

Review

Leveraging STING, Batf3 Dendritic Cells, CXCR3 Ligands, and Other Components Related to Innate Immunity to Induce A “Hot” Tumor Microenvironment That Is Responsive to Immunotherapy

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Simple Summary: The conceptualization of a “hot” tumor microenvironment has been characterized by mainly T cell infiltration, whereas, “cold” or “immune-deserted” tumors were seen as lacking T cells. However, the presence of antigen-presenting myeloid cells is equally important. In particular, Batf3-lineage DCs are highly efficient in priming effector T cells. Additionally, they can recruit T cells with CXCR3 ligands and re-activate dysfunctional T cells with co-stimulatory molecules in the tumor microenvironment.

Abstract: In a T-cell-inflamed phenotype, tumor eradication works best and is potentiated by immunotherapy such as checkpoint blockade. However, a majority of patients die despite receiving immunotherapy. One reason is insufficient T cell priming and infiltration in the tumor. Nature provides us with innate immune mechanisms in T-cell-inflamed tumors that we can adopt for more personalized immunotherapy strategies. Tumor sensing through innate signaling pathways and efficient antigen-presenting possess a significant role in bridging innate and adaptive immunity and generating a T-cell-inflamed tumor. One approach to strengthen these innate immune mechanisms is to deliver innate immune factors such as STING or activated DCs into the tumor microenvironment, in particular in patients resistant to checkpoint blockade. The low number of DCs in the tumor bed could potentially be increased with the growth factor FMS-like tyrosine kinase 3 ligand (Flt3L). CD103+ DCs are integral for three phases of anti-tumor immunity: priming, recruiting, and re-invigoration of effector T cells. Re-activation of dysfunctional T cells is achieved via co-stimulatory molecules such as the 4-1BB ligand. The presence of myeloid-cell-derived CXCL9 and CXCL10 in the tumor microenvironment can predict response to immunotherapy. We outline recent preclinical and clinical approaches to deliver these crucial components bridging innate and adaptive immunity into the tumor microenvironment.

Keywords: chemokines; tumor microenvironment; dendritic cells



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1. Introduction

A T-cell-inflamed tumor is associated with better outcome to immunotherapy in metastatic cancer [1–3]. In order to create a T-cell-inflamed tumor microenvironment, a close interaction of the innate immune system with T cells is warranted. Of particular importance is tumor sensing through the STING (STimulator of INterferon Genes) pathway in antigen-presenting cells (APCs). Among APCs, dendritic cells are the most efficient cells in internalizing and presenting tumor antigens on MHC class I molecules to CD8⁺ T cells. In particular, Batf3-lineage DCs (basic leucine zipper transcription factor ATF-like 3 expressing dendritic cells) are required for successful cross-priming of effector T cells in

the tumor draining lymph node [4,5]. DCs develop from bone-marrow-derived precursor cells and depend on the essential hematopoietic growth factor FMS-like tyrosine kinase 3 ligand (Flt3L). Tumor associated macrophages (TAMs) exhibiting an M1 phenotype are usually more abundant in the tumor microenvironment than DCs and can also present tumor antigens, though are generally less efficacious [6]. Myeloid cells are also involved in trafficking of tumor antigen specific T cells to the tumor microenvironment by releasing CXCR3-ligands. Whereas, Batf3 DCs can be recruited to the tumor bed through the chemokine (C-C motif) ligand 5 (CCL5) and XCL1 (also known as ATAC, lymphotactin, or SCM-1) produced by NK cells [7–9]. Sophisticated delivery systems for key innate immune factors such as bispecific antibodies, nanoparticles, fusion proteins, or viral vectors are on the edge of being translated into the clinic. Unwanted immune-related adverse events could be minimized by these targeted strategies. Ultimately, leveraging innate immunological pathways can lead to a more personalized immunotherapy. Future immunotherapy agents are likely to synergize with well-established antibodies for checkpoint blockade by enhancing infiltration with crucial immune cells such as activated Batf3 DCs and tumor-antigen-specific CD8⁺ T cells.

2. How Are Endogenous T Cells Successfully Recruited into The Tumor Microenvironment?

Non-T cell inflamed tumors have a set of immunogenic tumor antigens that are comparable to that of T cell inflamed tumors [10]. Thus, antigenicity is not the main reason for CTL infiltration. However, non-T cell inflamed tumors are lacking transcriptional markers for Batf3 DCs. Innate sensing of tumor derived factors is essential for the recognition of cancer by the immune system. One well-described signaling pathway is the STING pathway. Tumor infiltrating antigen presenting cells (APCs) can sense tumor-derived cytosolic DNA via cyclic-GMP-AMP synthase (cGAS) which activates the protein STING [5]. Activated STING results in the production of type I Interferons (IFNs) and maturation of Batf3 DCs downstream of the recruitment of TBK1 and the transcription factors IRF3 and NF- κ B [11] (Figure 1).

An alternate innate tumor sensing pathway involves the high-mobility-group box 1 (HMGB1) alarmin protein. This protein was secreted by dying tumor cells after radio- and chemotherapy and interacts with Toll-like receptor 4 (TLR4) and its adaptor MyD88 expressed by DCs [12,13]. The HMGB1 and TLR4 interaction improved antigen presentation by slowing down degradation of phagocytic cargo [12]. A subsequent report argues that TLR9 is involved sensing tumor-derived DNA, DC maturation, and CTL priming [13]. TLR9^{-/-} mice treated intratumorally with FITC-labeled E7 peptide and chemotherapy had significantly less FITC⁺ DCs in the tumor draining lymph nodes [14]. The CD11c⁺ DCs expressed less co-stimulatory molecules CD40 and CD80 compared to wild type (WT) mice. Furthermore, the frequency of CD8⁺ T cells was decreased in TLR9^{-/-} mice and they produced significantly less IFN- γ [14]. Matured CCR7 bearing CD103⁺ DCs take up cancer antigens and migrate through lymphatic vessels to the tumor draining lymph node following a chemokine gradient of CCL19 and CCL21 [15–17]. In the lymph node, CD103⁺ DCs cross-present internalized cancer antigens on MHC I molecules to naïve CD8⁺ T cells [18]. Tumor antigens are carried in small vesicles inside DCs that can also be transferred among DC subsets enabling also resident CD8 α ⁺ DCs to prime CD8⁺ T cells [19]. Recent results have shown that also CD11b⁺ conventional DCs can present tumor-derived antigens on MHC class I molecules upon interferon stimulation and thus contribute to anti-tumor CD8⁺ T cell immunity [20]. Activated CD8⁺ T cells expand clonally and then migrate through the blood stream to the tumor microenvironment. Integrin α 4 β 1 (VLA-4) and integrins α 4 and α L β 2 (LFA-1) on effector T cells bind to their endothelial ligands VCAM-1 and ICAM-1 which facilitates trans-endothelial migration and diapedesis [21]. Additionally, the interaction of LFA-1 and ICAM-1 contributes to the maturation of the cytotoxic immune synapse and effector function of CTLs [22]. CTLs can bind and kill their target cancer cells via specific T cell receptors which interact with the compatible antigen–MHC-I complex on the cancer cells [23]. Thus, new cancer antigens and DNA

are released from the dead cancer cells and the “cancer–immunity cycle” begins anew. Within the “cancer–immunity cycle” there are multiple points of possible pharmacological interventions (Figure 2).

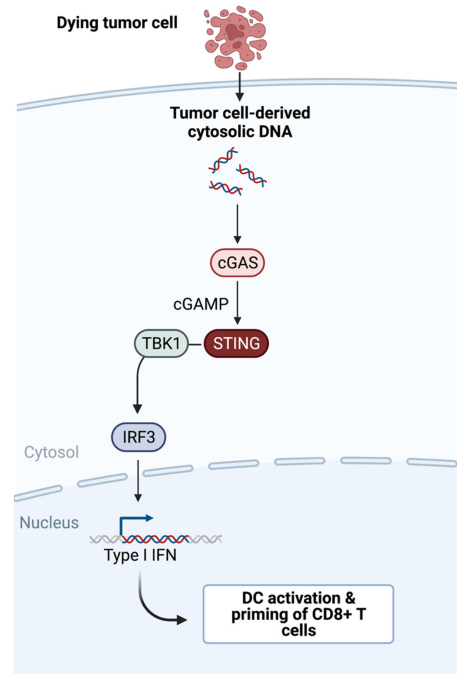


Figure 1. STING pathway: tumor-cell-derived DNA triggers the cGAS–cGAMP–STING signaling pathway and an innate immune response resulting in DC maturation and CD8+ T cell priming downstream of type I IFN production.

