytes (IELs), plasmablasts, and subsets of monocytes or macrophages (32). Knock-in within the *Zbtb46* gene has been used to target all cDCs. However, this gene is also expressed by endothelial cells and committed erythroid progenitors (14, 32–34). Since angiogenesis critically affects solid tumor development, experiments should be performed using bone marrow chimera mice generated by engrafting mutant bone marrow cells into a wild type (WT) recipient animal. *Batf3*<sup>-/-</sup> mice have been the most frequently used model to investigate whether cDC1 play a critical role in physiological processes. However, even in this model, complementary strategies are needed before drawing final conclusions, because *Batf3* is also expressed in cDC2 and effector CD4<sup>+</sup> T cells, and because it represses *Foxp3* expression in CD4<sup>+</sup> T cells leading to increased numbers of regulatory T cells (Treg) in knock-out mice (35, 36). In addition, the impact of *Batf3* inactivation on cDC1 homeostasis is less efficient in the *C57BL/6J* genetic background than in the *129svEv* one. Under inflammatory settings, the knock-out of *Batf3* can be compensated for cDC1 development, by the induction in DC precursors of the paralog genes *Batf* and *Batf2* (37–39). We have engineered mutant mouse models for cDC1 targeting based on the knock-in of Cre (42) or hDTR (43) into the *Gpr141b* (alias *A530099j19rik* or *Karma*)

gene, but these models also target mast cells (42). Finally, the *Xcr1* gene was targeted to generate mutant mouse models for specific, conditional or constitutive, cDC1 depletion, as well as for their genetic manipulation (42, 44, 46). The *Xcr1* gene is preserved in our models (42). In contrast, it is knocked-out in the other ones (46); hence, only heterozygous mice should be used for these models in order to avoid possible phenotypic effects due to a complete XCR1 deficiency. Besides cDC1, only a minute proportion of CD4<sup>+</sup> T cells are targeted in *Xcr1-Cre* mice (42). Although still imperfect, the mutant mouse models based on the manipulation of the *Xcr1* gene are the best to target cDC1 *in vivo*. In conclusion, none of the mutant mouse models used to date for cDC1 targeting are entirely specific and efficient, but some are better suited than others for this purpose. In any case, it is always important to use complementary methods to ensure that the phenotypes observed are only or mostly due to the manipulation of cDC1. For example, depleted mice should be replenished with wild-type cDC1 if possible. Alternately, results should be confirmed in other mutant models also targeting cDC1 but no other cell types in common.

## THE ROLE OF cDC1 IN CANCER IMMUNOSURVEILLANCE HAS NOT YET BEEN INVESTIGATED

Cancer development is a multistep process consisting in the accumulation of genetic mutations within a cell leading to increased or deregulated proliferation and survival, with clonal selection of neoplastic progeny (48). There is a strong contribution of the host immune responses in this dynamical process of tumor selection, which has been described as the three E of cancer immunoeediting: Elimination, Equilibrium and Escape of cancer cells (49). A failure of the immune system to eliminate all transformed cells early during their development is followed by an equilibrium state during which the immune system exerts a relentless pressure on surviving tumor cells, ultimately leading to tumor escape from the exhausted immune system. The initial elimination phase is therefore critical to restrict tumor growth very rapidly to prevent relapse or metastasis. Efficient recognition and elimination of transformed cells implies constant monitoring of the body by both the innate and adaptive immune systems, a process called cancer immunosurveillance. Upon monitoring spontaneous, carcinogen- or genetically-induced tumor development in mice bearing various immune deficiencies, critical roles in cancer immunosurveillance have been uncovered for  $\alpha\beta$  and  $\gamma\delta$  T cells, NKT cells and NK cells, as well as for the cytokines IFN- $\gamma$ , IFN-I, IL-12 and for the cytotoxic effector molecules Perforin and TRAIL (50). The role of cDC1 in cancer immunosurveillance remains to be assessed. However, a wealth of data has accumulated on their role in the spontaneous immune rejection of tumor grafts in mice, a popular surrogate model for immunosurveillance (**Table 2**) (35, 51, 54–58).

**TABLE 1** | Mouse models to deplete DCs, cDCs or cDC1 *in vivo*.

Mouse strain	Depleted cells	Gene also expressed in	Remarks	Expression profile references
CD11c-hDTR*	cDCs (pDCs?)	NK cells Effector/memory CTL Monocytes, macrophages Plasmablasts IELs	Off-target transgene expression leading to death upon multiple DT injections (Requiring to perform BM chimeras for prolonged depletion)	<b>(20)</b> (21–27) ImmGen Consortium
CD11c.DOG*	DCs	NK cells Effector/memory CTL Monocytes, Macrophages Plasmablasts IELs	hDTR expression only in CD11c <sup>+</sup> cells Prolonged DC depletion possible upon multiple DT injections Ovalbumin protein is expressed in DCs, resulting in extensive OT-I and OT-II proliferation after transfer	<b>(28)</b> (20–27) ImmGen Consortium
CD205-hDTR	cDC1 and LCs	Cortical thymic epithelium Tumor MoDC and cDC2	Death induced by DT injection Use of BM chimeras required to avoid death consecutive to depletion of radioresistant CD205 <sup>+</sup> cells	<b>(29)</b> (30, 31)
Zbtb46-hDTR*	cDC1 and cDC2	Endothelial cells Committed erythroid progenitors	Death induced by a single DT injection Use of BM chimeras required	<b>(32)</b> (14, 33)
Zbtb46-LSL-hDTR*	cDC1 and cDC2	Endothelial cells Committed erythroid progenitors	Allows prolonged cDC depletion upon multiple DT injections (Need to cross with a Cre strain)	<b>(34)</b> (14, 32, 33)
<i>Batf3</i> <sup>-/-</sup>	cDC1	cDC2 Eff (Th1) CD4 <sup>+</sup> T cells Other T cells?	cDC1 depletion is effective in <i>129/SvEv</i> mice but less in <i>C57BL/6</i> animals Intracellular pathogens infections or IL-12 injection restore cDC1 development Higher differentiation in Treg of <i>Batf3</i> <sup>-/-</sup> CD4 <sup>+</sup> T cells	<b>(35)</b> (14, 36–40)
Clec9a-hDTR*	cDC1	pDCs	Half of the pDCs are depleted	<b>(41)</b> , (42)
Karma-hDTR*	cDC1	Skin and PC Mast cells	Mast cells are targeted in addition to cDC1	<b>(43)</b> , (42)
XCR1-hDTR* (Kaisho)	cDC1		Deletion of the endogenous <i>Xcr1</i> gene Requiring the use of heterozygous mice	<b>(44)</b> , (42)
XCR1-hDTR** (Dalod)	cDC1		Fate mapping of a minute proportion of CD4 <sup>+</sup> T cells	(14, 43, 45), <b>(42)</b>
XCR1-DTA*** (Kaisho)	cDC1		Deletion of the endogenous <i>Xcr1</i> gene Requiring the use of heterozygous mice	(14, 43) <b>(46)</b> , (45)
XCR1-DTA*** (Dalod)	cDC1		Fate mapping of a minute proportion of CD4 <sup>+</sup> T cells	(14, 43, 45), <b>(42)</b>

**Bold:** First publication. \*Mouse models expressing the Cre DNA recombinase under the same gene promoter have been generated. \*\**Xcr1-Cre;Rosa26-LSL-hDTR* mice; \*\*\**Xcr1-Cre;Rosa26-LSL-DTA* mice.

## BATF3<sup>-/-</sup> MICE FAIL TO REJECT SYNGENEIC TUMOR GRAFTS, SUGGESTING A CRITICAL ROLE FOR cDC1 IN SPONTANEOUS ANTITUMOR IMMUNE DEFENSES

Most tumor cells are not able to directly prime naïve T cells, due to their low expression of MHC class I and co-stimulation molecules or to their acquisition of immunosuppressive functions such as high expression of ligands for immune checkpoint receptors. Thus, induction of CTL responses against most tumors requires accessory cells able to take-up, process and present exogenous tumor Ag in association with MHC-I molecules, a process known as cross-presentation. cDCs are highly efficient in initiating and globally orchestrating adaptive immunity, due to their professional capacities to simultaneously deliver all necessary signals to T cells, namely

Ag presentation as signal 1, co-stimulation as signal 2, and cytokines as signal 3 (59). Mouse and human cDC1 excel at activating CTLs, due to their higher capacity to cross-present cellular Ag as compared to other types of Ag-presenting cells (11). It seemed therefore logical that cDC1 should play a critical role in anti-tumor immunity (**Table 2**). Kenneth Murphy's group was the first to confirm this hypothesis, by showing loss of spontaneous rejection of transplantable tumors in *Batf3*<sup>-/-</sup> mice (35). Contrary to their WT counterparts, *Batf3*<sup>-/-</sup> cDCs failed to induce proliferation of OT-I cells when co-cultured with cells loaded with the OVA protein, suggesting that cross-presentation of cellular Ag indeed constitutes one of the critical, non-redundant functions of cDC1. Several other studies have since reported similar results, altogether using a variety of transplantable tumors (**Table 2**). These studies strongly support a critical role for cDC1 in spontaneous antitumor immune defenses. However, a possible role of the loss of *Batf3* expression in cDC2 or in effector T cells has not been ruled out. In addition,

**TABLE 2 |** Tumor cell lines spontaneously rejected in immunocompetent hosts.

Tumor rejection	IFN-I-dependent	IFN-I-independent
Lost in <i>Batf3</i> <sup>-/-</sup> mice	1969 (51–53) 1773RS100 (35) B16.SIY (54) d38m2 (51) d42m1 (51) GAR4.GR1 (51) H31m1 (35, 51) <b><i>Ptgs1/Ptgs2</i><sup>-/-</sup> BRAF<sup>V600E</sup></b> (55, 56)	
ND	<u>7835</u> (51) <u>MC-57.SIY</u> (54, 57, 58) P198 (51)	F515 (51)

**Bold:** NK cell dependent rejection. **Underlined:** NK cell independent rejection. **Fibrosarcoma:** 1969, 1773RS100, 7835, d38m2, d42m1, F515, GAR4.GR1, H31m1, MC-57.SIY. **Melanoma:** B16.SIY, *Ptgs1/Ptgs2*<sup>-/-</sup> BRAF<sup>V600E</sup>. **Mastocytoma:** P198. **129/SvEv background:** 1773RS100, d38m2, d42m1, F515, GAR4.GR1, H31m1. **C57BL6/J background:** 1969, 7835, B16.SIY, MC-57.SIY, *Ptgs1/Ptgs2*<sup>-/-</sup> BRAF<sup>V600E</sup>. **DBA/2 background:** P198.

*Batf3*<sup>-/-</sup> mice can still achieve partial tumor control and mount tumor-specific CTL response under low-dose tumor challenge (35, 51, 54), which might be explained either by the incomplete cDC1 loss or by partial redundancy between cDC1 and other cell types for the cross-presentation of cellular Ag and the induction of antitumor adaptive immunity. Further studies are warranted to address these issues.

