

Review

Turning enemies into allies—reprogramming tumor-associated macrophages for cancer therapy

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SUMMARY

Checkpoint blockade therapies that target inhibitory receptors on T cells have revolutionized clinical oncology. Antibodies targeting CTLA-4 or the PD-1/PD-1 ligand (PD-L1) axis are now successfully used alone or in combination with chemotherapy for numerous tumor types. Despite the clinical success of checkpoint blockade therapies, tumors exploit multiple mechanisms to escape or subvert the anti-tumor T cell response. Within the tumor microenvironment, tumor-associated macrophages (TAMs) can suppress T cell responses and facilitate tumor growth in various ways, ultimately debilitating clinical responses to T cell checkpoint inhibitors. There is therefore significant interest in identifying biologicals and drugs that target immunosuppressive TAM within the tumor microenvironment and can be combined with immune checkpoint inhibitors. Here, we review approaches that are currently being evaluated to convert immunosuppressive TAM into immunostimulatory macrophages that promote T cell responses and tumor elimination.

INTRODUCTION

Macrophages provide a first line of defense during infections and other tissue insults through multiple mechanisms: (1) phagocytosis of pathogens and damaged cells; (2) production of bactericidal nitric oxide (NO) and reactive oxygen species (ROS); (3) secretion of chemokines that recruit other immune cells; and (4) secretion of cytokines, such as interleukin-12 (IL-12), that activate CD8 T cells, T helper 1 (TH1) cells, and natural killer (NK) cells. Moreover, macrophages express Fc receptors for immunoglobulin G (IgG) that enable phagocytosis and killing of targets opsonized by IgG through antibody-dependent phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC), respectively.^{1,2} Conversely, macrophages also contribute to resolution of immune responses, tissue repair, and restoration in several ways: (1) secreting soluble factors that attenuate immune responses, such as IL-10, transforming growth factor β (TGF- β), prostaglandin E2 (PGE2), and tryptophan catabolites generated by indoleamine 2,3-dioxygenase (IDO); (2) releasing chemokines that attract Treg cells; (3) promoting angiogenesis through VEGF secretion; and (4) producing metabolites generated by arginase I that promote wound healing.^{1,3} Although immune defense and tissue repair functions of macrophages were originally framed within the dual M1-M2 paradigm, it is now recognized that macrophage programs are complex and heterogeneous, depending on context.^{1,4}

Beyond their contribution to host defense and tissue repair, macrophages are also one of the most abundant immune cell populations in human tumors and mouse tumor models.^{1,5,6} The origins, phenotypes, and functions of tumor-associated macrophages (TAMs) are heterogeneous, depending on the type of tumor and the microenvironment in which tumors develop.¹ TAMs can derive from tissue-resident