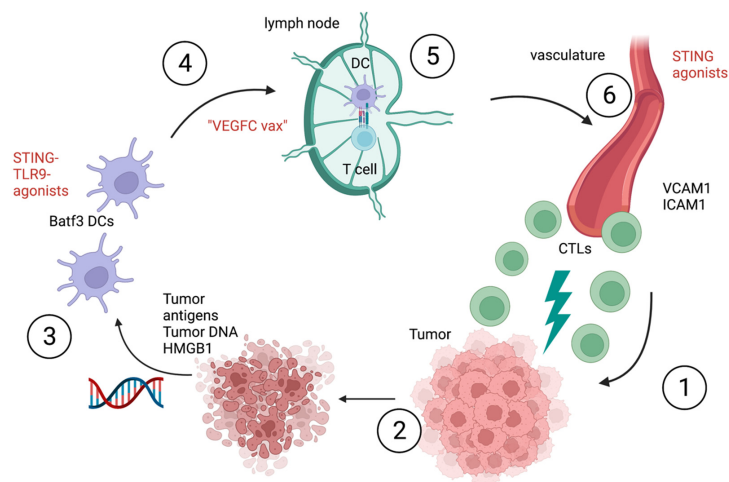


Figure 2. “Cancer–immunity cycle”. 1: CTL-mediated tumor killing; 2: tumor antigen release, release of HMGB1 and tumor-derived DNA; 3: tumor antigen uptake by Batf3 DCs, tumor-derived DNA and HMGB1 sensing via STING, TLR9 and TLR4, activation and maturation of Batf3 DCs; 4: migration of antigen-presenting Batf3 DCs through lymphatic vessels to the tumor-draining lymph node following a chemokine gradient of the CCR7-ligands CCL19 and CCL21; 5: DCs prime CD8+ T cells via a MHC-I–antigen complex, clonal expansion of tumor antigen specific T cells; 6: CTLs migrate via blood and exit the vessels into the tumor. Crawling along the endothelial wall and diapedesis are facilitated through the adhesion molecules VCAM1 and ICAM1. Possible interventions: STING–, TLR9– agonists and “VEGFC vax” enhancing cross-priming and vasculature normalization.

Clinical Applications

Clinical applications built on the foundation of this work include a phase 1b study, a combination of Pembrolizumab with vidutolimod, a TLR9-agonist, given intratumorally to 44 patients with resistance to prior anti-PD-1 treatment resulted in durable response in $\frac{1}{4}$ of the treated patients underlined by an increased IFN γ production [24]. In vivo and in vitro data have shown that STING agonists such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA) or cyclic dinucleotide (CDN)-based STING agonists support DC maturation, facilitate CD8⁺ T cell cross-priming, and increase production of type I IFNs, TNF- α , and other innate cytokines [25]. However, DMXAA is not able to bind human STING [26]. Intratumoral administration of STING was favored in the past because of metabolic instability of CDN-based STING agonists [27]. Two recent reports propose alternate administration routes for the more stable STING agonists named MSA-2 and SR-717 configured in a closed confirmation [28,29]. MSA-2 is administered orally and SR-717 systemically. Both agonists achieved cross-priming and tumor control in mice. Additionally, exosome delivery mechanisms for STING agonists are also in clinical development; this approach may help to overcome the direct cytotoxicity observed with initial clinical STING agonists [30]. Clinical trials for intravenous application of STING are ongoing (NCT04420884, NCT04096638, NCT04609579 and NCT04592484). Apart from DCs, tumor endothelial cells can also produce type I IFNs upon STING activation [31]. Endothelial STING expression correlated with CTL infiltration into the tumor in murine and human tissue and better overall survival [32]. In the mouse model, intratumorally administered STING upregulated genes for the vascular adhesion molecules Vcam and Icam, decreased hypoxia, reduced blood vessel density, and induced blood vessel maturation through increased pericyte coverage [32]. Other reports described tumor vasculature destruction through either high IFN- β -levels injected into the tumor or through STING agonists via TNF- α -production [25,33]. Overall, STING agonists in the right concentration can suppress angiogenesis/malformation and contribute to vascular normalization. Tumor antigen specific T cells need functional blood vessels within the tumor in order to migrate from the tumor draining lymph node to the tumor microenvironment. Conversely, antigen-loaded migratory DCs travel via lymph vessels from the tumor bed to the tumor draining lymph nodes. B16 melanoma bearing mice with blocked vascular endothelial growth factor receptor 3 (VEGFR3) signaling and a lack of lymphatic vessels showed significantly less trafficking of DCs to the draining lymph nodes, reduced immune cell infiltrates, and inflammatory cytokines in the tumor microenvironment [34]. Inducing lymph angiogenesis with a vaccine overexpressing VEGF-C (“VEGFC vax”) resulted in increased infiltration of CD8 α ⁺ cross-presenting DCs and CD8⁺ T cells and hence better tumor control in combination with anti-PD-1 in a B16 melanoma mouse model [35].

3. Chemokines in The Tumor Microenvironment

Another important mechanism for immune cell infiltration into the tumor microenvironment is chemoattraction through chemokines. CXCL9, CXCL10, and CXCL11 (in humans) bind to the CXCR3-receptor on T cells. CXCL9 and CXCL10 enhanced migration of T cells in vivo and in vitro and were associated with T-cell-inflamed tumors in a gene expression analysis [2]. Type I IFN induces the expression of CXCL9 and CXCL10 [36]. Pre-clinically, Batf3 DCs were characterized as a predominant source for CXCL9 and CXCL10 [4]. The presence of Batf3 DCs, CXCL9 and CXCL10 in the tumor microenvironment independently correlated with T cell infiltration [4]. In melanoma patients, baseline levels of CXCL9, CXCL10, and CXCL11 were associated with response to anti-PD-1 therapy [37]. According to single-cell RNA sequencing data from patients with melanoma, head and neck, and lung cancers who underwent checkpoint blockade therapy, these chemokines were primarily produced by macrophages [37]. DCs such as CD103⁺ DCs were the second strongest cellular source for CXCL9/10/11 among the measured immune cells. In vitro, melanoma cell lines M537 produced CXCL9 and CXCL10 which was associated CD8⁺ effector T cells [2]. In a recently published work with in situ staining of RNA transcripts

and proteins in baseline biopsies of melanoma patients, we found that the major cellular source of CXCL10 was indeed CD45+ immune cells in humans [38]. However, cancer cells were also able to produce CXCL10 to a lesser extent in some patients before commencing therapy with anti-PD-1. The baseline CXCL10 production of CD45+ immune cells and Sox10+ both correlated significantly with response to immunotherapy [38].

In vitro, cancer cells also produced CCL4, which led to the migration of CD103+ DCs [8]. The corresponding receptor on DCs is CCR5 [39]. Tumor-derived CCL4 can recruit CD103+ into the tumor microenvironment. Conversely, activated B-catenin signaling and ATF3 transcription suppressed CCL4 transcription [8]. CCL4 expression levels were increased significantly in T-cell-infiltrated tumors [2], arguing for successful cross-priming and recruitment of T cells by Batf3 DCs. In a murine model, XCL1 and CCL5 were mainly produced by NK cells and attracted conventional type 1 dendritic cells (cDC1) into the tumor microenvironment [7] (Figure 3). XCL1 binds to its chemokine receptor XCR1 which is expressed on Batf3 DCs [40,41]. In humans, the gene signature of XCL1 was associated with better survival and correlated with a NK cell and a cDC1 signature [7].