## INSIGHTS INTO HOW cDC1 COULD PROMOTE PROTECTIVE SPONTANEOUS ANTITUMOR IMMUNITY

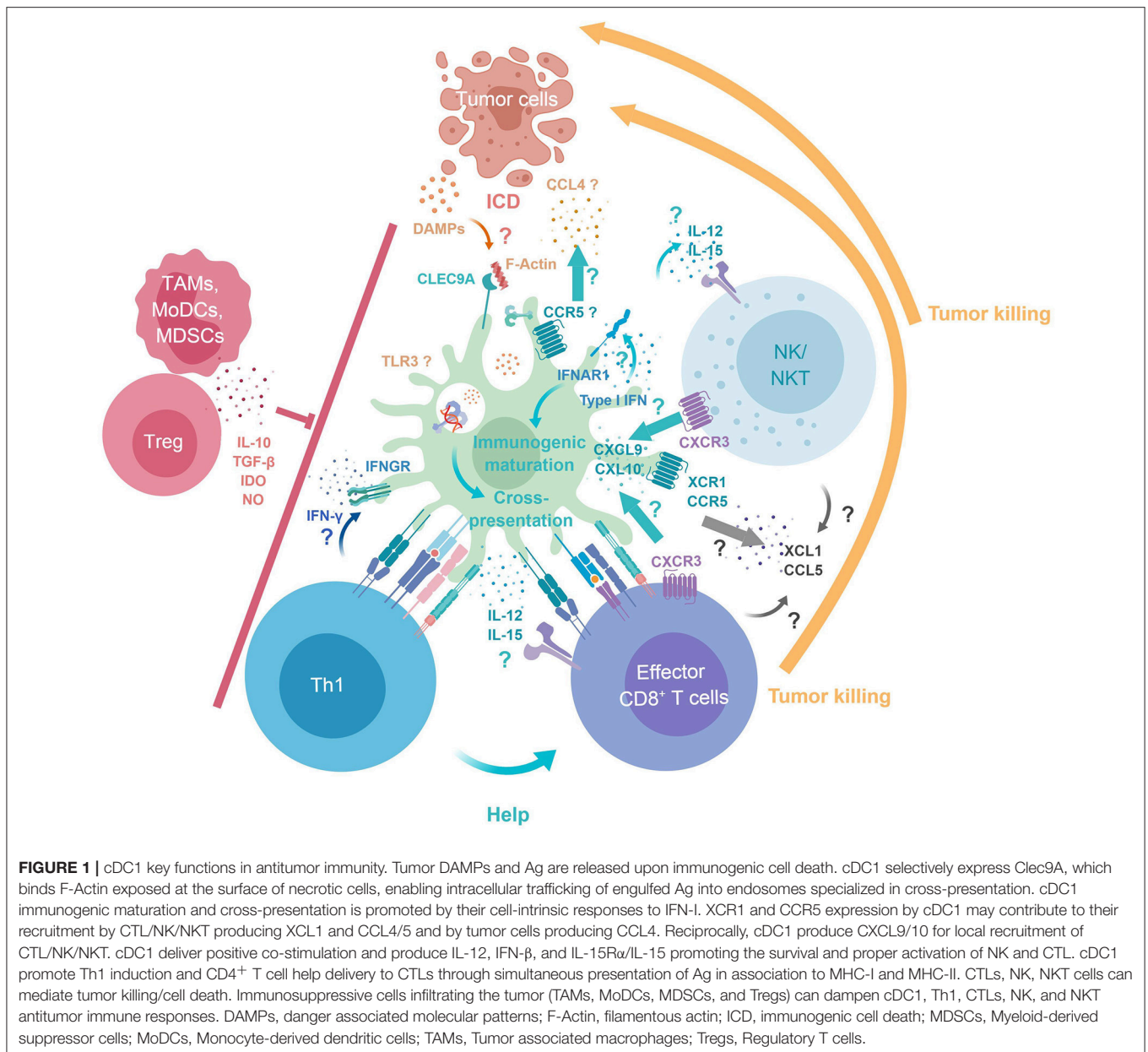
### Cross-Presentation by cDC1 Is Necessary but Not Sufficient for Immune Control of a Regressor Fibrosarcoma

The importance of cross-presentation in cancer immunology has been extensively reviewed (60). Very recently, the WDFY4 molecule, a member of the BEACH (Beige and Chediak-Higashi) domain-containing family of proteins, was reported to be specifically required for cross-presentation of cell-associated Ag by cDC1, and for cDC1-dependant immune control of the highly immunogenic 1969 regressor fibrosarcoma (52). The demonstration of a cell-intrinsic requirement of WDFY4 in cDC1 for immunity against cancer was achieved by comparing tumor growth between *Wdfy4*<sup>-/-</sup>:WT vs. *Wdfy4*<sup>-/-</sup>:*Batf3*<sup>-/-</sup> mixed bone marrow chimera mice. Importantly, *Wdfy4*<sup>-/-</sup> cDC1 were not compromised in their abilities to produce IL-12 and to present Ag in association with MHC class II molecules for CD4<sup>+</sup> T cell activation. *Wdfy4*-deficient cDC1 appeared to be selectively impaired in their ability to cross-present Ag but not in other functions also required for efficient CTL priming and expansion. To our knowledge, this study is the first to demonstrate that a specific defect in cDC1 cross-presentation *in vivo* leads to a failure of mice

to control spontaneously tumor growth. Further studies are warranted to confirm these data and extend it to other preclinical tumor models. A major role of *Batf3* in cDC1 is to sustain their expression of *Irf8*. Consistent with this, the development of cDC1 and their ability to cross-present cell-associated Ag are rescued in *Batf3*<sup>-/-</sup> animals transgenic for *Irf8*. Nevertheless, these mice still fail to control the growth of a regressor fibrosarcoma, likewise to *Batf3*<sup>-/-</sup> animals. Thus, in addition to cross-presentation, other functions of cDC1 are also necessary for the promotion of protective antitumor immunity but remain to be identified (53). Moreover, both in mice and humans, cDC2 and pDCs can also perform cross-presentation of cell-associated Ag, under specific conditions of stimulation, less efficiently than cDC1 (11). Hence, we propose that cDC1 play a critical role in antitumor immunity not only due to their strong cross-presentation activity but rather because they uniquely combine several key features that are not simultaneously expressed together in other cell types, as detailed below (Figure 1).

### Proposed Key Features Underlying cDC1 Non-redundant Role in Anti-tumor Immunity

First, the expression of XCR1 and CCR5 by cDC1 may enable their local recruitment by cytotoxic lymphocytes producing the ligands for these chemokine receptors, XCL1 and CCL4/5 (45, 55, 61–63). Second, reciprocally, cDC1 ability to produce high levels of CXCL9/10 may promote local recruitment of effector and memory CTLs expressing CXCR3 (43, 57, 64). Third, cDC1 can deliver positive co-stimulation signals. Fourth, cDC1 are a major source of IL-12, IFN-β, and IL-15, thereby promoting the survival and proper activation of NK, NKT cells and CTLs (43, 65–69). In a model of lung metastasis, cDC1 were the major source of IL-12, which was critical to control metastasis in a NK cell- and IFNγ-dependent manner (66). Fifth, cDC1 can promote Th1 induction (70–72) and favor CD4<sup>+</sup> T cell help delivery to CTL through simultaneous presentation of Ag in association to MHC-I and MHC-II (73, 74). Depending on the cues that they receive during their activation at the time of Ag processing and presentation, DCs will polarize into different functions during their maturation (75, 76). At steady state, during their homeostatic activation, DCs acquire the ability to induce immune tolerance by causing the death, anergy or polarization into regulatory functions of self-reactive T cells, a process referred to as DC tolerogenic maturation. On the contrary, in proper activating contexts, DCs undergo an immunogenic maturation by acquiring the combined expression of activating co-stimulation molecules and cytokines leading to the induction of strong Ag-specific effector lymphocyte responses. The immunogenic maturation of cDC1 is promoted by IFN-I (51, 54, 68, 75), including through cross-talk with pDCs as a major source of these cytokines (77). Cell-intrinsic responses of cDC1 to IFN-I appear to be critical for spontaneous tumor rejection by enhancing their cross-presentation capacity (51, 54), and perhaps also their trans-presentation of IL-15 which promotes the proliferation and effector differentiation of CTLs



(68). However, cross-presentation was not totally abolished in *Ifnar1*<sup>-/-</sup> DCs (51, 54). Upon exposure to high doses of Ag *in vitro*, cross-presentation was even as efficient in *Ifnar1*<sup>-/-</sup> DCs as in WT DCs. Although, in spontaneously rejected tumor grafts, the cellular source of IFN-I was identified as expressing CD11c, IFN-β production was not altered in *Batf3*<sup>-/-</sup> mice. Further investigations are required to assess the roles of different types of DCs in CTL activation and in the production of, or responses to, IFN-I, during spontaneous tumor control. In summary, cDC1 constitute a versatile and efficient platform for CTL activation by uniquely bridging several components of innate and adaptive immune responses in a manner promoting mutually beneficial cross-talk (Figure 1). However, further studies are warranted to determine whether the different mechanisms detailed above are

each critical for the protective antitumor functions of cDC1, as well as their respective importance.

### When and Where Are cDC1 Functions Exerted During Cancer Immunosurveillance?

Intra-tumoral cDC1 have been suggested to be crucial for *in situ* maintenance of the effector functions of pre-activated CTLs (65). cDC1 promote memory CTL recall upon secondary infections (43). In an experimental model of established immune memory, only tumors that could be infiltrated by both cDC1 and CTLs were spontaneously controlled (57). While conditional cDC1 depletion was not performed in these

settings to functionally confirm the importance of cDC1 for the reactivation of antitumor CTLs, this point was addressed in another study examining the reactivation of adoptively transferred antitumor central memory CTLs into WT vs. *Batf3*<sup>-/-</sup> recipient mice (78). In conclusion, cDC1 might not only be required for the initiation of adaptive immunity against intracellular pathogens or tumors but all along the life cycle of CTLs, including for their maintenance in the tumor as well as for the generation and recall of memory to prevent relapse or metastases.

Several studies have suggested that T cell priming in the tumor draining lymph node is required to mount anti-tumor immunity (79, 80). A study showed that tumor-associated cDC1 bearing intact tumor Ag traffic to the draining lymph node to prime naïve CTLs in a CCR7-dependent manner (80). However, *Ccr7* knock-out had little impact on tumor growth (80). Moreover, CTL priming, activation, proliferation and effector function acquisition in tumor was observed when T cell egress from lymph nodes was blocked (81) or in mice lacking lymph nodes (82). Although these experimental settings could alter cDC1 and lymphoid cell trafficking (83, 84), they nevertheless show that the activation of antitumor adaptive immunity can occur directly at the tumor site (82), possibly in tertiary lymphoid structures developing locally (85). In any case, for efficient tumor rejection without relapse or metastases, systemic immunity is likely important in addition to *in situ* responses, as recently appreciated in the context of immunotherapy (86).

## Proposed Model of cDC1 Role in Antitumor Immunity

Based on the knowledge discussed in the previous sections, we propose a putative model of the mechanisms through which cDC1 promote the rejection of syngeneic tumor grafts in preclinical mouse models (Table 2) and may physiologically contribute to cancer immunosurveillance (Figure 2). cDC1 take up cell-associated Ag in the tumor after immunogenic cell death, undergo immunogenic maturation, and traffic to the tumor-draining lymph node. There, cDC1 prime naïve CTLs and polarize them toward protective effector functions. CTLs expand and migrate to tumor, where they can be attracted by chemokines secreted locally by cDC1. The tumor-associated cDC1 also sustain infiltrating CTL protective functions (expansion, maintenance and memory recall), and might also prime naïve CTLs *in situ*.

## FAILURE OF IMMUNOSURVEILLANCE: ARE cDC1 DIRECT TARGETS OF TUMOR ESCAPE MECHANISMS?

We propose a classification of tumors (Hot/Warm/Cold/Icy) according to their immunogenicity, their cDC1 infiltration, maturation and phenotype, and the characteristics of the antitumor CTL response.