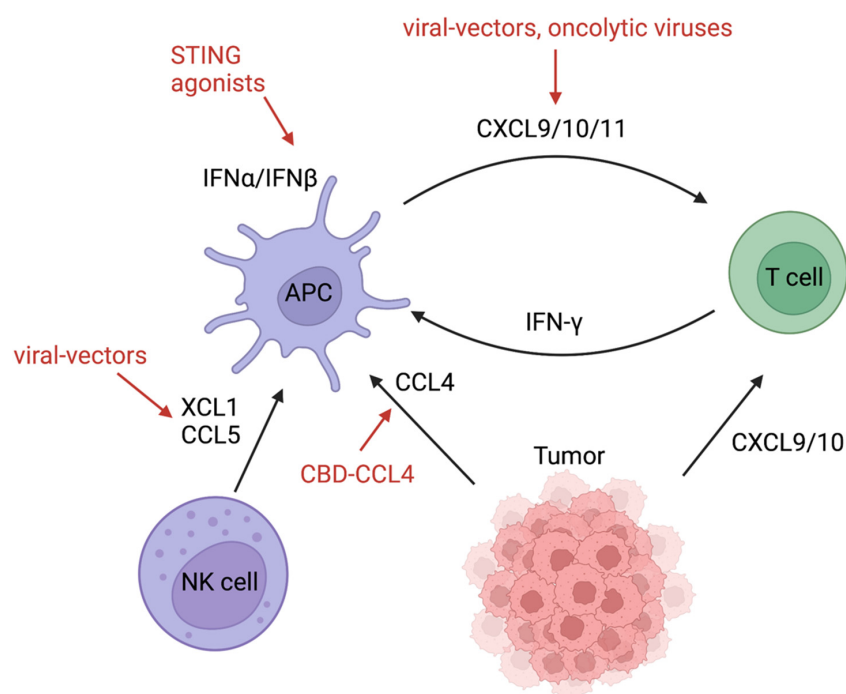


Figure 3. Chemoattraction in the tumor microenvironment. Tumor-derived CCL4 can attract Batf3 DCs and CXCL9/10 can recruit cytotoxic T cells to the tumor microenvironment. Natural Killer Cells attract Batf3 DCs via XCL1 and CCL5. CCL4 can be administered with a fusion protein consisting of CCL4 and the collagen-binding domain (CBD) of von Willebrand factor and XCL1 with a viral-vector. Batf3 DCs can produce CXCL9/10/11 and recruit CTLs. In turn, IFN- γ produced by CTLs can stimulate Batf3 DCs. Batf3 DCs are activated by STING agonists which can contribute to CXCL9/10/11 production via IFN α/β . CXCL9/10/11 can be induced by oncolytic viruses or delivered with the help of virus-based vectors.

3.1. Inducing Chemokines

Injecting a virus-based vector expressing XCL1 and soluble Flt3L intratumorally improved tumor control in MC38- and B16-bearing mice through the accumulation of cross-presenting Batf3 DCs in the tumor draining lymph node [42]. Synergistic effects with anti-PD-1, anti-CD137, or anti-CTLA-4 mAbs were described. Interestingly, Batf3 knockout mice were missing these anti-tumoral effects. Another group exploited a different strategy to deliver the DC-chemoattractant CCL4 into the tumor microenvironment. Instead of injecting a viral-vector into the tumor directly, a fusion protein consisting of

CCL4 and the collagen-binding domain (CBD) of von Willebrand factor were administered intravenously [43]. Increased infiltration with CD103+ DCs and CD8+ T cells was noted in the tumor microenvironment. By binding the collagen of the tumor stroma specifically, off-target side effects were not observed in this B16 melanoma mouse model. Combination therapy of CBD-CCL4 and checkpoint blockade improved survival significantly [43]. Interestingly, blocking only CXCR3 led to a loss of tumor control through this combination therapy, underpinning the importance of Batf3 DCs and CXCR3 ligands for the recruitment of CTLs and tumor killing. The CXCR3 ligands CXCL9 and CXCL10 also have been successfully delivered via viral-vectors in colon and melanoma mouse models [44,45]. The intra-tumoral delivery resulted in increased CTL infiltration and better tumor control. CXCL9 delivery with a viral-vector was combined with the immunostimulatory factor OX40 ligand (OX40L)/tumor necrosis factor superfamily member 4 (TNFSF4) and anti-PD-1 therapy [45]. Furthermore, oncolytic viruses can function as strong inducers of CXCL9 and 10 in the tumor microenvironment [46,47]. One group designed an oncolytic virus overexpressing CXCL11 and proved increased frequency of tumor infiltrating lymphocytes in a murine tumor model [48]. The viral enhancement of the CXCR3 ligands expression worked synergistically with checkpoint blockade in all animal models [46–48]. Clinical trials are starting to leverage these concepts to treat human cancers. A current trial makes use of an oncolytic virus which actively expresses an FAP-TAc antibody, CXCL9, CXCL10, and IFN α (NCT04053283). CXCL10 can be indirectly upregulated by endogenous DNA sensing pathways such as STING. This has been shown inversely by blocking IFNAR1 which downregulated CXCL10 expression in vitro in a colon cancer model [49]. In vitro bone-marrow-derived DCs expressed significantly more CXCL9 upon STING activation [5]. CXCL9/10 expression downstream of STING activation is mediated in a IFN α /IFN- β -dependent fashion. Thus, in vitro experiments have shown that IFN- β treatment induced CXCL10 expression of melanoma cells [50] and DCs stimulated with IFN α /IFN- β produced significant amounts of CXCL9 and CXCL10 [41]. Blockade with an anti-PD-1 antibody enhanced proliferation of T cells and thereby increased expression of IFN- γ in the tumor microenvironment which in turn increased production of CXCL10 (Interferon gamma-induced protein 10) [51].

3.2. Adverse Effects Caused by Chemokines

Systemic upregulation of CXCL9/10/11 can contribute to inflammation and autoimmunity such as Alopecia areata, Vitiligo, autoimmune arthritis, type 1 diabetes, or adult-onset Still's disease [52–56]. Similarly, patients with checkpoint inhibitor colitis showed an upregulation of CXCL9 and CXCL10 in the myeloid compartment measured with single-cell RNA sequencing [57]. In vitro migration studies underlined that low concentrations of CXCL9 and CXCL10 produced by tumor endothelial cells promoted chemotaxis. However, high concentrations of CXCL9 and 10 produced by endothelial cells induced trans-endothelial migration (chemo-repulsion) of melanoma cells [58], arguing for a dose-dependent effect of the chemokine gradients. Therefore, targeted chemokine delivery has to be performed with caution. Nonetheless, it has potential to expand efficacy of checkpoint blockade when administered in the correct time, dosage, and directly into the tumor microenvironment.

4. Interaction of Batf3 DCs and CTLs in The Tumor Microenvironment

In vitro data showed that Batf3 DCs are involved in repriming CTLs in the tumor microenvironment [59]. A second interaction of adoptively transferred already primed CTLs with Batf3 DCs in the tumor microenvironment was needed for more effective tumor control. However, Batf3 DCs within the tumor microenvironment are rare. The significance of these rare tumor-infiltrating DCs is underscored by a TCGA analysis which revealed that CD103+ DC transcripts correlated with better overall survival across cancers [59]. Interestingly, a Flt3L-expressing B16 melanoma tumor model facilitated the expansion of the sparse CD103+ DC in the tumor [59], arguing for a potential therapeutic role of Flt3L

in non-inflamed tumors. NK cells were identified as a major source for Flt3L in the tumor microenvironment in a melanoma mouse model with a Flt3l-reporter mouse [60]. When NK cells were depleted, significantly decreased amounts of CD103⁺ were found in the tumor draining lymph node and tumor. The importance of Flt3L has been successfully demonstrated in humans using intratumoral recombinant Flt3L along with radiotherapy and an innate immune agonist. In this trial, patients with indolent lymphomas non-responsive to immune checkpoint inhibition developed de novo CD8⁺ T cell tumor infiltrates, and clinical responses were observed [61]. This approach highlights the potential role of Flt3L therapy to mobilize cross-presenting DCs to generate a CD8⁺ T cell response in cold tumors. DCs are not only crucial for successful priming and activation but also production of co-stimulatory molecules. One important example for activation of CTLs is characterized by the binding of the 4-1BB ligand located on APCs to the 4-1BB (CD137) receptor on T cells [62]. Co-stimulation through this interaction leads to cytotoxicity, proliferation, and activation of CD8 T cells [63]. The co-stimulation via 4-1BB is particularly important in Batf3 DCs because they are the APCs responsible for the most efficient cross-priming of tumor antigens to CTLs [36]. This is shown by less effective cross-priming in Batf3^{-/-} mice stimulated with an anti-CD137-monoclonal antibody (mAb) alone or together with an anti-PD-1 mAb [64]. As opposed to WT mice, treatment did not result in higher frequencies of tumor-antigen-specific CTLs and subsequently anti-tumor control. Furthermore, CTLs in Batf3^{-/-} mice showed much lower levels of PD-1 and Batf3^{-/-} DCs were less proliferative and produced less IFN γ in vitro [64]. Noteworthy, recombinant IL-12 repetitively administered into tumor lesions which generally boosts the CTL effector function was not able to restore the anti-tumor effect of anti-CD137-mAb in Batf3^{-/-} mice [64,65]. However, cultured Flt3L-derived DCs injected into Batf3-low BrafV600E/Pten^{-/-}/CAT-STA tumor-bearing mice tumors achieved a response to checkpoint blockade by restoring the interaction between CD103⁺ dendritic cells and CTLs [8]. Hence, one therapeutic avenue is to deliver DCs to the tumor microenvironment or tumor-antigen-loaded DCs directly into the lymph node in particular in non-inflamed tumors. In the presence of a tumor, infiltrating DCs blocking CD47 had additional beneficial effects in a hepatocellular carcinoma model [66]. It led to the activation of CD103⁺ DCs, secretion of IL12 and CXCL9, and recruitment of NK cells.

Delivering DCs into The Tumor Microenvironment

In mice, nanoparticles loaded with tumor antigens were successfully phagocytosed by DCs in vitro [67]. Mice injected with nanoparticle-DCs induced more tumor-antigen-specific CD4⁺ or CD8⁺ T cells in the draining lymph nodes. The CTLs produced more IFN- γ and subsequently a better tumor control and survival were achieved. Pre-conditioning the DCs might be another key element for DC-vaccination in cancer. The strongest adjuvant for DC-vaccination among all TLR agonists was polyinosinic:polycytidylic acid (polyI:C) which stimulates TLR3 [68]. It caused the strongest production of proinflammatory cytokines, e.g., CXCL10 or bioactive IL12p70 and infiltration with tumor-antigen-specific CTLs. In vivo experiments have proven that DC-derived IL12 increases IFN- γ production by CTLs [69]. In a small clinical trial, tumor-antigen-specific IL-12p70-producing DCs that were activated with CD40L/IFN- γ have been used to assess antigen-specific immunity [70]. Clinical response in 3 out of 7 patients correlated with more CTLs and higher amounts of IFN- γ and IL12p70. Lacking IL12p70 was explained with a defect in the IL12p35 pathway. Noteworthy, ex vivo IL-12 expression could be restored by adding the TLR3 agonist poly I:C and the TLR8 agonist R848 as adjuvants [70]. Targeted IL-12 expression has the potential of preventing systemic toxicity. Activating and expanding DCs in vivo with Flt3L and a polyI:C and injecting CD141⁺ DCs intratumorally could add efficacy to the anti-tumoral effect of anti-PD-1 therapy in a humanized melanoma mouse model [71]. The next important step for efficient tumor cell killing is activating CTLs in the tumor microenvironment. The costimulatory molecule 4-1BB is specifically expressed on inactive antigen-specific CTLs in the tumor microenvironment but not in the lymph node [72],

arguing for the necessity of intratumoral activation of T cells via 4-1BB ligand expressed on Batf3 DCs or other APCs or 4-1BB agonists (Figure 4) [73]. However, clinical studies with the anti-human 4-1BB (h4-1BB)-agonistic mAbs urelumab or utomilumab ended with severe hepatotoxicity or low efficacy, respectively [74]. Preclinically, bispecific antibodies that target the costimulatory molecule 4-1BB and tumor antigen or tumor stroma prevented a systemic adverse event and resulted in increased infiltration with CD8+ T cells producing IFN- γ and Granzyme b [63]. Another bispecific antibody targeting B7-H3 and 4-1BB worked synergistically with anti-PD-1 blockade by increasing the frequency of CTLs and eliminating the murine tumors without systemic adverse events [75]. Cell therapy approaches using 4-1BBL expressing erythrocytes are also in development. This approach restricts 4-1BBL expression to intravascular spaces only and may improve tolerability [76]. Additionally, bi-specific antibodies for 4-1BB with PD-L1 are in development with the goal to restrict 4-1BB agonism only to sites of PD-L1 expression (Table 1). These approaches may improve the therapeutic window for 4-1BB agonists to allow safe delivery of this promising therapy.

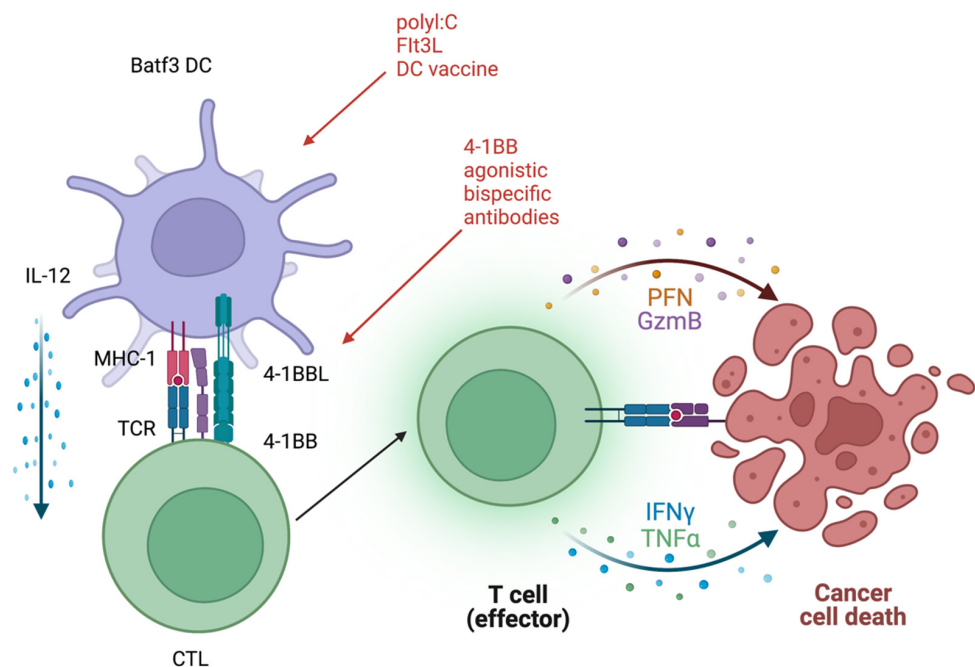


Figure 4. Batf3 DC-CTL interaction tumor microenvironment. In the tumor microenvironment, repriming of CTLs via Batf3 DCs is mandatory for efficient tumor eradication. Inactive CTLs need co-stimulation with 4-1BB ligand (4-1BBL). Batf3 DCs can provide this signal. Alternatively, 4-1BB-agonists such as bispecific antibodies targeting 4-1BB could be used. Batf3 DCs also secrete IL-12 in order to generate CTL effector function and IFN- γ production. To expand the low frequency of Batf3 DCs in the tumor microenvironment, vaccines delivering DCs combined with IL12 are on the verge of being translated in the clinic. Before administration, DCs have to be activated and expanded with polyI:C or Flt3L ex vivo.

Table 1. Induction and delivery of components related to innate immune that synergize with immunotherapy: summary of recent preclinical and clinical studies.

Components	Function	Model/Species	Delivery Route/Therapeutic Agent	Year/Citation
STING	sensing tumor-derived DNA, DC maturation and CTL priming	mouse	systemic administration of SR-717 in a “closed” conformation	2020 [28]
		mouse	oral administration of MSA-2 in a “closed” conformation	2020 [29]

Table 1. Cont.

Components	Function	Model/Species	Delivery Route/Therapeutic Agent	Year/Citation
		mouse	engineered extracellular vesicle exogenously loaded with cyclic dinucleotide	2021 [30]
		human	intravenous infusion of TAK-676	ongoing NCT04420884
		human	intravenous infusion of SB 11285	ongoing NCT04096638
		human	intravenous infusion of SNX281	ongoing NCT04609579
		human	intratumoral injection of CDK-002	ongoing NCT04592484
TLR9	sensing tumor-derived DNA, DC maturation and CTL priming	human	intratumoral injection of Vidutolimod	2021 [24]
		mouse	intratumoral injection CpG oligodeoxynucleotide (TLR9 ligand) and an antibody against OX40	2022 [77]
VEGF	local lymphangiogenesis, immune cell trafficking, and CTL activation	mouse	injection of “VEGF ₁₂₁ vax”	2021 [35]
TLR3+ Flt3L+ CD141+ DCs	DC activation and expansion	Humanized mouse	intratumoral injection of human CD141+ DCs, activated/expanded with Flt3L and polyinosinic:polycytidylic acid (TLR3 agonist)	2021 [71]
Flt3L+ Poly-ICLC+ Radiotherapy	DC expansion and maturation	Human	intratumoral Flt3L, Poly-ICLC plus low-dose radiotherapy	ongoing NCT01976585
XCL1+ Flt3L	DC recruitment and expansion	mouse	intratumoral injection of XCL1 and SFlt3L encoded in recombinant Semliki Forest virus-derived vectors	2018 [42]
CCL4	DC recruitment	mouse	intravenous administration of a fusion protein of CCL4 and the collagen-binding domain of von Willebrand factor	2019 [43]
CXCL9/10/11	CTL recruitment	mouse	CXCL9 and OX40L	2020 [45]
		mouse	intravenous injection of oncolytic vesicular stomatitis virus encodes CXCL9	2020 [47]
		mouse	genetically engineered mesenchymal stem cells producing CXCL10	2018 [78]
		humanized mouse	injection of CXCL10 producing SynNotch T cells	2021 [79]
		mouse	intravenous delivery of CXCL9/10/11 plasmids by nanoparticles	2022 [80]
		human	NG-641 is an oncolytic adenoviral vector which expresses a FAP-Tac antibody together with an immune enhancer module (CXCL9/CXCL10/IFN α).	ongoing NCT04053283
B7-H3+ 4-1BB	T cell checkpoints, stimulation leads to CD8+ T expansion	human	intravenous injection of bispecific antibody targeting B7-H3 and 4-1BB	2021 [75]
		human	bi-specific mAb for PD-L1 and 4-1BB	ongoing NCT03809624 [81]

Table 1. Cont.