“Hot” tumors are characterized as strongly infiltrated by effector CTLs. They are spontaneously controlled by the immune

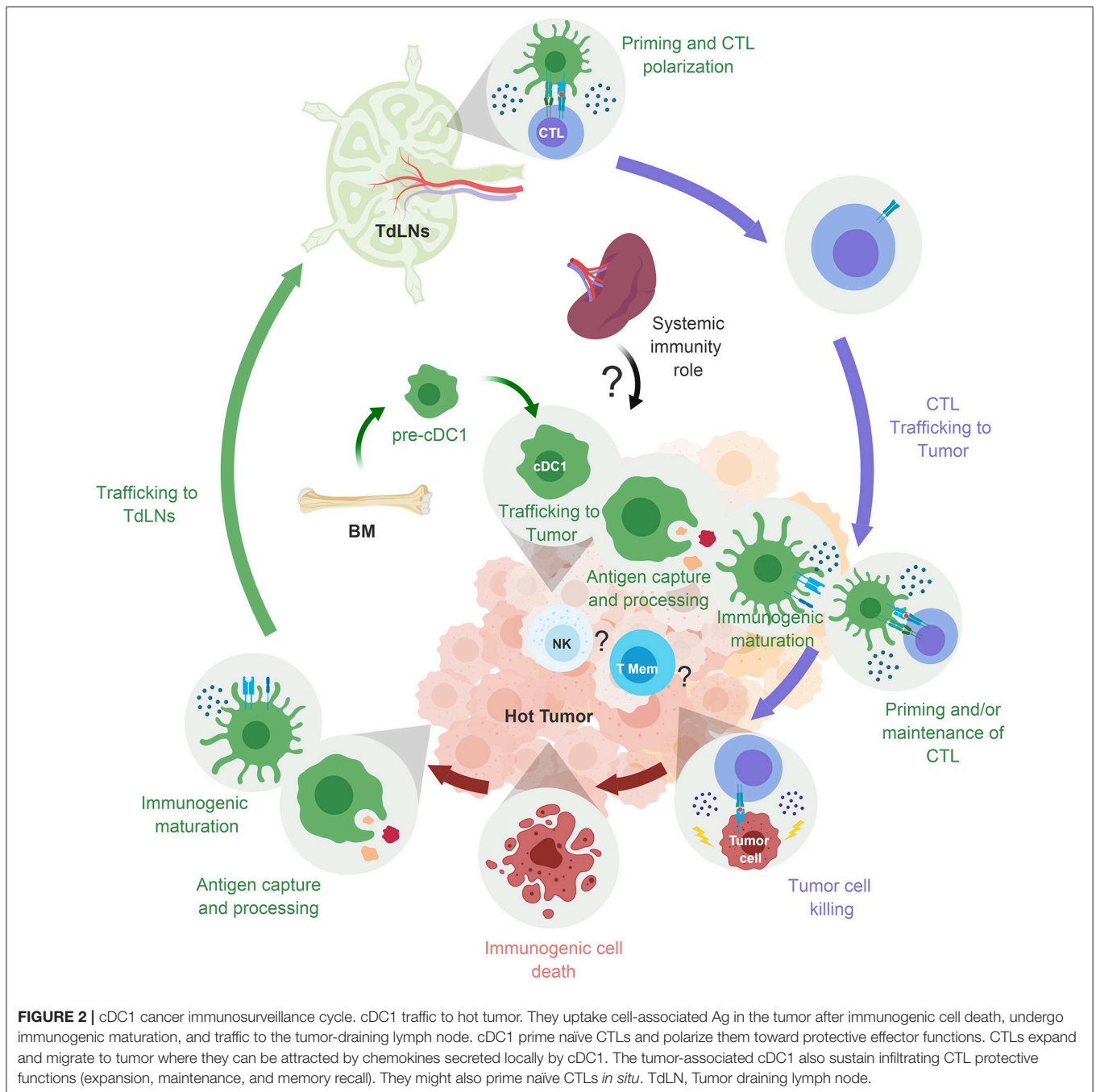
system (Figure 2). They include the syngeneic cancer cell lines used to study spontaneous rejection of tumor grafts (Table 2).

“Warm” tumors express tumor neoAg and are infiltrated by cDC1 and CTLs (Figure 3 Right). Experimental studies in mice suggest that the correlation between high CTL numbers and increased cDC infiltration in tumors is due to a positive feedback loop between these two cell types mutually promoting their local recruitment and survival. It is not clear how this process is initiated, i.e., which cell type is recruited first to the tumor site. This might depend on the combination of tumor type and host characteristics. “Warm” tumors are ultimately not controlled by the immune system, due to their late selection for harboring immune escape mechanisms, such as intrinsic impairment of Ag processing and presentation (87, 88) or induction of CTL exhaustion (89). In these tumors, cDC1 could have undergone immunogenic maturation but may present Ag to CTL in a manner contributing to their chronic activation and exhaustion, e.g., through engagement of checkpoint receptors such as PD-1 or CTLA4.

“Cold” tumors are weakly immunogenic and poorly infiltrated but induce some level of adaptive immunity (Figure 3 Top). In such tumors, cDC1 could also be direct targets of immune escape mechanisms, such as local production of factors inhibiting DC differentiation or promoting tolerogenic over immunogenic maturation. Those factors include TGF- $\beta$ , IL-10, IL-6, CSF-1, and VEGF (90). Although cDC1 are proposed to contribute to central and peripheral tolerance (75, 91, 92), whether they can be hijacked by tumors to promote local immunosuppression has not been rigorously investigated.

“Icy” tumors are not immunogenic *per se*, are not infiltrated by T cells and fail to induce immune responses (Figure 3 Left). Those tumors have evaded or hijacked innate immunity in a manner preventing immune cell infiltration at very early stages of the cancer immunoediting process (87). cDC1 can be direct targets of these early immune escape mechanisms. In melanoma, the WNT/ $\beta$ -Catenin signaling pathway prevents the recruitment of cDC1 and CTLs into the tumor, at least in part by inhibiting the local production of CCL4 and CXCL9 (57, 93). CCL4 contributes to the recruitment of cDC1 through their CCR5 chemokine receptor (93). CXCL9 helps promoting the recruitment of both pre-cDC1 (94) and memory/effector T cells (57), through CXCR3. Another mechanism of evasion of innate immunity by melanoma is tumor-intrinsic elevated COX activity leading to PGE<sub>2</sub> production and downstream inhibition of NK cell, cDC1 and CTL infiltration (55, 56), by disrupting the XCL1/XCR1 and CCL5/CCR5 chemotactic axes. Impairment of CTL infiltration into the tumor is proposed to occur downstream of the failure of cDC1 recruitment (55, 56).

In brief, cDC1 are direct targets of tumor escape mechanisms since the tumor microenvironment can modulate all of the processes necessary to promote their protective antitumor functions. It can determine the tolerogenic vs. immunogenic nature of tumor cell death (95–97), control the expression of the growth factors and chemokines promoting local recruitment, differentiation, expansion and survival of cDC1 or their progenitors (55, 93, 98), dampen cDC1 production of activating



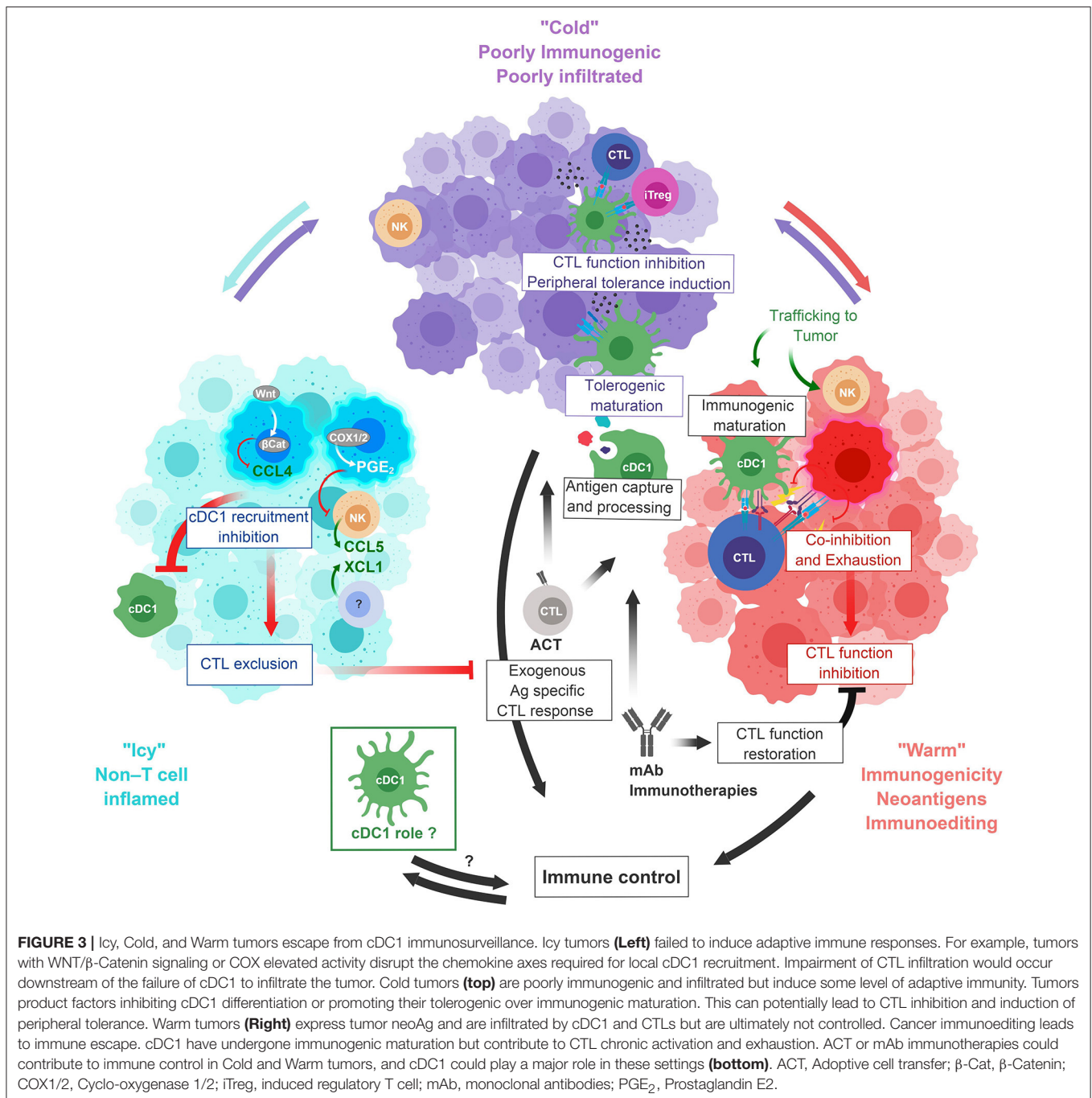
cytokines (56, 67), and inhibit their maturation or even polarize it toward tolerance (99, 100).

## STUDIES OF THE NATURAL ROLE OF cDC1 IN IMMUNOTHERAPIES

In the last two decades, cancer treatments have successfully shifted from only targeting the cancer itself to also manipulating the immune system, with the aim to boost or induce *de novo*

protective antitumor cellular immune responses, mainly CTLs but also NK and NKT cells. These novel treatments called immunotherapies encompass different strategies. Here we will specifically discuss studies performed in experimental settings mirroring the two types of immunotherapies that have shown the best clinical benefits in cancer patients. First, we will focus on treatments providing exogenous effector cells through ACT of autologous antitumor CTLs, after their expansion and activation *in vitro* (eventually combined with genetic engineering for CAR T cells), i.e., CTL ACT. Second, we will discuss mAb





**FIGURE 3** | Icy, Cold, and Warm tumors escape from cDC1 immunosurveillance. Icy tumors (**Left**) failed to induce adaptive immune responses. For example, tumors with WNT/β-Catenin signaling or COX elevated activity disrupt the chemokine axes required for local cDC1 recruitment. Impairment of CTL infiltration would occur downstream of the failure of cDC1 to infiltrate the tumor. Cold tumors (**top**) are poorly immunogenic and infiltrated but induce some level of adaptive immunity. Tumors product factors inhibiting cDC1 differentiation or promoting their tolerogenic over immunogenic maturation. This can potentially lead to CTL inhibition and induction of peripheral tolerance. Warm tumors (**Right**) express tumor neoAg and are infiltrated by cDC1 and CTLs but are ultimately not controlled. Cancer immunoediting leads to immune escape. cDC1 have undergone immunogenic maturation but contribute to CTL chronic activation and exhaustion. ACT or mAb immunotherapies could contribute to immune control in Cold and Warm tumors, and cDC1 could play a major role in these settings (**bottom**). ACT, Adoptive cell transfer; β-Cat, β-Catenin; COX1/2, Cyclo-oxygenase 1/2; iTreg, induced regulatory T cell; mAb, monoclonal antibodies; PGE<sub>2</sub>, Prostaglandin E2.

immunomodulation (mAIM) to block checkpoint receptors on CTLs or NK cells (101–106), i.e., ICB, which has proven more efficient than conventional chemotherapies or radiotherapies in several cancer types, with better overall responses, and, most strikingly, significantly increased long-term survival (107). ACT or ICB monotherapy promotes durable disease control only in 30% of the patients. While they dramatically improve the response rate in patients with metastatic melanoma, ICB bi-therapies cause significant adverse effects and toxicities, with high incidences of autoimmune manifestations (108, 109).