Components	Function	Model/Species	Delivery Route/Therapeutic Agent	Year/Citation
4-1BBL+ IL15	IFN- γ production, promote CD8+ T and NK cell proliferation	human	intravenous infusion of RTX-240 (genetically engineered red blood cells that express 4-1BBL and IL-15/IL-15TP)	2021 [76]
IL-12	induction of Th1 anti-tumor responses	Human	intra-tumoral injection of IL-12 encoding mRNA with PD-L1 antibody	ongoing NCT03946800 [82]

5. Conclusions

Learning from the innate immune system and functional endogenous T cell priming, activation and recruitment in the tumor microenvironment can offer various leverage points to improve immunotherapy. Attempts to bring innate immune DNA sensing agonists, DC- and T-cell-chemoattracting factors or co-stimulatory molecules into the clinic are ongoing. Challenges lie in finding the right formulation, doses, delivery route, and combination for efficient agonists without causing systemic adverse events. Immune related adverse events could potentially be overcome by innovatively engineered delivery-systems such as nanoparticles or engineered oncolytic viruses. All the aforementioned strategies facilitate optimal activation and recruitment of CTLs into the tumor environment and, therefore, work well in conjunction with existing immunotherapies such as checkpoint blockade or adoptive cell therapy.

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References

- Fridman, W.H.; Pagès, F.; Sautès-Fridman, C.; Galon, J. The Immune Contexture in Human Tumours: Impact on Clinical Outcome. *Nat. Rev. Cancer* **2012**, *12*, 298–306. [[CrossRef](#)] [[PubMed](#)]
- Harlin, H.; Meng, Y.; Peterson, A.C.; Zha, Y.; Tretiakova, M.; Slingluff, C.; McKee, M.; Gajewski, T.F. Chemokine Expression in Melanoma Metastases Associated with CD8⁺ T-Cell Recruitment. *Cancer Res.* **2009**, *69*, 3077–3085. [[CrossRef](#)] [[PubMed](#)]
- Tumeh, P.C.; Harview, C.L.; Yearley, J.H.; Shintaku, I.P.; Taylor, E.J.M.; Robert, L.; Chmielowski, B.; Spasic, M.; Henry, G.; Ciobanu, V.; et al. PD-1 Blockade Induces Responses by Inhibiting Adaptive Immune Resistance. *Nature* **2014**, *515*, 568–571. [[CrossRef](#)] [[PubMed](#)]
- Spranger, S.; Dai, D.; Horton, B.; Gajewski, T.F. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer Cell* **2017**, *31*, 711–723.e4. [[CrossRef](#)]
- Woo, S.-R.; Fuertes, M.B.; Corrales, L.; Spranger, S.; Furdyna, M.J.; Leung, M.Y.K.; Duggan, R.; Wang, Y.; Barber, G.N.; Fitzgerald, K.A.; et al. STING-Dependent Cytosolic DNA Sensing Mediates Innate Immune Recognition of Immunogenic Tumors. *Immunity* **2014**, *41*, 830–842. [[CrossRef](#)]
- Liu, J.; Geng, X.; Hou, J.; Wu, G. New Insights into M1/M2 Macrophages: Key Modulators in Cancer Progression. *Cancer Cell Int.* **2021**, *21*, 389. [[CrossRef](#)]
- Böttcher, J.P.; Bonavita, E.; Chakravarty, P.; Bles, H.; Cabeza-Cabrero, M.; Sammicheli, S.; Rogers, N.C.; Sahai, E.; Zelenay, S.; Reis e Sousa, C. NK Cells Stimulate Recruitment of CDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. *Cell* **2018**, *172*, 1022–1037.e14. [[CrossRef](#)]
- Spranger, S.; Bao, R.; Gajewski, T.F. Melanoma-Intrinsic β -Catenin Signalling Prevents Anti-Tumour Immunity. *Nature* **2015**, *523*, 231–235. [[CrossRef](#)]
- Guilliams, M.; Ginhoux, F.; Jakubzick, C.; Naik, S.H.; Onai, N.; Schraml, B.U.; Segura, E.; Tussiwand, R.; Yona, S. Dendritic Cells, Monocytes and Macrophages: A Unified Nomenclature Based on Ontogeny. *Nat. Rev. Immunol.* **2014**, *14*, 571–578. [[CrossRef](#)]