Understanding the mechanisms controlling responsiveness to ACT or ICB is thus a prerequisite before complementing these immunotherapies by adjuvant treatments able to further improve the rate and duration of remission for cancer patients. One hypothesis to explain patient non-response to immunotherapies is an impairment of the accessory cells needed to promote CTL reactivation and to sustain their effector functions, rather than cell-intrinsic defects in the CTLs themselves. In this scenario, cDC1 are likely candidates, based on their critical role in promoting the spontaneous rejection of tumors in preclinical

mouse models, and on their unique functional features endowing them with a high efficiency for nurturing cytotoxic cells all along their life cycle.

## Role of cDC1 in Promoting CTL ACT Efficacy

The ACT procedure the most commonly used so far consists in isolating endogenous CTLs from a cancer patient, expanding them *in vitro* through tumor Ag-specific re-stimulation under conditions allowing reversal of exhaustion, and then re-infusing them into the host. By using autologous cells for the treatment, this strategy alleviates any side effects that could arise in allogeneic settings. However, one major issue is that only few cancer types respond to this treatment. This might be due to immune escape mechanisms in the tumor limiting locally CTL access to the activating signals necessary to prevent their exhaustion and promote their proliferation, sustained activation and survival. Preclinical mouse models have been used to address this issue, aiming at determining whether professional Ag cross-presentation in the context of positive co-stimulation and delivery of specific cytokines is necessary for ACT efficacy. Since cDC1 excel at this combination of functions (**Figure 1**), they could promote ACT efficacy. Indeed, injection of diphtheria toxin in ACT recipient *Zbtb46-DTR* mice significantly decreased their response to immunotherapy. cDC1 but not cDC2 from tumor-engrafted control mice were shown to cross-present tumor Ag and produce IL-12 *ex vivo*. Thus, it was concluded that cDC1 are necessary for ACT efficacy in these experimental settings (65). However, opposite results were recently reported under similar experimental conditions, showing a lack of cDC requirement for ACT success (110). Differences between the experimental set-up of these two studies might explain their different conclusions, since only the second study used bone marrow chimera mice rather than directly *Zbtb46-DTR* animals, which is necessary to rule out any impact of loss of *Zbtb46* expression in other cells than cDCs (33). Therefore, additional studies are necessary to determine whether cDCs are required for maximal ACT efficacy, and how. If those studies unravel specific pathways that can be potentiated, this could allow designing of a “DC adjuvant” therapy for ACT, which might broaden its success rate to more patients and for additional cancer types.

## Role of cDC1 in Promoting Responses to mAIM

In the course of a normal immune response, Ag-presenting cells regulate their expression of ligands for T cell activating vs. inhibitory co-receptors. This contributes to fine tune the intensity and kinetics of the adaptive immune response, in order to balance efficient immune control of pathogens with the risk of developing an immunopathology due to an excessive T cell activation. Tumors can hijack this process by expressing ligands for T cell inhibitory receptors leading to premature termination/exhaustion of CTL responses (48, 107). This tumoral immune evasion strategy can be overcome by mAIM through

infusion of mAbs capable of either inhibiting the engagement of T cell inhibitory co-receptors (i.e., ICB mAbs) or mimicking the engagement of T cell activating co-receptors (co-stimulation activating mAbs) [listed in (106)]. These mAbs can be used as monotherapy or bi-therapy. The ICB mAbs the most commonly used in clinics are directed against programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4). Although their use has dramatically improved patient survival for different types of cancer, their precise mode of action is still a matter of debate. The mechanisms underlying lack of response in the majority of patients remain elusive. Preclinical mouse models have been used to address this issue and showed that treatment efficacy is abrogated in cDC1-deficient *Batf3*<sup>-/-</sup> animals (**Table 3**) (56, 57, 65, 69, 117, 118). This is the case for anti-CTLA4 (117) or anti-PD-L1 (79) monotherapies, for a bi-therapy combining anti-PD-1 and CTLA4 mAbs (69), and for a bi-therapy combining the anti-PD-1 mAb with the co-stimulation activating anti-CD137 mAb (118). However, these studies did not determine whether the lack of mAIM-dependent tumor control in *Batf3*<sup>-/-</sup> mice was due to a lack of antitumor CTL priming at the time of tumor engraftment, before immunotherapy, or to a failure of mAIM at inducing the reactivation of previously primed but exhausted antitumor CTLs, at the time when the immunotherapy was administered. Efficient activation of anti-tumor CTLs, for proliferation and acquisition of effector functions, requires cross-presentation of tumor-associated Ag, activating co-stimulation and delivery of specific cytokines from accessory cells. As described previously, cDC1 excel at simultaneously delivering all these signals (**Figure 1**). In particular, one of the critical functions of cDC1 during mAIM immunotherapies may be to deliver IL-12 (69). In addition, cDC1 may also be a major source of CXCL9/10 (43) for recruiting activated or memory CTLs into the tumors (57, 93) (**Figures 1, 2**).

## Current Limitations, Controversies or Unknowns

Many of the conclusions drawn above are based on the use of *Batf3*<sup>-/-</sup> mice, or on the assumption that cDC1 are the main source of the cytokines or chemokines promoting response to mAIM therapies, without formal demonstration of this point by functional inactivation of candidate functions selectively in cDC1. Moreover, the respective importance of Ag cross-presentation vs. delivery of specific activating co-stimulation or cytokine signals by cDC1 has not been delineated yet under immunotherapies condition. Thus, further studies are required to confirm and extend these analyses, by using other mutant mouse models allowing specific cDC1 depletion or selective manipulation of each of their candidate functions.

## HARNESSING cDC1 FUNCTIONS TO IMPROVE IMMUNOTHERAPIES AGAINST CANCER

In parallel of developing immunotherapies to directly boost lymphocyte effector responses against tumor cells, the

**TABLE 3 |** Studies in mouse preclinical cancer models investigating the impact of cDC1 depletion on the protective effects of various immunotherapies not designed to directly target these cells.

Model of cDC1 depletion	Tumor models	Therapy used	Outcome of cDC1 depletion	Remarks	References
CD11c-hDTR <i>Batf3</i> <sup>-/-</sup>	B16gp100 (Melanoma)	Drug: Ad. <i>flisagr170</i> MOA: Immune chaperone inducing NF- $\kappa$ B activity	Beneficial effect lost upon DT injection and in <i>Batf3</i> <sup>-/-</sup> mice	DT injection resulted in only a partial loss of protection	(111)
<i>Batf3</i> <sup>-/-</sup> OD11c-hDTR	B16-EGFR-S1Y (Melanoma)	Drug: anti-EGFR-IFN $\beta$ MOA: IFN-I delivery to mAb resistant tumor enhance immune response	Beneficial effect lost upon DT injection but not in <i>Batf3</i> <sup>-/-</sup> mice		(112)
<i>Batf3</i> <sup>-/-</sup>	Braf <sup>V600E</sup> (Melanoma)	Drug: Aspirin/anti-PD-1 MOA: COX inhibition allows a more efficient mAIM therapy	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice	CXCL9/10 source to be further investigated	(56)
<i>Batf3</i> <sup>-/-</sup>	B16 (Melanoma) 4T1.2-Neu (Breast Cancer)	Drug: DC-targeted XBP1 plasmid/Tumor Ag MOA: XBP1 enhances DC cross-presenting capacity	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice		(113)
<i>Batf3</i> <sup>-/-</sup>	B16F10 (Melanoma)	Drug: IFN- $\alpha$ /TA99+FcIL-2 MOA: IFN- $\alpha$ given after Ag uptake by cDC1 promotes their immunogenic maturation	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice		(114)
<i>Batf3</i> <sup>-/-</sup>	B16 (Melanoma)	Drug: BRAFI/anti-PD-L1/poly(I:C)/Flt3-L MOA: Sequentially administered combination therapy targeting DC, CTL and the tumor itself	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice	<i>Batf3</i> <sup>-/-</sup>	(79)
<i>Batf3</i> <sup>-/-</sup>	BP-S1Y (Melanoma) BPC-S1Y (Melanoma) MC-57.S1Y (Fibrosarcoma)	Drug: Flt3-L-BM-DC/anti-CTLA-4/anti-PD-L1 MOA: Intratumoral injection of BM-DCs induce CTL recruitment	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice	CXCL9/10 source to be further investigated	(57)
<i>Batf3</i> <sup>-/-</sup>	B16-mCD20+ (Melanoma)	Drug: anti-Olec9A-IFN $\alpha$ 2/TNF/Doxorubicin/anti-PD-L1/anti-CTLA-4/anti-OX40 MOA: IFN-I targeting to cDC1 through Olec9A mAb	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice		(115)
Olec9a-hDTR <i>Batf3</i> <sup>-/-</sup> CD11c-hDTR	4T1 (Breast Cancer) B16F10 (Melanoma) B16-OVA (Melanoma)	Drug: poly(I:C) /MSU+M.smeg MOA: MSU+M.smeg and poly(I:C) stimulate cDC1 immunogenic maturation through TLR3. poly(I:C) promotes MoDC recruitment	Poly(I:C) but no MSU+M.smeg beneficial effect preserved upon DT injection in <i>Batf3</i> <sup>-/-</sup> mice		(116)
<i>Batf3</i> <sup>-/-</sup>	d42m1-T3 (Fibrosarcoma)	Drug: anti-CTLA-4 MOA: Injection of anti-CTLA-4 allowing tumor rejection	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice		(117)
<i>Batf3</i> <sup>-/-</sup>	B16F10 (Melanoma)	Drug: anti-PD-1/anti-CD137 MOA: Immunostimulatory mAbs used in combination enhance antitumor immunity	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> mice	IL-12 source to be further investigated	(118)
<i>Batf3</i> <sup>-/-</sup>	MC-38 (Colon Carcinoma) AT-3ovadim CD73+ (Breast Carcinoma)	Drug: anti-PD-1/anti-CTLA-4 MOA: Reactivation of endogenous CTLs and depletion of Treg	Beneficial effect lost in <i>Batf3</i> <sup>-/-</sup> , <i>I12p35</i> <sup>-/-</sup> and <i>I12p40</i> <sup>-/-</sup> mice		(69)
Zbtb46-hDTR	EG-7 (Thymoma)	Drug: <i>in vitro</i> pre-activated Ag specific CTL MOA: Ag-specific CTL infiltrate the tumor and delay its growth	Beneficial effect lost upon DT injection	These two studies drew diverging conclusion on cDC1 implication. Further studies are required.	(65)
Zbtb46-hDTR	B16-OVA (Melanoma)	Drug: <i>in vivo</i> pre-activated Ag specific CTL MOA: Ag-specific CTL delay the tumor growth	Beneficial effect preserved upon DT injection		(110)

MOA : Mode of action.

community has put much effort in trying to elaborate vaccines to ignite or reactivate endogenous antitumor immune responses in patients. Among all Ag presenting cells identified so far, cDC1 are the only ones to express selectively unique cell surface markers, such as CLEC9A or XCR1, which enables their specific targeting with mAb *in vivo*. Intratumor injection of bone marrow-derived DCs highly enriched in cDC1 increased local CTL infiltration and improved response to ICB (57). Therefore, in combination with other immunotherapies, cDC1 represent a very good candidate immune cell type to mobilize with off-the-shelf compounds for boosting patient antitumor immunity.