10. Spranger, S.; Luke, J.J.; Bao, R.; Zha, Y.; Hernandez, K.M.; Li, Y.; Gajewski, A.P.; Andrade, J.; Gajewski, T.F. Density of Immunogenic Antigens Does Not Explain the Presence or Absence of the T-Cell-Inflamed Tumor Microenvironment in Melanoma. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E7759–E7768. [[CrossRef](#)]
11. Flood, B.A.; Higgs, E.F.; Li, S.; Luke, J.J.; Gajewski, T.F. STING Pathway Agonism as a Cancer Therapeutic. *Immunol. Rev.* **2019**, *290*, 24–38. [[CrossRef](#)]
12. Apetoh, L.; Ghiringhelli, F.; Tesniere, A.; Obeid, M.; Ortiz, C.; Criollo, A.; Mignot, G.; Maiuri, M.C.; Ullrich, E.; Saulnier, P.; et al. Toll-like Receptor 4-Dependent Contribution of the Immune System to Anticancer Chemotherapy and Radiotherapy. *Nat. Med.* **2007**, *13*, 1050–1059. [[CrossRef](#)]
13. Kang, T.H.; Mao, C.-P.; Lee, S.Y.; Chen, A.; Lee, J.-H.; Kim, T.W.; Alvarez, R.D.; Roden, R.B.S.; Pardoll, D.; Hung, C.-F.; et al. Chemotherapy Acts as an Adjuvant to Convert the Tumor Microenvironment into a Highly Permissive State for Vaccination-Induced Antitumor Immunity. *Cancer Res.* **2013**, *73*, 2493–2504. [[CrossRef](#)]
14. Kang, T.H.; Mao, C.-P.; Kim, Y.S.; Kim, T.W.; Yang, A.; Lam, B.; Tseng, S.-H.; Farmer, E.; Park, Y.-M.; Hung, C.-F. TLR9 Acts as a Sensor for Tumor-Released DNA to Modulate Anti-Tumor Immunity after Chemotherapy. *J. Immunother. Cancer* **2019**, *7*, 260. [[CrossRef](#)] [[PubMed](#)]
15. Loef, E.J.; Sheppard, H.M.; Birch, N.P.; Dunbar, P.R. Live-Cell Microscopy Reveals That Human T Cells Primarily Respond Chemokinetically Within a CCL19 Gradient That Induces Chemotaxis in Dendritic Cells. *Front. Immunol.* **2021**, *12*, 863. [[CrossRef](#)] [[PubMed](#)]
16. Martín-Fontecha, A.; Sebastiani, S.; Höpken, U.E.; Ugucioni, M.; Lipp, M.; Lanzavecchia, A.; Sallusto, F. Regulation of Dendritic Cell Migration to the Draining Lymph Node: Impact on T Lymphocyte Traffic and Priming. *J. Exp. Med.* **2003**, *198*, 615–621. [[CrossRef](#)]
17. Roberts, E.W.; Broz, M.L.; Binnewies, M.; Headley, M.B.; Nelson, A.E.; Wolf, D.M.; Kaisho, T.; Bogunovic, D.; Bhardwaj, N.; Krummel, M.F. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. *Cancer Cell* **2016**, *30*, 324–336. [[CrossRef](#)] [[PubMed](#)]
18. Corrales, L.; Matson, V.; Flood, B.; Spranger, S.; Gajewski, T.F. Innate Immune Signaling and Regulation in Cancer Immunotherapy. *Cell Res.* **2017**, *27*, 96–108. [[CrossRef](#)] [[PubMed](#)]
19. Ruhland, M.K.; Roberts, E.W.; Cai, E.; Mujal, A.M.; Marchuk, K.; Beppler, C.; Nam, D.; Serwas, N.K.; Binnewies, M.; Krummel, M.F. Visualizing Synaptic Transfer of Tumor Antigens among Dendritic Cells. *Cancer Cell* **2020**, *37*, 786–799.e5. [[CrossRef](#)]
20. Duong, E.; Fessenden, T.B.; Lutz, E.; Dinter, T.; Yim, L.; Blatt, S.; Bhutkar, A.; Wittrup, K.D.; Spranger, S. Type I Interferon Activates MHC Class I-Dressed CD11b+ Conventional Dendritic Cells to Promote Protective Anti-Tumor CD8+ T Cell Immunity. *Immunity* **2022**, *55*, 308–323.e9. [[CrossRef](#)]
21. Shulman, Z.; Cohen, S.J.; Roediger, B.; Kalchenko, V.; Jain, R.; Grabovsky, V.; Klein, E.; Shinder, V.; Stoler-Barak, L.; Feigelson, S.W.; et al. Transendothelial Migration of Lymphocytes Mediated by Intraendothelial Vesicle Stores Rather than by Extracellular Chemokine Depots. *Nat. Immunol.* **2012**, *13*, 67–76. [[CrossRef](#)]
22. Franciszkiwicz, K.; Le Floc’h, A.; Boutet, M.; Vergnon, I.; Schmitt, A.; Mami-Chouaib, F. CD103 or LFA-1 Engagement at the Immune Synapse between Cytotoxic T Cells and Tumor Cells Promotes Maturation and Regulates T-Cell Effector Functions. *Cancer Res.* **2013**, *73*, 617–628. [[CrossRef](#)] [[PubMed](#)]
23. Chen, D.S.; Mellman, I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* **2013**, *39*, 1–10. [[CrossRef](#)]
24. Ribas, A.; Medina, T.; Kirkwood, J.M.; Zakharia, Y.; Gonzalez, R.; Davar, D.; Chmielowski, B.; Campbell, K.M.; Bao, R.; Kelley, H.; et al. Overcoming PD-1 Blockade Resistance With CpG-A Toll-Like Receptor 9 Agonist Vidutolimod in Patients With Metastatic Melanoma. *Cancer Discov.* **2021**, *11*, 2998–3007. [[CrossRef](#)] [[PubMed](#)]
25. Corrales, L.; Glickman, L.H.; McWhirter, S.M.; Kanne, D.B.; Sivick, K.E.; Katibah, G.E.; Woo, S.-R.; Lemmens, E.; Banda, T.; Leong, J.J.; et al. Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep.* **2015**, *11*, 1018–1030. [[CrossRef](#)] [[PubMed](#)]
26. Conlon, J.; Burdette, D.L.; Sharma, S.; Bhat, N.; Thompson, M.; Jiang, Z.; Rathinam, V.A.K.; Monks, B.; Jin, T.; Xiao, T.S.; et al. Mouse, but Not Human STING, Binds and Signals in Response to the Vascular Disrupting Agent 5,6-Dimethylxanthenone-4-Acetic Acid. *J. Immunol.* **2013**, *190*, 5216–5225. [[CrossRef](#)]
27. Gajewski, T.F.; Higgs, E.F. Immunotherapy with a Sting. *Science* **2020**, *369*, 921–922. [[CrossRef](#)]
28. Chin, E.N.; Yu, C.; Vartabedian, V.F.; Jia, Y.; Kumar, M.; Gamo, A.M.; Vernier, W.; Ali, S.H.; Kissai, M.; Lazar, D.C.; et al. Antitumor Activity of a Systemic STING-Activating Non-Nucleotide CGAMP Mimetic. *Science* **2020**, *369*, 993–999. [[CrossRef](#)]
29. Pan, B.-S.; Perera, S.A.; Piesvaux, J.A.; Presland, J.P.; Schroeder, G.K.; Cumming, J.N.; Trotter, B.W.; Altman, M.D.; Buevich, A.V.; Cash, B.; et al. An Orally Available Non-Nucleotide STING Agonist with Antitumor Activity. *Science* **2020**, *369*, eaba6098. [[CrossRef](#)]
30. Jang, S.C.; Economides, K.D.; Moniz, R.J.; Sia, C.L.; Lewis, N.; McCoy, C.; Zi, T.; Zhang, K.; Harrison, R.A.; Lim, J.; et al. ExoSTING, an Extracellular Vesicle Loaded with STING Agonists, Promotes Tumor Immune Surveillance. *Commun. Biol.* **2021**, *4*, 1–17. [[CrossRef](#)]
31. Demaria, O.; De Gassart, A.; Coso, S.; Gestermann, N.; Di Domizio, J.; Flatz, L.; Gaide, O.; Michielin, O.; Hwu, P.; Petrova, T.V.; et al. STING Activation of Tumor Endothelial Cells Initiates Spontaneous and Therapeutic Antitumor Immunity. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15408–15413. [[CrossRef](#)] [[PubMed](#)]