## Specific Targeting of cDC1 for Vaccination Purposes

Many preclinical studies in various mouse models have demonstrated the efficacy of *in vivo* targeting of Ag specifically to cDC1 in combination with the administration of a proper adjuvant for priming or reactivating adaptive immunity, leading to a rapid yet long term immune protection against infections by intracellular pathogens or against tumors (119) (Table S1). Adjuvants used to induce a beneficial inflammation promoting an immunogenic environment to prevent or counterbalance tumor immunosuppressive functions include the Toll-Like-Receptor ligands LPS, Imiquimod, CpG or Poly(I:C). Other adjuvants include drugs which directly stimulate accessory lymphocytes, such as  $\alpha$ GalCer for NKT cell activation (120, 121), or agonistic anti-CD40 antibodies which mimic the helper signal delivered by CD4<sup>+</sup> T cells to DCs for promoting their production of the lymphocyte activating cytokines IL-12 and IL-15/IL-15R $\alpha$  (68, 122, 123). Vaccine formulation including naked DNA (124, 125), porous polymer matrices (126), or oil in water nano-emulsion (127) are intrinsically immunogenic. Vaccination based on macroporous polymer matrices encapsulating tumor lysates, GM-CSF and CpG, were quite effective in attenuating tumor growth (126), although not targeting specifically cDC1. DEC-205 has been by far the cell surface marker the most used to target cDC1 *in vivo* (Table S1). However, it is not specific of cDC1 since it is expressed on Langerhans cells in the epidermis, on all migratory DC in lymph nodes (128) and it is highly upregulated on various DC subsets in tumors (30). The same issue applies to CD40. This raises the question of the respective roles of cDC1 vs. other types of DC in the protection conferred by vaccines based on *in vivo* Ag delivery through DEC-205 or CD40. Indeed, tumor Ag delivery to pDCs or cDC2 by using anti-BST2 (129) or anti-DCIR2 mAb (130) respectively, or administration of tumor Ag-pulsed pDC (131), are highly efficient in conferring protection against cancer. This shows that not only cDC1 but also cDC2 or pDCs can induce protective antitumor immunity, providing that Ag is delivered to these cells through adequate endocytic receptors in the presence of proper maturation signals. Interestingly, immunization with tumor-associated exogenous cDC1 or cDC2 prior to tumor engraftment revealed complementary functions of these two DC types (30). In a model of challenge with Lewis Lung carcinoma, only cDC2 vaccination led to reduced tumor growth rate and weight, correlating with reduced tumor

infiltration by myeloid-derived suppressor cells, functional polarization of tumor-associated macrophages toward a M1-like antitumor phenotype, and promotion of Th17 rather than Treg CD4<sup>+</sup> T cell responses (30). cDC1 were confirmed to be more efficient than cDC2 for the induction of antitumor CTL responses, which protected against a challenge with B16 melanoma (30).

In summary, even though other DC types can be successfully harnessed for cancer vaccines in mouse preclinical models, many studies showed that *in vivo* targeting of cDC1 is highly efficient for the activation of antitumor CTL responses able to induce complete tumor rejection in prophylactic settings and to delay significantly tumor progression or metastasis in therapeutic settings (Table S1). The efficacy of DC-targeted vaccines depends on three critical parameters: (i) the mode of delivery of the Ag, (ii) the nature of the Ag, and (iii) the nature of the adjuvant. Targeting Ag to cell surface receptors trafficking into late endosomes or lysosomes promotes more efficient cross-presentation by human cDC1 as compared to cDC2, whereas both cell types can mediate this function upon Ag delivery to early endosomes (132). The route of administration of the vaccine should be carefully determined depending on the necessity to induce mucosal and/or systemic immunity according to the type of cancer involved (86, 133). Once activated, tumor-associated DCs have the capacity to migrate to tumor-draining lymph nodes to prime T cells (80) and may rather favor a local antitumor immunity. There is also evidence that CTL priming can occur directly in the tumor (82). Hence, intra- or peritumoral administration of cDC1-targeted Ag for solid tumors may be the best way to enhance priming of CTLs both inside the tumor, and through migration of tumor-associated DCs to the draining lymph node. The tumor Ag should be well selected as the immune system can be almost irreversibly tolerized against certain self Ag (134). Some adjuvants are more efficient in promoting a beneficial inflammatory microenvironment in the tumor, linked to their ability to induce IFN-I. It might be desirable to include adjuvants that directly engage cDC1 since exposure to inflammatory mediators in the absence of direct signaling by pattern recognition receptors might not be sufficient to promote immunogenic DC maturation (135).

## Mobilizing cDC1 Functions in Combination Immunotherapies

In most of the preclinical models discussed above, cDC1-targeting therapeutic vaccines delay tumor progression or metastasis, or even promote a better tumor control over a long time, but fail by themselves in inducing complete tumor rejection. However, combining strategies mobilizing cDC1 with current immunotherapies, in particular with ICB, should promote the induction of long lasting protective antitumor immunity in more patients, and should more generally improve the objective response rate, the response duration and the overall survival of patients. In preclinical mouse models of immunotherapies, the antitumor effects of various off-the-shelf treatments were shown to require cDC1 functions (Table S1), and a variety of strategies were specifically designed to harness

cDC1 against cancer (**Table 4**). Hereafter, we discuss how these studies advanced our understanding of when, where and how to mobilize cDC1 functions in combination immunotherapies.

Upon immunotherapy, cDC1 are increased in the tumor very early after the beginning of the treatment, and have left the tumor in favor of cDC2 during the phase of immunotherapy-induced rejection of the tumor (86). Therefore, the location and timing of cDC1 booster administration in combination with immunotherapies are likely to be determinant for treatment success.

One way to attempt improving the response of cancer patients to immunotherapy is to boost the ability of their cDC1 to cross-present tumor Ag (60). Combined administration in mice of mAbs directed against tumor Ag with a stabilized form of IL-2 enhances antitumor immunity in a cDC1-dependent manner (**Table 4**) (114, 139). This is because antibody-dependent cell-mediated cytotoxicity provokes an immunogenic tumor cell death favoring the up-take and cross-presentation by cDC1 of tumor cell fragments. Indeed, tumor cell lysates or tumor plasma membrane vesicles may represent the best sources of Ag for cross-presentation, because they include a constellation of neoAg. Tumor Ag cross-presentation by cDC1 can also be triggered upon administration of tumor Ag coupled to mAb directed against cDC1 surface markers (**Table S1**).

Cross-presentation of tumor Ag by cDC1 must occur simultaneously to their immunogenic maturation such that they can deliver all of the signals required for the efficient priming of naïve CTLs or the reactivation of exhausted CTLs, including proper co-stimulation, activating cytokines, chemokines and CD4<sup>+</sup> T cell help, in the tumor bed or upon migration to the draining lymph node. This implies administrating the good adjuvant at the right time and in the proper place. TLR3, CpG, or STING agonist adjuvants promoting a strong production of IFN-I are especially efficient at promoting antitumor immunity, even more upon peritumoral rather than systemic delivery (79, 140–142). To further promote the beneficial anti-tumor activity of IFN-I and limit their deleterious side effects, a synthetic mutated IFN $\alpha$ 2 has been engineered and coupled to anti-Clec9a mAb, allowing delivery of IFN-I activity specifically on cDC1. The administration of this cDC1-targeted adjuvant synergizes with mAIM, chemotherapy, or with low dose of TNE, resulting in a regression or a long-lasting protection against melanoma and breast carcinoma in the absence of toxic effects (115). Targeting IFN-I on tumor cells also improves the antitumor effects of mAIM (112, 114, 143), in part through direct effects on cDC1 and/or cDC2 (143) but also more generally by modulating the responses of many other immune cells in the tumor microenvironment. Importantly, to promote protective antitumor immunity, IFN-I must be delivered simultaneously to, or shortly after, the tumor Ag. Indeed, IFN-I-induced cDC1 maturation strongly decreases their phagocytic capacity and thus prevents their ability to cross-present if occurring before tumor Ag uptake (114).

IL-12 production by cDC1 is proposed to significantly contribute to their protective antitumor activity, at least in part by promoting Th1 response and activating IFN- $\gamma$  production by NK cells and CTLs. Administration of recombinant IL-12

in combination or not with mAIM therapy displayed anti-metastatic (66) or immunotherapy-induced antitumor effect (118) in WT animals (**Table 4**). However, interestingly, these potentiating effect of the mAIM therapy was lost in *Batf3*<sup>-/-</sup> mice (118), showing that IL-12 administration is not sufficient to replace the antitumor functions of cDC1.

Another function of cDC1 that could be exploited for boosting current immunotherapies is their ability to respond to the chemoattractant XCL1, due to their specific expression of the chemokine receptor XCR1. At steady state, high levels of the *Xcl1* transcript are detected in NK cells, NKT cells and memory CTLs. Upon activation, *Xcl1* expression is further upregulated in these cells and induced in effector CTLs, which promotes the recruitment of cDC1 into inflamed tissues in close contact to XCL1-producing cells, leading to a cross-talk amplifying the responses of both cell types (77). Therefore, intra-tumoral delivery of XCL1 seemed a promising strategy to enhance local recruitment of cDC1 in order to harness their protective functions in combination immunotherapies. Certain types of melanoma or colon carcinoma tumors engineered to express high amount of XCL1 harbored a higher cDC1 infiltration and were rejected faster or grew more slowly in WT but not in *Batf3*<sup>-/-</sup> mice, as compared to control tumors. However, this process was inhibited in tumors producing PGE2, due in part to the ability of this molecule to decrease XCR1 expression in cDC1 (55). This study illustrates well the necessity not only to mobilize cDC1 in combination immunotherapies, but at the same time to dampen the immunosuppressive pathways targeting cDC1 functions in the tumor microenvironment. Hence, in addition to directly targeting CTL and cDC1 functions, combined immunotherapies should probably include means to counteract the tumor immunosuppressive pathways acting indirectly on these cells, such as inhibiting  $\beta$ -catenin, PGE<sub>2</sub> or adenosine receptor signaling (55, 57, 93, 144), or depleting/reprogramming the tumor-associated mononuclear phagocytes endowed with immunosuppressive functions including macrophages, MDSCs and pDCs (67, 145, 146).

Because cDC1 are the rarest subset of Ag presenting cells in tumors (30) and their numbers have been shown to decrease in the course of certain immunotherapies (86), strategies aiming at harnessing their functions for cancer treatment should include methods to promote their expansion *in vivo*. Tumor-infiltrating NK and T cells upregulate FLT3-L, which seems to contribute to the local expansion of tumor cDC1 (62), and most likely cDC2. Administration of recombinant FLT3-L to tumor-bearing mice as a supportive treatment to mAIM immunotherapy reinforces CTL infiltration and activation in the tumor (137), and the combined administration of FLT3-L and poly(I:C) which respectively support cDC1 expansion and activation significantly improved antitumor mAIM immunotherapy in mice (79, 118) (**Table 4**). Alternatively, large quantities of cDC1 could be injected peritumorally simultaneously to ICB administration, in order to further promote the priming of naïve CTLs toward neoAg or the reactivation of endogenous antitumor CTL responses. This should be achievable since recent studies showed that large numbers of fully functional cDC1 can be

**TABLE 4 |** Anti-tumor off-the shelf therapies relying on cDC1 functions.

Mode of action	Therapy	Putative "cDC1 booster" and protocol of administration	Type of tumors	Depend on	Does not depend on	Effects on cells	Impact of cDC1 depletion	References
Promoting maturation	Cytokine therapy (Fc-IL-2)	IFN-1 delivery, 48h after induction of ADDC against tumor upon administration of an anti-tumor mAb	B16-F10 DD- Her2/neu breast cancer RM9 prostate cancer	CD8 $\alpha$ + cells CSF1R+ cells IFN- $\gamma$	IFN- $\gamma$ -production by CTLs Batf3-independent	$\uparrow$ CTL activation $\uparrow$ cDC1 maturation and tumor uptake Early influx of neutrophils $\uparrow$ production of chemokines	Delayed control in <i>Batf3</i> <sup>-/-</sup> mice	(114)
Blocking checkpoint inhibitors on cDC1 (and putatively on other cells)	Chemotherapy	anti-TIM3 antagonist mAb before chemotherapy	PPMTV-mCherry	IFN-1 IL-12 IFN- $\gamma$ CXCR3 CD8 $\alpha$ + cells	n.d.*	Potentiated CTL activation No effect on IL-12 production by cDC1 $\uparrow$ chemokine secretion by cDC	$\uparrow$ growth in <i>Batf3</i> <sup>-/-</sup> and <i>Ilgax-Cre; Irfg</i> <sup>fl/fl</sup> mice or in Zbtb46-hDTR BM chimera	(64)
Providing cytokine support for T cell reactivation and polarization	Chemotherapy	anti-galectin9 mAb before chemotherapy (Gal9 = Tim3 ligand)	PPMTV-mCherry	CXCR3 CD8 $\beta$ + T cells	n.d.	n.d.	n.d.	(64)
Expansion of cDC	mAIM (Anti-CD137)	Recombinant IL-12 after mAIM (mimicking boosting of IL-12 production by cDC1)	MC38 s.c	n.d.	n.d.	$\uparrow$ tumor control	no effect of IL-12 in <i>Batf3</i> <sup>-/-</sup> mice	(118)
	mAIM (anti-PD-L1)  mAIM (anti-PD-1 or anti-CD137) Radiation	FLT3-L (for 9 consecutive days) + 2 inj. Poly(I:C)  Hydrodynamically injected iv on the day of engraftment 1 after inject. Poly(I:C) i.t. 7d Poly(I:C) 1 d before radiation	B16 BRAF <sup>v600E</sup> ; PTEN melanoma B16-OVA s.c. LLC-OVA s.c. (BALB/c)	n.d.  n.d.	n.d.  n.d.	$\uparrow$ CTL activation and infiltration in tumor  n.d.	n.d.  $\uparrow$ growth in <i>Batf3</i> <sup>-/-</sup> mice	(79)  (118)
	mAIM (anti-PD-L1 + anti-CTLA-4)	FLT3-L 1/week for 4 weeks	RCC Xenograft: Renca cells s.c. (BALB/c)	n.d.	n.d.	$\uparrow$ CTL activation in LN, spleen and tumor $\uparrow$ CXCL10 and IFN- $\beta$	$\uparrow$ growth in <i>Batf3</i> <sup>-/-</sup> mice	(136)
						$\uparrow$ CD103 <sup>+</sup> cell and CTL tumor infiltration $\uparrow$ CTL activation	n.d.	(137)

All tumor models were engrafted in C57BL/6J unless otherwise specified. LCC, Lewis Lung Carcinoma; RCC, Renal Cell Carcinoma; Fc, fragment, crystallizable region of murine immunoglobulin G2a (IgG2a);  $\uparrow$ increased; n.d., not determined.  
\*Of note: anti-TIM3 alone exerts its effect independently of CD11c+ cells (138).

generated *in vitro* from hematopoietic progenitors cultured with FLT3-L on feeder cells expressing the Notch ligand Delta-like 1 (147, 148).

In summary, several studies have attempted to improve the response to cancer chemotherapies, radiotherapies or mAIM immunotherapies by combining these treatments with putative or known cDC1 boosters (Table S1 and Table 4). In all cases, tumor progression was greatly dampened in parallel with enhanced CTL activation and sometimes with a documented increased maturation of cDC1. In many studies, this beneficial effect was shown to be abrogated in *Batf3*<sup>-/-</sup> mice. These studies in mouse preclinical models of combined immunotherapies strongly enforce the hypothesis that harnessing cDC1 functions in cancer patients should improve their response rate and long-term survival to already existing immunotherapies including ICB, and show how this could be achieved.

## WHAT FUNCTIONAL SPECIFICITIES MAKE HUMAN cDC1 GOOD CANDIDATE AG-PRESENTING CELLS FOR THE PROMOTION OF PROTECTIVE ANTI-TUMOR IMMUNITY?

### Comparative Genomics Established Overall Homology Between Mouse and Human cDC1

A striking overall homology between human and mouse cDC1 was established through cross-species comparative genomics of several immune cell types (14, 149–153). This provided a very strong incentive to investigate the role of human cDC1 in antitumor immunity, considering the body of evidence discussed above supporting a critical role of mouse cDC1 in promoting NK- and CTL-mediated tumor control in preclinical cancer models.

### Conservation of Key Characteristics Proposed to Underlie Mouse cDC1 Protective Role Against Cancer

A number of shared and distinctive features of mouse and human cDC1 are summarized in Table 5 (11, 14, 15, 61, 63, 79, 147–150, 154–162, 165–174), with their possible relevance for immune defense against cancer. Globally, the combination of features proposed to endow mouse cDC1 with their unique efficacy to promote protective anti-tumor immunity is well conserved in human cDC1. Differences in cross-presentation efficacy appear to be more subtle between human than mouse DC subsets (155, 175). Of note, however, a consensus has emerged from various studies that human cDC1 are more efficient than other DC types for the cross-presentation of cell-associated Ag (15, 45, 63, 155–157), likewise to the situation in the mouse. Human cDC1 were reported by several teams not to produce IL-12 (150, 167). However, other studies have shown that under optimal conditions of stimulation human cDC1 can produce this cytokine to levels equivalent or higher than those made by cDC2 or MoDCs (147, 156, 168, 169, 173, 174).

## Current Limitations, Controversies or Unknowns

One study has recently reported that human cDC1 do not migrate efficiently from the parenchyma of non-lymphoid tissues to their draining lymph nodes (176). This bears important implications for vaccination or immunotherapies if it is confirmed.

The mechanisms that make human cDC1 especially efficient for cross-presentation of cell-associated Ag are still not understood. One of the main limitations to address this issue, and more generally to study the functions of human cDC1 and their molecular regulation, is their rarity and fragility.

## WHAT EVIDENCES EXIST THAT HUMAN cDC1 CORRELATE WITH A BETTER OUTCOME IN CANCER PATIENTS AND WHAT CAN BE INFERRED FROM THESE STUDIES REGARDING THEIR PROTECTIVE MODE OF ACTION?

### A Higher Expression of cDC1 Gene Signatures in Tumors Correlates With a Better Clinical Outcome

#### State-of-the-Art in Assessing cDC1 Infiltration From Whole Tumor Tissue Gene Expression Profiles

Several public datasets are available with gene expression profiles of whole tumor tissue from large cohorts of patients with well documented clinical characteristics. Increasing numbers of teams are querying this gold mine to test whether higher expression in tumors of gene signatures specific for various cell types or biological pathways are associated with a better or worse clinical outcome. Such analyses could allow high throughput testing of the possible relationship between overall survival and tumor infiltration by specific cell types in a given activation state. Such analyses would then allow focusing further studies on the most promising observations, to test whether they are confirmed by using immunohistochemistry or flow cytometry to directly measure the frequency of specific combinations of immune cell types and activation states in the tumors. However, there is currently no consensus on which gene signatures are the most specific and robust for each immune cell type of interest. In particular, until very recently, to assess the prognostic value of DC infiltration into the tumors, the gene signatures used were those from *in vitro* derived MoDCs. The extent of DC infiltration into tumors as computationally inferred in these studies had no significant prognostic value for overall patient survival, or was even associated to an increased hazard risk (177–181). However, based on the known major differences between MoDCs and cDCs (14–16) and on the beneficial role of mouse cDC1 in antitumor immunity, further studies were needed to assess whether higher infiltration of human tumor by other DC types, in particular cDC1, could be associated with a better clinical outcome.

In the last four years, from the few studies performed to address this issue, a consensus has been emerging that

**TABLE 5 |** Shared and distinctive features of mouse and human cDC1.

Feature	Present in mouse cDC1	Present in human cDC1	Relevance to anti-tumor immunity	References
Dependency on IRF8 and NOTCH signaling for differentiation	YES	YES	Not applicable	(147, 148, 154)
High efficiency for cellular Ag cross-presentation	YES	YES	Cross-presentation of tumor-associated Ag	(15, 45, 63, 155–157)
Expression of CLEC9A	YES, shared with pDCs	YES	Intracellular routing of engulfed tumor Ag in endosomes specialized in cross-presentation	(158–160)
Higher efficacy for cytosolic export of engulfed proteins	YES, specific to cDC1	YES, shared with other DC types	Cross-presentation of tumor Ag	(155, 161)
Alkaline endosomes	YES, specific to cDC1	YES, shared with cDC2	Limits the degradation of endocytosed tumor Ag to favor their cross-presentation	(155, 162)
Selective high expression of <i>RAB11A</i> , <i>RAB7B</i> , <i>RAB43</i> and <i>SEPT3</i>	YES	YES	Small RAB GTPases with documented or putative role in promoting Ag cross-presentation	(14, 163–165)
<i>GCSAM</i> ( <i>GCET2</i> ), <i>CLNK</i> , <i>SNX22</i> and <i>WDFY4</i> expression	YES, Clnk expression shared with NK and mast cells	YES, CLNK expression specific to cDC1	WDFY4 involved in cross-presentation; other gene functions in cDC1 unknown	(11, 14, 52, 165)
CADM1 (IGSF4A) expression	YES	YES	CTL activation?	(166)
Specific expression of XCR1	YES	YES	Local recruitment of cDC1 by, or stabilization of their interactions with, NK cells and CTLs	(14, 45, 61, 63)
High TLR3 expression and specific production of IFN- $\beta$ and IFN- $\lambda$ s upon TLR3 triggering	YES IL-12 induced as well	YES, high IL-12 production observed in some but not all studies	<ul style="list-style-type: none"> <li>Putative source of IFN-<math>\beta</math>/<math>\lambda</math>s in tumors, promoting DC maturation and CTL activation?</li> <li>Therapeutic target to promote immunogenic inflammation in combined immunotherapies</li> </ul>	(15, 79, 147, 150, 156, 167–170)
TLR9 and TLR11 expression and production of IL-12 upon their triggering	YES, shared with other DC subsets for TLR9	NO, TLR9 not expressed in human cDC1, no TLR11 ortholog in humans	Not applicable in humans	(171, 172)
TLR8 expression and production of IL-12 upon its triggering	NO, loss of TLR8 ligand binding in mice	YES, under adequate conditions of stimulation	<ul style="list-style-type: none"> <li>Local recruitment and activation of CTL and NK cells</li> <li>Therapeutic target to promote immunogenic inflammation in combined immunotherapies</li> </ul>	(147, 173, 174)

higher expression of cDC1 transcriptomic fingerprints in various tumors correlates with a better clinical outcome (**Figure 4**, green cells, in the bold rectangle).