32. Yang, H.; Lee, W.S.; Kong, S.J.; Kim, C.G.; Kim, J.H.; Chang, S.K.; Kim, S.; Kim, G.; Chon, H.J.; Kim, C. STING Activation Reprograms Tumor Vasculatures and Synergizes with VEGFR2 Blockade. *J. Clin. Investig.* **2019**, *129*, 4350–4364. [[CrossRef](#)] [[PubMed](#)]
33. Spaapen, R.M.; Leung, M.Y.K.; Fuertes, M.B.; Kline, J.P.; Zhang, L.; Zheng, Y.; Fu, Y.-X.; Luo, X.; Cohen, K.S.; Gajewski, T.F. Therapeutic Activity of High-Dose Intratumoral IFN- β Requires Direct Effect on the Tumor Vasculature. *J. Immunol.* **2014**, *193*, 4254–4260. [[CrossRef](#)]
34. Lund, A.W.; Wagner, M.; Fankhauser, M.; Steinskog, E.S.; Broggi, M.A.; Spranger, S.; Gajewski, T.F.; Alitalo, K.; Eikesdal, H.P.; Wiig, H.; et al. Lymphatic Vessels Regulate Immune Microenvironments in Human and Murine Melanoma. *J. Clin. Investig.* **2016**, *126*, 3389–3402. [[CrossRef](#)]
35. Sasso, M.S.; Mitrousis, N.; Wang, Y.; Briquez, P.S.; Hauert, S.; Ishihara, J.; Hubbell, J.A.; Swartz, M.A. Lymphangiogenesis-Inducing Vaccines Elicit Potent and Long-Lasting T Cell Immunity against Melanomas. *Sci. Adv.* **2021**, *7*, eabe4362. [[CrossRef](#)] [[PubMed](#)]
36. Fuertes, M.B.; Woo, S.-R.; Burnett, B.; Fu, Y.-X.; Gajewski, T.F. Type I Interferon Response and Innate Immune Sensing of Cancer. *Trends Immunol.* **2013**, *34*, 67–73. [[CrossRef](#)]
37. House, I.G.; Savas, P.; Lai, J.; Chen, A.X.Y.; Oliver, A.J.; Teo, Z.L.; Todd, K.L.; Henderson, M.A.; Giuffrida, L.; Petley, E.V.; et al. Macrophage-Derived CXCL9 and CXCL10 Are Required for Antitumor Immune Responses Following Immune Checkpoint Blockade. *Clin. Cancer Res.* **2020**, *26*, 487–504. [[CrossRef](#)]
38. Reschke, R.; Yu, J.; Flood, B.A.; Higgs, E.F.; Hatogai, K.; Gajewski, T.F. Immune Cell and Tumor Cell-Derived CXCL10 Is Indicative of Immunotherapy Response in Metastatic Melanoma. *J. Immunother. Cancer* **2021**, *9*, e003521. [[CrossRef](#)]
39. Aliberti, J.; Reis e Sousa, C.; Schito, M.; Hieny, S.; Wells, T.; Huffnagle, G.B.; Sher, A. CCR5 Provides a Signal for Microbial Induced Production of IL-12 by CD8 Alpha+ Dendritic Cells. *Nat. Immunol.* **2000**, *1*, 83–87. [[CrossRef](#)]
40. Dorner, B.G.; Dorner, M.B.; Zhou, X.; Opitz, C.; Mora, A.; Güttler, S.; Hutloff, A.; Mages, H.W.; Ranke, K.; Schaefer, M.; et al. Selective Expression of the Chemokine Receptor XCR1 on Cross-Presenting Dendritic Cells Determines Cooperation with CD8+ T Cells. *Immunity* **2009**, *31*, 823–833. [[CrossRef](#)]
41. Fuertes, M.B.; Kacha, A.K.; Kline, J.; Woo, S.-R.; Kranz, D.M.; Murphy, K.M.; Gajewski, T.F. Host Type I IFN Signals Are Required for Antitumor CD8+ T Cell Responses through CD8 α + Dendritic Cells. *J. Exp. Med.* **2011**, *208*, 2005–2016. [[CrossRef](#)] [[PubMed](#)]
42. Sánchez-Paulete, A.R.; Teijeira, Á.; Quetglas, J.I.; Rodríguez-Ruiz, M.E.; Sánchez-Arráez, Á.; Labiano, S.; Etxeberria, I.; Azpilikueta, A.; Bolaños, E.; Ballesteros-Briones, M.C.; et al. Intratumoral Immunotherapy with XCL1 and SFlt3L Encoded in Recombinant Semliki Forest Virus-Derived Vectors Fosters Dendritic Cell-Mediated T-Cell Cross-Priming. *Cancer Res.* **2018**, *78*, 6643–6654. [[CrossRef](#)]
43. Williford, J.-M.; Ishihara, J.; Ishihara, A.; Mansurov, A.; Hosseinchi, P.; Marchell, T.M.; Potin, L.; Swartz, M.A.; Hubbell, J.A. Recruitment of CD103+ Dendritic Cells via Tumor-Targeted Chemokine Delivery Enhances Efficacy of Checkpoint Inhibitor Immunotherapy. *Sci. Adv.* **2019**, *5*, eaay1357. [[CrossRef](#)] [[PubMed](#)]
44. Antonicelli, F.; Lorin, J.; Kurdykowski, S.; Gangloff, S.C.; Le Naour, R.; Sallenave, J.M.; Hornebeck, W.; Grange, F.; Bernard, P. CXCL10 Reduces Melanoma Proliferation and Invasiveness in Vitro and in Vivo: CXCL10 and Melanoma Progression. *Br. J. Dermatol.* **2011**, *164*, 720–728. [[CrossRef](#)]
45. Yin, P.; Gui, L.; Wang, C.; Yan, J.; Liu, M.; Ji, L.; Wang, Y.; Ma, B.; Gao, W.-Q. Targeted Delivery of CXCL9 and OX40L by Mesenchymal Stem Cells Elicits Potent Antitumor Immunity. *Mol. Ther.* **2020**, *28*, 2553–2563. [[CrossRef](#)] [[PubMed](#)]
46. Cervera-Carrascon, V.; Quixabeira, D.C.A.; Santos, J.M.; Havunen, R.; Zafar, S.; Hemminki, O.; Heiniö, C.; Munaro, E.; Siurala, M.; Sorsa, S.; et al. Tumor Microenvironment Remodeling by an Engineered Oncolytic Adenovirus Results in Improved Outcome from PD-L1 Inhibition. *Oncol Immunology* **2020**, *9*, 1761229. [[CrossRef](#)]
47. Eckert, E.C.; Nace, R.A.; Tonne, J.M.; Evgin, L.; Vile, R.G.; Russell, S.J. Generation of a Tumor-Specific Chemokine Gradient Using Oncolytic Vesicular Stomatitis Virus Encoding CXCL9. *Mol. Ther. Oncolytics* **2019**, *16*, 63–74. [[CrossRef](#)]
48. Liu, Z.; Ravindranathan, R.; Kalinski, P.; Guo, Z.S.; Bartlett, D.L. Rational Combination of Oncolytic Vaccinia Virus and PD-L1 Blockade Works Synergistically to Enhance Therapeutic Efficacy. *Nat. Commun.* **2017**, *8*, 14754. [[CrossRef](#)]
49. Mowat, C.; Mosley, S.R.; Namdar, A.; Schiller, D.; Baker, K. Anti-Tumor Immunity in Mismatch Repair-Deficient Colorectal Cancers Requires Type I IFN-Driven CCL5 and CXCL10. *J. Exp. Med.* **2021**, *218*, e20210108. [[CrossRef](#)]
50. Kobayashi, H.; Nobeyama, Y.; Nakagawa, H. Tumor-Suppressive Effects of Natural-Type Interferon- β through CXCL10 in Melanoma. *Biochem. Biophys. Res. Commun.* **2015**, *464*, 416–421. [[CrossRef](#)]
51. Peng, W.; Liu, C.; Xu, C.; Lou, Y.; Chen, J.; Yang, Y.; Yagita, H.; Overwijk, W.W.; Lizée, G.; Radvanyi, L.; et al. PD-1 blockade enhances T cell migration to tumors by elevating IFN- γ inducible chemokines. *Cancer Res.* **2012**, *72*, 5209–5218. [[CrossRef](#)] [[PubMed](#)]
52. Antonelli, A.; Ferrari, S.M.; Corrado, A.; Ferrannini, E.; Fallahi, P. CXCR3, CXCL10 and Type 1 Diabetes. *Cytokine Growth Factor Rev.* **2014**, *25*, 57–65. [[CrossRef](#)] [[PubMed](#)]
53. Boniface, K.; Jacquemin, C.; Darrigade, A.-S.; Dessarthe, B.; Martins, C.; Boukhedouni, N.; Vernisse, C.; Grasseau, A.; Thiolat, D.; Rambert, J.; et al. Vitiligo Skin Is Imprinted with Resident Memory CD8 T Cells Expressing CXCR3. *J. Invest. Dermatol.* **2018**, *138*, 355–364. [[CrossRef](#)]
54. Han, J.H.; Suh, C.-H.; Jung, J.-Y.; Ahn, M.-H.; Han, M.H.; Kwon, J.E.; Yim, H.; Kim, H.-A. Elevated Circulating Levels of the Interferon- γ -Induced Chemokines Are Associated with Disease Activity and Cutaneous Manifestations in Adult-Onset Still's Disease. *Sci. Rep.* **2017**, *7*, 46652. [[CrossRef](#)]

55. Ito, T. Recent Advances in the Pathogenesis of Autoimmune Hair Loss Disease Alopecia Areata. *Clin. Dev. Immunol.* **2013**, *2013*, 348546. [[CrossRef](#)] [[PubMed](#)]
56. Loos, T.; Dekeyser, L.; Struyf, S.; Schutyser, E.; Gijssbers, K.; Gouwy, M.; Fraeyman, A.; Put, W.; Ronsse, I.; Grillet, B.; et al. TLR Ligands and Cytokines Induce CXCR3 Ligands in Endothelial Cells: Enhanced CXCL9 in Autoimmune Arthritis. *Lab. Investig.* **2006**, *86*, 902–916. [[CrossRef](#)] [[PubMed](#)]
57. Luoma, A.M.; Suo, S.; Williams, H.L.; Sharova, T.; Sullivan, K.; Manos, M.; Bowling, P.; Hodi, F.S.; Rahma, O.; Sullivan, R.J.; et al. Molecular Pathways of Colon Inflammation Induced by Cancer Immunotherapy. *Cell* **2020**, *182*, 655–671.e22. [[CrossRef](#)]
58. Amatschek, S.; Lucas, R.; Eger, A.; Pflueger, M.; Hundesberger, H.; Knoll, C.; Grosse-Kracht, S.; Schuett, W.; Koszik, F.; Maurer, D.; et al. CXCL9 Induces Chemotaxis, Chemorepulsion and Endothelial Barrier Disruption through CXCR3-Mediated Activation of Melanoma Cells. *Br. J. Cancer* **2011**, *104*, 469–479. [[CrossRef](#)]
59. Broz, M.L.; Binnewies, M.; Boldajipour, B.; Nelson, A.E.; Pollack, J.L.; Erle, D.J.; Barczak, A.; Rosenblum, M.D.; Daud, A.; Barber, D.L.; et al. Dissecting the Tumor Myeloid Compartment Reveals Rare Activating Antigen-Presenting Cells Critical for T Cell Immunity. *Cancer Cell* **2014**, *26*, 638–652. [[CrossRef](#)]
60. Barry, K.C.; Hsu, J.; Broz, M.L.; Cueto, F.J.; Binnewies, M.; Combes, A.J.; Nelson, A.E.; Loo, K.; Kumar, R.; Rosenblum, M.D.; et al. A Natural Killer/Dendritic Cell Axis Defines Responsive Tumor Microenvironments in Melanoma. *Nat. Med.* **2018**, *24*, 1178–1191. [[CrossRef](#)]
61. Hammerich, L.; Marron, T.U.; Upadhyay, R.; Svensson-Arvelund, J.; Dhainaut, M.; Hussein, S.; Zhan, Y.; Ostrowski, D.; Yellin, M.; Marsh, H.; et al. Systemic Clinical Tumor Regressions and Potentiation of PD1 Blockade with in Situ Vaccination. *Nat. Med.* **2019**, *25*, 814–824. [[CrossRef](#)] [[PubMed](#)]
62. Chen, L.; Flies, D.B. Molecular Mechanisms of T Cell Co-Stimulation and Co-Inhibition. *Nat. Rev. Immunol.* **2013**, *13*, 227–242. [[CrossRef](#)] [[PubMed](#)]
63. Claus, C.; Ferrara, C.; Xu, W.; Sam, J.; Lang, S.; Uhlenbrock, F.; Albrecht, R.; Herter, S.; Schlenker, R.; Hüsser, T.; et al. Tumor-Targeted 4-1BB Agonists for Combination with T Cell Bispecific Antibodies as off-the-Shelf Therapy. *Sci. Transl. Med.* **2019**, *11*, eaav5989. [[CrossRef](#)] [[PubMed](#)]
64. Sánchez-Paulete, A.R.; Cueto, F.J.; Martínez-López, M.; Labiano, S.; Morales-Kastresana, A.; Rodríguez-Ruiz, M.E.; Jure-Kunkel, M.; Azpilikueta, A.; Aznar, M.A.; Quetglas, J.I.; et al. Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti-PD-1 Monoclonal Antibodies Requires Batf3-Dependent Dendritic Cells. *Cancer Discov.* **2016**, *6*, 71–79. [[CrossRef](#)]
65. Ruffell, B.; Chang-Strachan, D.; Chan, V.; Rosenbusch, A.; Ho, C.M.T.; Pryer, N.; Daniel, D.; Hwang, E.S.; Rugo, H.S.; Coussens, L.M. Macrophage IL-10 Blocks CD8+ T Cell-Dependent Responses to Chemotherapy by Suppressing IL-12 Expression in Intratumoral Dendritic Cells. *Cancer Cell* **2014**, *26*, 623–637. [[CrossRef](#)]
66. Wang, S.; Wu, Q.; Chen, T.; Su, R.; Pan, C.; Qian, J.; Huang, H.; Yin, S.; Xie, H.; Zhou, L.; et al. Blocking CD47 Promotes Anti-Tumor Immunity through CD103+ Dendritic Cell-NK Cell Axis in Murine Hepatocellular Carcinoma Model. *J. Hepatol.* **2022**, S0168-8278(22)00179-9. [[CrossRef](#)]
67. Cho, N.-H.; Cheong, T.-C.; Min, J.H.; Wu, J.H.; Lee, S.J.; Kim, D.; Yang, J.-S.; Kim, S.; Kim, Y.K.; Seong, S.-Y. A Multifunctional Core-Shell Nanoparticle for Dendritic Cell-Based Cancer Immunotherapy. *Nat. Nanotech* **2011**, *6*, 675–682. [[CrossRef](#)]
68. Bogunovic, D.; Manches, O.; Godefroy, E.; Yewdall, A.; Gallois, A.; Salazar, A.M.; Marie, I.; Levy, D.E.; Bhardwaj, N. TLR4 Engagement during TLR3-Induced Proinflammatory Signaling in Dendritic Cells Promotes IL-10-Mediated Suppression of Antitumor Immunity. *Cancer Res.* **2011**, *71*, 5467–5476. [[CrossRef](#)]
69. Henry, C.J.; Ornelles, D.A.; Mitchell, L.M.; Brzoza-Lewis, K.L.; Hiltbold, E.M. IL-12 Produced by Dendritic Cells Augments CD8+ T Cell Activation through the Production of the Chemokines CCL1 and CCL17. *J. Immunol.* **2008**, *181*, 8576–8584. [[CrossRef](#)]
70. Carreno, B.M.; Magrini, V.; Becker-Hapak, M.; Kaabinejadian, S.; Hundal, J.; Petti, A.A.; Ly, A.; Lie, W.-R.; Hildebrand, W.H.; Mardis, E.R.; et al. A Dendritic Cell Vaccine Increases the Breadth and Diversity of Melanoma Neoantigen-Specific T Cells. *Science* **2015**, *348*, 803–808. [[CrossRef](#)]
71. Lee, Y.S.; O'Brien, L.J.; Walpole, C.M.; Pearson, F.E.; Leal-Rojas, I.M.; Masterman, K.-A.; Atkinson, V.; Barbour, A.; Radford, K.J. Human CD141+ Dendritic Cells (CDC1) Are Impaired in Patients with Advanced Melanoma but Can Be Targeted to Enhance Anti-PD-1 in a Humanized Mouse Model. *J. Immunother. Cancer* **2021**, *9*, e001963. [[CrossRef](#)] [[PubMed](#)]
72. Williams, J.B.; Horton, B.L.; Zheng, Y.; Duan, Y.; Powell, J.D.; Gajewski, T.F. The EGR2 Targets LAG-3 and 4-1BB Describe and Regulate Dysfunctional Antigen-Specific CD8+ T Cells in the Tumor Microenvironment. *J. Exp. Med.* **2017**, *214*, 381–400. [[CrossRef](#)] [[PubMed](#)]
73. Ziblat, A.; Horton, B.; Higgs, E.; Hatogai, K.; Gajewski, T. 328 Batf3 dendritic cells and 4-1BB/4-1BB ligand axis are required at the effector phase within the tumor microenvironment for anti-PD-L1 efficacy. *J. Immunotherapy Cancer* **2021**, *9*, A354. [[CrossRef](#)]
74. Chester, C.; Sanmamed, M.F.; Wang, J.; Melero, I. Immunotherapy Targeting 4-1BB: Mechanistic Rationale, Clinical Results, and Future Strategies. *Blood* **2018**, *131*, 49–57. [[CrossRef](#)] [[PubMed](#)]
75. You, G.; Lee, Y.; Kang, Y.-W.; Park, H.W.; Park, K.; Kim, H.; Kim, Y.-M.; Kim, S.; Kim, J.-H.; Moon, D.; et al. B7-H3×4-1BB Bispecific Antibody Augments Antitumor Immunity by Enhancing Terminally Differentiated CD8+ Tumor-Infiltrating Lymphocytes. *Sci. Adv.* **2021**, *7*, eaax3160. [[CrossRef](#)]
76. Hamid, O.; Luke, J.; Spira, A.; Kuesters, G.; Siencylo, I.; Gordon, G.; Johnson, M. A Phase 1 Trial of RTX-240, an Allogeneic Engineered Red Blood Cell With Cell-Surface Expression of 4-1BBL and Trans-Presented IL-15, in Patients With Advanced Solid Tumors. *Cancer Res.* **2021**, *81*, 615. [[CrossRef](#)]

77. Hong, W.X.; Sagiv-Barfi, I.; Czerwinski, D.K.; Sallets, A.; Levy, R. Neoadjuvant Intratumoral Immunotherapy with TLR9 Activation and Anti-OX40 Antibody Eradicates Metastatic Cancer. *Cancer Res.* **2022**, *82*, 1396–1408. [[CrossRef](#)]
78. Mirzaei, H.; Salehi, H.; Oskuee, R.K.; Mohammadpour, A.; Mirzaei, H.R.; Sharifi, M.R.; Salarinia, R.; Darani, H.Y.; Mokhtari, M.; Masoudifar, A.; et al. The Therapeutic Potential of Human Adipose-Derived Mesenchymal Stem Cells Producing CXCL10 in a Mouse Melanoma Lung Metastasis Model. *Cancer Lett.* **2018**, *419*, 30–39. [[CrossRef](#)]
79. Xia, M.; Chen, J.; Meng, G.; Shen, H.; Dong, J. CXCL10 Encoding SynNotch T Cells Enhance Anti-Tumor Immune Responses without Systemic Side Effect. *Biochem. Biophys. Res. Commun.* **2021**, *534*, 765–772. [[CrossRef](#)]
80. Ma, Y.; Liu, Y.; Zhi, Y.; Wang, H.; Yang, M.; Niu, J.; Zhao, L.; Wang, P. Delivery of CXCL9/10/11 Plasmid DNAs Promotes the Tumor-Infiltration of T Cells and Synergizes with PD1 Antibody for Treating Lung Cancer. *Cancer Nanotechnol.* **2022**, *13*, 10. [[CrossRef](#)]
81. Kinkead, H.; Macedo, C.; Sanabria, A.; Cyprus, G.; Pandit, R.; Kalabus, J.; Becklund, B.; Sulzmaier, F.; Timmer, J.; Deveraux, Q.; et al. 12 Key Pharmacokinetic and Pharmacodynamic Parameters That Correlate with the Anti-Tumor Activity of a Bispecific PD-L1 Conditional 4–1BB Agonist. *J. Immunother. Cancer* **2021**, *9* (Suppl. S2). [[CrossRef](#)]
82. Hamid, O.; Hellman, M.; Carneiro, B.; Marron, T.; Subbiah, V.; Mehmi, I.; Eyles, J.; Dubois, V.; Ridgway, B.; Hamid, O.; et al. 19O Preliminary Safety, Antitumor Activity and Pharmacodynamics Results of HIT-IT MEDI1191 (MRNA IL-12) in Patients with Advanced Solid Tumours and Superficial Lesions. *Ann. Oncol.* **2021**, *32*, S9. [[CrossRef](#)]