In the case for breast cancer (BRCA), a good prognosis of a higher tumor infiltration by cDC1 has been documented independently by 4 studies (55, 65, 66, 182), altogether interrogating three patient cohorts [TCGA, METABRIC, and the meta-cohort generated by Györfy et al. (183)]. A higher expression of the cDC1 transcriptomic signature in tumor was at least as powerful a predictor of prolonged patient survival to cancer as that of the CTL signature (55, 182). Transcriptomic fingerprints or genes associated to certain other immune cell types including cDC2, pDCs or monocytes/macrophages did not have a positive prognostic value (**Figure 4**, gray or red cells). This supports the hypothesis of a specific protective role of high infiltration of breast tumors by cDC1, rather than the alternate hypothesis that differential levels of cDC1 gene expression in tumors reflect differences in their overall leukocyte infiltration

and lead recapitulates the known different clinical outcome of “Hot” or “Warm” vs. “Cold” or “Icy” tumors. However, more studies are warranted to address this issue. For triple negative breast cancer (TNBC), the positive prognostic value of higher cDC1 infiltration in tumors was even better than for all types of BRCA or for luminal BRCA. This was observed in three studies, encompassing altogether the analyses of two patient cohorts (55, 65, 66).

Similar analyses were performed for other types of cancer. For head and neck squamous cell carcinoma (HNSC) and lung adenocarcinoma (LUAD), a higher expression of the cDC1 transcriptomic signature in tumor was also associated to a better clinical outcome by at least two independent studies (55, 62, 80). This was also the case for skin cutaneous melanoma (SKCM) (**Figure 4**) (47, 72), on two distinct patient cohorts, TCGA and the cohort described by Boguonovic et al. (184). In addition, for metastatic melanoma, the specific positive prognostic value of high cDC1 infiltration in the



cancer types <sup>1</sup>	cDC1+other MNP <sup>2</sup>				cDC1e cells <sup>3</sup>				cDC1e or CLEC9A				cDC1-attracting chemokines				Inferred cell type infiltration, or gene high expression, prognostic value <sup>2</sup>										# patients	study reference	dataset
	cDC1e cells <sup>3</sup>	cDC1e or CLEC9A	cDC1-attracting chemokines	dCD2	PDC	Mono/Mac	CD8 T cells	NK cells	CD4 T cells	FLT3LG	CCR7	CXCL9	LAMP3	GZMB	CD68	CD14	HAVCR2 (TM3)												
12 different	+	+	+																			3802	65	TCGA					
BRCA	+	+																				845							
HNSC	+	+																				303							
HNSC	+	+																				355							
LUAD	+	+																				1098							
BRCA	+	+																				528							
HNSC	+	+																				528							
LUAD	+	+																				585							
SKCM	+	+																				470							
BRCA	+	+																				3853							
BRCA, TNBC	+	+																				255							
Metastoma, metastatic				NS	NS	NS	+	+														38	62, 8	184					
Metastoma				(AM) <sup>4</sup>	(AM) <sup>4</sup>	(AM) <sup>4</sup>	(AM) <sup>4</sup>	+														454	62	TCGA					
BRCA, Luminal					+				+													1043	182	METABRIC					
BRCA, TNBC				NS	NS	NS	NS	NS														259							
BRCA																													
TNBC										+																			
Gastric cancer																													
LUAD																													
BRCA																						379							
BRCA, Luminal A												+	+																
BRCA, Luminal B												(AM) <sup>5</sup>	(AM) <sup>5</sup>									1933							
BRCA, Her2+												NS	NS									1149	64	GSE20194, GSE20271					
BRCA, basal												+	+									251							
BRCA, basal												+	+									618							

cDC1, CD8 T cell, NK cell and other gene signature content<sup>5</sup>

- \*cDC1: *KIT, CCR7, BATF3, FLT3, ZBTB46, IRF9, BTLA, MYCL1, CLEC9A*.
- \*other MNP: 17 genes encompassing *ABC3, CSAR1, LYME1, MERTK, MRC1 (CD206), SIGLEC1 (CD168), STAB1, CD8 T cells: 222 genes encompassing CCL5, CD3D, CD3E, CD3G, CD3Z, FLT3LG, GZMK, ITK, KLKB1, KLRG1, KLRK1, LCK, TRA, TRB* (see Palmer C. et al., BMC Genomics, 2006).
- \*CD8: *CLEC9A, XCR1, CLNK* and *BATF3*.
- \*DC1-specific genes: *CLEC9A*.
- \*CD1-attracting chemokines: *XCL1, XCL2* and *CCL5*.
- \*CD8 T cell: *CD8A, CD8B* and *CD3E*.
- \*NK cell: *NCR1, NCR3, KLRB1, CD160, PRF1*.
- \*Mononuclear phagocyte-specific gene: *CD68*.
- \*Mono/Mac-specific gene: *CD14*.
- \*cDC1: *KIT, CCR7, BATF3, FLT3, ZBTB46, IRF9, BTLA, MYCL1*.
- \*NK cell: *ONLY, KLRC3, KLRF1, KLRF1, NCR1*.
- \*DC maturation & migration to draining lymph node: *COR7*.
- \*DC local differentiation/ survival factor: *FLT3LG*.
- \*NK cell: *GNLY, KLRC3, KLRF1, KLRF1, NCR1*, each tested independently.

The Luminal BRCA cDC1e cell UP signature (signature of DAPI<sup>+</sup>CD45<sup>+</sup>Lin<sup>-</sup>CD11c<sup>-</sup>HLA-DR<sup>+</sup>CD14<sup>-</sup>CD1c<sup>+</sup> cells isolated from the tumor bed and shown to be enriched in cDC1) includes 88 genes, encompassing 6 genes (7%) known to be selectively expressed in cDC1 (*CLNK, CLEC9A, XCR1, GCSAML, ENPP3, KIT*) and one gene (1%) known to be expressed in NK and/or T cells (*GNLY*).

The TNBC cDC1e cell UP signature includes 24 genes, encompassing 2 genes (8%) known to be selectively expressed in cDC1 (*SNX22* and *CLEC9A*) and 10 genes (42%) known to be selectively expressed in NK/T cells (*IL2RB, SH2D1B, LAY1, SKAP1, PRF1, GZMK, ITK, LCK, APAP12, CLEC2D*).

- \*cDC1: *IRF9, BATF3, ZBTB46, CCR7, IL2A* and *IL2B*, each tested independently in BRCA, *IRF9, BATF3* and *CD103* each tested independently in TNBC, *IRF9* only in gastric cancer and LUAD.
- \*NK cell: *NCR1, NK62D, CD39, IFNG*, each tested independently.

\*CD8<sup>+</sup> T cells: *CD8A*.

\*Cytotoxic effector immune cells: *GZMB*.

\*mature immunogenic DCs: *LAMP3, CXCL9*.

\*Immune checkpoint receptor expressed on cDC1 and other cells: *HAVCR2* (encoding TIM3).

BRCA, breast cancer; HNSC, head and neck squamous cell carcinoma; LUAD, lung adenocarcinoma; SKCM, skin cutaneous melanoma; TNBC, triple negative breast cancer. <sup>1</sup>Unless alternative methods (AM) are mentioned, Kaplan-Meier plots for overall survival were computed, comparing 2 patient groups parsed based on gene expression values or transcriptional signature scores, at median value, or as upper versus lower quartile, or at 50% stringency threshold. Positive (+) prognostic values are highlighted in green, negative (-) ones in red, non significant (NS) ones in gray, and not determined ones in white.

<sup>2</sup>The cDC1+other MNP signature score was computed as z-score of the signature Log<sub>2</sub> Ratio. Log<sub>2</sub> Ratio=Log<sub>2</sub>(mean expression value across cDC1 genes/mean expression value across other MNP genes). Z-score Ratio=(Log<sub>2</sub> Ratio - mean(Log<sub>2</sub> Ratio across patients))/(standard deviation(Log<sub>2</sub> Ratio across patients)).

<sup>3</sup>The cell population signature used were computed from healthy mouse spleen immune cell type gene expression profiling datasets and mouse gene symbols converted into those of their human orthologs.

<sup>4</sup>cDC1e cells are cells sorted from the tumors as DAPI<sup>+</sup>CD45<sup>+</sup>Lin<sup>-</sup>CD11c<sup>-</sup>HLA-DR<sup>+</sup>CD14<sup>-</sup>CD1c<sup>+</sup> and shown to be enriched for cDC1 based on the comparison of the expression of hallmark cell type genes between this cell population and the other cell populations isolated from the same tumors (pDCs, cDC2 and Mono/Mac).

<sup>5</sup>Micro-bulk RNA sequencing was performed on each of these cell populations from several patients suffering from Luminal BRCA or TNBC. UP and DOWN specific transcriptional signatures were derived for each of these cell populations and used for the Kaplan-Meier plot analyses on another, independent, cohort of Breast Cancer patient, applying the cell population signatures derived from one type of cancer onto the whole tumor gene expression profiles of patients suffering from the TNBC dataset, and Luminal BRCA cDC1e cell signature applied on the Luminal BRCA dataset.

<sup>6</sup>Genes that were used in at least two independent studies are shown in bold blue font when used for the same cell type and in bold orange font when used for different cell types or purposes.

<sup>7</sup>An alternative method (AM) was used to assess the prognostic value of the infiltration of the tumor by different immune cell types. Statistical analyses were performed between responder versus non-responder patients for the percent of the different types of mononuclear phagocytes within HLA-DR<sup>+</sup> cells or for the percent of other myeloid cell types or of lymphocyte types within CD45<sup>+</sup> cells.

<sup>8</sup>An alternative method (AM) was used to assess the prognostic value of higher expression of candidate genes in tumors. Chi-square was used to compute the significance of differences between responder versus non-responder patients for the percent of the different types of mononuclear phagocytes within HLA-DR<sup>+</sup> cells or for the candidate gene.

**FIGURE 4 |** Synthesis of various studies aiming at evaluating the prognosis value of the tumor infiltration by different immune cell types based on the analysis of the whole tumor gene expression profiles.

tumor bed was confirmed by flow cytometry analyses, whereas no significant prognostic value was observed for many other cell types including cDC2, pDCs, Mono/Mac and most surprisingly CTLs (62) (**Figure 4**). Finally, for LUAD, single cell RNA sequencing and paired CyTOF analyses of tumors and their neighboring normal lung tissue showed that cDC1 were significantly reduced in tumors, contrasting to increased numbers of macrophages in an immunosuppressive state and of cDC2/MoDCs (185). This study further supports the previously proposed hypothesis that the balance between cDC1 vs. cells of the monocyte/macrophage/neutrophil lineages in the tumor leukocyte infiltrate strongly determines the degree of local immunosuppression (65, 93).

### Limitations, Controversies, or Unknowns

All of the above studies suggest that, in a variety of human cancers, intra-tumoral cDC1 abundance correlates with a better clinical outcome. However, further studies are required to confirm these results, to extend these types of analyses to other types of cancer, and to deepen our understanding of the underlying mechanisms.

A first issue that clearly stands out in **Figure 4** is the lack of a consensus definition of the transcriptomic fingerprints used for each immune cell type across studies, not only for cDC1 but even for CTLs or NK cells. Indeed, there is relatively little overlap between the gene signatures used for the same cell types across studies (blue names in **Figure 4**). Several genes used in some of the cDC1 transcriptomic fingerprints are known to have a promiscuous expression across many cell types (55). *CCR7* and *ITGAE* (CD103) are expressed on all mature DC types and on T cell subsets. *BATF3* and *ZBTB46* are shared with cDC2, and *FLT3* with cDC2, pDCs and hematopoietic progenitors. *IRF8* is highly expressed in pDCs and certain types of monocytes or macrophages. *THBD* (CD141/BDCA3) can be expressed on cDC2, pDCs, MoDCs and non-immune cell types. One study undertook the “tour de force” of profiling by microbulk RNAseq all the distinct mononuclear phagocyte types that they could identify in, and isolate from, BRCA or TNBC, in order to generate the cell-type specific transcriptomic signatures the most relevant to the cancer types studied (182). However, in this study, the TNBC gene signature of the cell population enriched in cDC1 (cDC1e) (182) encompasses only 8% of genes known to be selectively expressed in cDC1 but 42% of genes known to be expressed in NK cells. This raises the question of the interpretation of the positive prognostic value of that signature. It might not only reflect the infiltration of cDC1 but also that of NK cells, in consistency with other analyses in an independent study (55). Indeed, depending on individual samples, the cDC1e population encompassed 50–95% of other cells than cDC1. Using CD16 and CD56 for excluding NK cells from cDC1e cells might have been insufficient, since the strongly activated human NK cells expressing the highest levels of *XCL1* and *XCL2* express neither of these cell surface markers (55). More generally, it is likely that the much higher infiltration of TNBC by lymphocytes, as compared to luminal BRCA, led to major differences in the cell types other

than cDC1 that were included in the cDC1e cell population between these two types of cancers. This could confound interpretation of the results of the enrichment analysis of these signatures.

There is a need to define better transcriptomic signatures for human immune cell types, allowing to more rigorously computationally deconvolute the extent of their infiltration in tumors and its eventual correlation with the clinical outcome. One strategy to achieve this aim is to select genes which show high selective expression in the targeted immune cell type across tissues and activation conditions, as well as between human and mouse (14, 45, 153). An alternative strategy could be to perform single cell RNA sequencing from tumor samples, in order to define the transcriptomic signatures specific to various combinations of relevant immune cell types and activation states in the most unbiased way. This strategy would also alleviate the potential confounding effect of cross-contamination between populations as can occur with microbulk gene expression profiling studies (11, 182). Moreover, it will generate transcriptomic signatures specific to the combination of the cell types and of the cancer studied. Indeed, it has been reported that using gene signatures derived from another tissue does not always work adequately to computationally deconvolute the immune cell type composition of tumors, due to differential imprinting of cells in distinct local microenvironments (181, 182).

A second issue is the necessity to include signatures of various types of immune cells, to ensure that the better prognostic value observed in the patients whose tumors harbor higher levels of the genes specific to the candidate immune cell type is not merely a reflect of a higher overall leukocyte infiltration. Indeed, the goal is not just to compare globally “Hot” or “Warm” vs. “Cold” tumors. Rather, it is to pinpoint which immune cell types specifically promote tumor control, or on the contrary contribute to local immunosuppression, in order to identify how to best manipulate the tumor infiltrate, for the benefits of cancer patients, through combined immunotherapies. Thus, the cell types considered to be functionally and/or developmentally the most closely related to the candidate one should be included, for example cDC2 or MoDCs for cDC1, NK cells and  $\gamma\delta$  T cells for  $\alpha\beta$  T cells. In addition, one should also include cell types expected to have no, or opposite, impact on tumor growth, for example neutrophils, macrophages and regulatory T cells which are considered as promoting immunosuppression.

A third issue is to deepen our understanding of when, where and how cDC1 promote tumor control. In several studies, the inferred higher cDC1 infiltration in tumors was correlated with higher inferred infiltrations of CTLs or NK cells, and with higher expression of *FLT3L*, *XCL1*, *XCL2*, *CCL4*, *CCL5*, *LAMP3*, *CCR7*, *CXCL9*, *CXCL10*, and *CXCL11* (**Figure 4**) (55, 57, 62, 64, 80, 93). These observations need further independent confirmation through the analysis of other cohorts of patients, and by using complementary methodologies including immunohistochemistry, CyTOF or single cell RNA sequencing to measure the correlation between cDC1 infiltration into the tumors and the status of antitumor

**TABLE 6** | Completed clinical trials targeting cDC1.

Study start	Brief title	Condition	NCT identifier	Intervention	Phase	Results	References
2006	Peritumoral injection of CpG B with or without GM-CSF for treating patients with stage II Melanoma	Stage II melanoma, planned to undergo sentinel lymph node procedure	Not applicable	Preoperative local injection of either: <ul style="list-style-type: none"> <li>• GM-CSF + CpG B</li> <li>• CpG B</li> <li>• saline around primary tumor excision site</li> </ul>	II	Combined CpG/GM-CSF administration selectively increased cDC1 frequencies and cross-presenting capacity in SLN. cDC1 matured locally upon instruction by GM-CSF and pDCs type I IFN. CpG induced Th1 skewing and increased NK cell and antitumor CTL frequencies in SLN. Higher IL-10 production and Treg activity in SLN. Decreased metastasis in SLN from patients who received CpG.	(186, 187)
2009	A study of CDX-1401 (DEC205/NY-ESO-1) in patients with malignancies known to express NY-ESO-1	Advanced malignancies refractory to available therapies	NCT00948961	CDX-1401 + Resiquimod ± Poly(I:C:LC)	I/II	Induction of humoral and cellular immunity to NY-ESO-1. Disease stabilization in 13 of 45 patients. Tumor regression in 2 patients. Objective tumor regression in 6 of 8 patients who received ICB after CDX-1401.	(185)
2014	CDX-1401 (DEC205/NY-ESO-1) and Poly(I:C:LC) vaccine therapy with or without CDX-301 in treating patients with stage IIB–IV melanoma	Resected melanoma	NCT02129075	CDX-1401 + Poly(I:C:LC) ± rhuFLT3-L (CDX-301) pre-treatment	II	Higher tumor-specific immune responses observed in subjects who received FLT3-L	(188, 189)

SLN, Sentinel lymph nodes.

NK and CTL responses. In any case, these studies support our proposed model of a critical positive cross talk between cDC1, cytotoxic lymphocytes and CD4<sup>+</sup> T cells for promoting effective antitumor immunity (**Figure 2**). Finally, it would be of utmost interest to extend to cohorts of patients benefiting from various types of immunotherapies these analyses aiming at deconvoluting the gene expression profiles of whole tumor tissue into immune cell type composition. This should help determining whether the clinical response can be predicted from cDC1 infiltration in the lesions, and to adapt the treatments accordingly for example by combining to ICB the use of drugs promoting cDC1 recruitment and activation into the tumors of patients when this process is defective (**Figure 3**).

### Efficacy of Immunotherapeutic Protocols That May Preferentially Target/Harness Human cDC1

A few clinical trials have already been conducted using treatment protocols that have been proposed to preferentially target/harness human cDC1 (**Table 6**) (186, 187, 190). They gave encouraging results, which further supports the rationale of specifically targeting human cDC1 for the design of novel combined immunotherapies against cancer (189).

### WHAT TOOLS ARE AVAILABLE TO STUDY AND MANIPULATE HUMAN cDC1 FOR THE BENEFITS OF CANCER PATIENTS?

Building on the conservation of cDC1 molecular makeup and functions between mouse and human, similar tools have been generated in both species to specifically target these cells for immunotherapy against cancer. This should accelerate translation from mouse preclinical studies to human clinical trials. Hence, most of the tools and approaches that have been detailed in the section on mouse experimental models (**Figure 3** and **Tables 3, 4**) could be implemented in humans, as briefly summarized below.

#### Generation and Study of Novel *in vitro* Models of Human DC Types

To overcome the roadblock of the rarity of human cDC1 and of their fragility upon *ex vivo* isolation, we and others recently developed optimized *in vitro* culture systems to generate high numbers of cDC1, cDC2 and pDCs from CD34<sup>+</sup> hematopoietic progenitors (15, 147, 148). These novel *in vitro* models will allow rigorous comparison of the functions of the different human DC types, dissection of their molecular regulation, and better understanding of their cross talk. Further adaptations of these protocols are warranted to derive *in vitro* autologous cDC1

from the circulating CD34<sup>+</sup> cells of patients, load them with Ag and mature them, under conditions compatible for clinical use in vaccination or immunotherapy. It should be noted that encouraging results have been obtained with clinical trials of autologous ACT of *ex vivo* loaded and matured pDCs and cDC2 in melanoma patients, which seem superior to MoDCs to prime or boost endogenous CTL responses against the tumor. This emphasizes that, as in mice, cDC1 are not the only DC type that could be successfully harnessed for combined immunotherapy in cancer patients (6, 7, 191, 192).

## Means to Specifically Deliver Ag and Maturation Signals to Human cDC1

Considering their conserved specific expression pattern on mouse and human cDC1, and the very encouraging results obtained in mouse preclinical models, the CLEC9A and XCR1 receptors are the best candidates for Ag, or Ag+adjuvant cargo, delivery to human cDC1, using recombinant ligands (193, 194) or monoclonal antibodies. A combination of TLR3- and TLR8-specific agonists is desirable to promote an immunogenic maturation associated with the production of both IL-12 and IFN- $\beta/\lambda$  (Table 6) (195). Targeting delivery of IFN-I activity to cDC1 is another very promising adjuvant based on the proof-of-principle published in mice (Tables 3, 5). Additional means could be envisioned to favor the cross-talk between cDC1 and NK or NK T cells (196), e.g., use of NK cell immune checkpoint blockers (101–105) or targeted delivery to cDC1 of activating antigenic ligands for NK T cells (120).

## Means to Promote cDC1 Differentiation, Survival and Local Recruitment in the Tumor Bed

Systemic injection of FLT3-L could promote cDC1 differentiation and survival (79). Local delivery of XCL1 could promote their recruitment in the tumor bed. In patients responding to checkpoint blockade inhibitors, these functions might be achieved upon local NK and CTL activation for FLT3-L, XCL1, and CCL4/5 production (55).

## Blockade of cDC1 Checkpoint Inhibitors

A systematic analysis of immune factor checkpoint expression on human DC types is ongoing in order to investigate which ones could be reasonable candidates as components of combined immunotherapies targeting both CTLs and DCs (197).

## CONCLUDING REMARKS

Lately, cDC1 have been in the spotlight of many studies investigating in mice the immune mechanisms driving tumor rejection, spontaneously or upon immunotherapy. All these studies converge toward a hub role of cDC1 in providing the initial priming, or in sustaining the activation, of antitumor T and NK cell responses. These advancements in our understanding of the role of cDC1 in antitumor immunity have been made possible by the recent blossoming of genetic tools

allowing cDC1 manipulation. However, so far, most conclusions have been drawn from results obtained under experimental conditions that were not solely targeting cDC1, whether it was the use of genetically engineered mouse models or of mAb directing against cell surface markers. In fact, to be protective against immunosuppressive tumors such as those treated in the clinic, the immune response is necessarily complex and multi-parametric. More and more observations pinpoint that, in addition to cDC1, other DCs, type 1 CD4<sup>+</sup> T cells, and sometimes neutrophils are also central in promoting protective antitumor immunity, whereas Treg or type 17 CD4<sup>+</sup> T cells, monocytes and macrophages may rather play immunosuppressive roles. Further studies using models allowing conditional depletion of cDC1 will be critical in rigorously investigating whether cDC1 functions are instrumental at the time when immunotherapies are delivered. These studies will definitely settle the currently prevailing hypothesis that cDC1 functions, when specifically boosted, could provide great support to boost patient responses to currently used anticancer immunotherapies.

In human tumors, enrichment of genetic signatures described as cDC1-specific is associated with a good prognosis and a better clinical outcome in a several cancers, including luminal and TN breast cancer. These correlative analyses should be extended to additional types of cancer and to different patient treatment regimen. It is possible that the extent of cDC1 infiltration in the tumor fluctuates over time following the development or the suppression of an efficient antitumor immune response, as observed in mice during immunotherapy (86), and that cDC1 infiltration may not be protective against all types of cancer. Still, the perspective of exploiting cDC1 to improve current immunotherapies is extremely encouraging, and completion of cDC1-targeting vaccine clinical trials in human will surely help in gaining insight into their importance in cancer.

## AUTHOR CONTRIBUTIONS

All authors wrote the manuscript, contributed to manuscript revision, read and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2019.00009/full#supplementary-material>

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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