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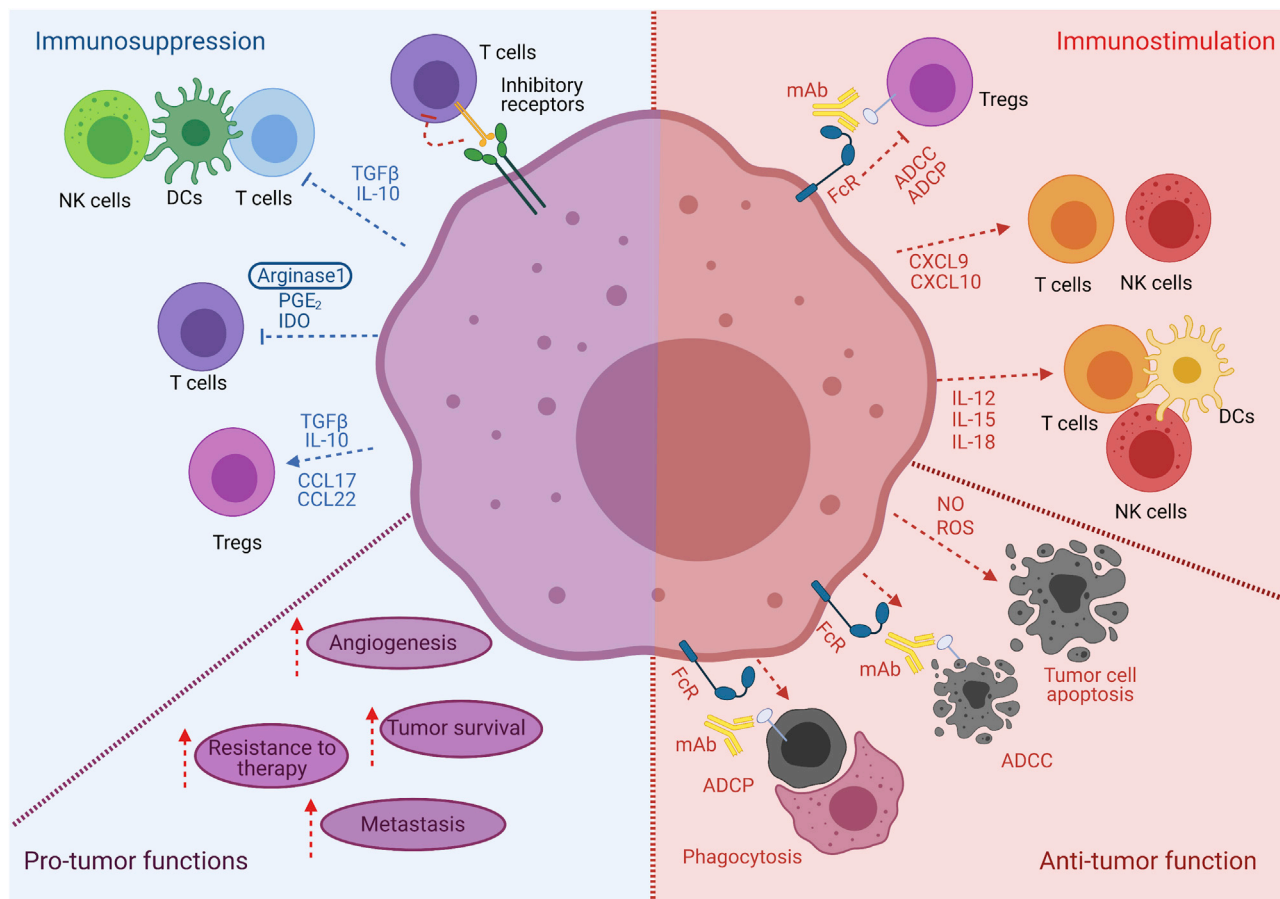


Figure 1. Pro-tumor and anti-tumor functions of TAMs

Upper left: immunosuppressive functions. TAMs can inhibit T cell, dendritic cells (DCs), and natural killer (NK) cell activation through the immunosuppressive cytokines IL-10 and TGF- β ; TAMs also suppress anti-tumor T cell functions through inhibitory receptor-ligand interactions, production of indoleamine 2,3-dioxygenase (IDO), release of arginase I catabolites, and PGE₂. Moreover, TAMs contribute to differentiation and recruitment of regulatory T cells (Treg cells) via IL-10, TGF β , CCL17, and CCL22. Upper right: immunostimulatory functions. TAMs can activate the anti-tumor immune response by recruiting and activating T cells, NK cells, and DCs through the chemokines CXCL9 and CXCL10 and the pro-inflammatory cytokines IL-12, IL-15, and IL-18. TAMs can eliminate Treg cells via antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) during immunotherapy with monoclonal antibodies targeting Treg cells. Lower left: direct pro-tumor functions. TAMs can promote resistance to therapy, angiogenesis, tumor cell survival, and metastatization. Lower right: direct anti-tumor activities. TAMs can directly kill tumor cells via phagocytosis, ADCP, ADCC, release of nitric oxide (NO), and reactive oxygen species (ROS).

macrophages that seed tissues during fetal hematopoiesis and persist through self-renewal, as well as peripheral blood monocytes that infiltrate the tumor.^{7–14} Tumor-imprinted monocytes, known as monocyte-related myeloid-derived suppressor cells (MDSCs), which differentiate into suppressive macrophages, have also been identified in patients and in tumor bearing mice.¹⁵ In general, the phenotypic and functional profile of TAMs parallel those observed during tissue repair, which promote suppression of anti-tumor immune responses, tumor growth, vascularization, and metastatization (Figure 1).¹ In agreement with a predominant pro-tumorigenic impact, TAM infiltration is often associated with a poor prognosis and abbreviated survival for many different types of human cancer (e.g., breast, bladder, ovarian, and gastric cancer and hematological malignancies).^{16–20} Thus, therapeutic strategies have been devised to either limit macrophage recruitment into the tumor or deplete macrophages already present in it. Blockade of CCR2 can limit recruitment of monocytes into the tumor,^{6,21,22} whereas inhibitors of the receptor for CSF1

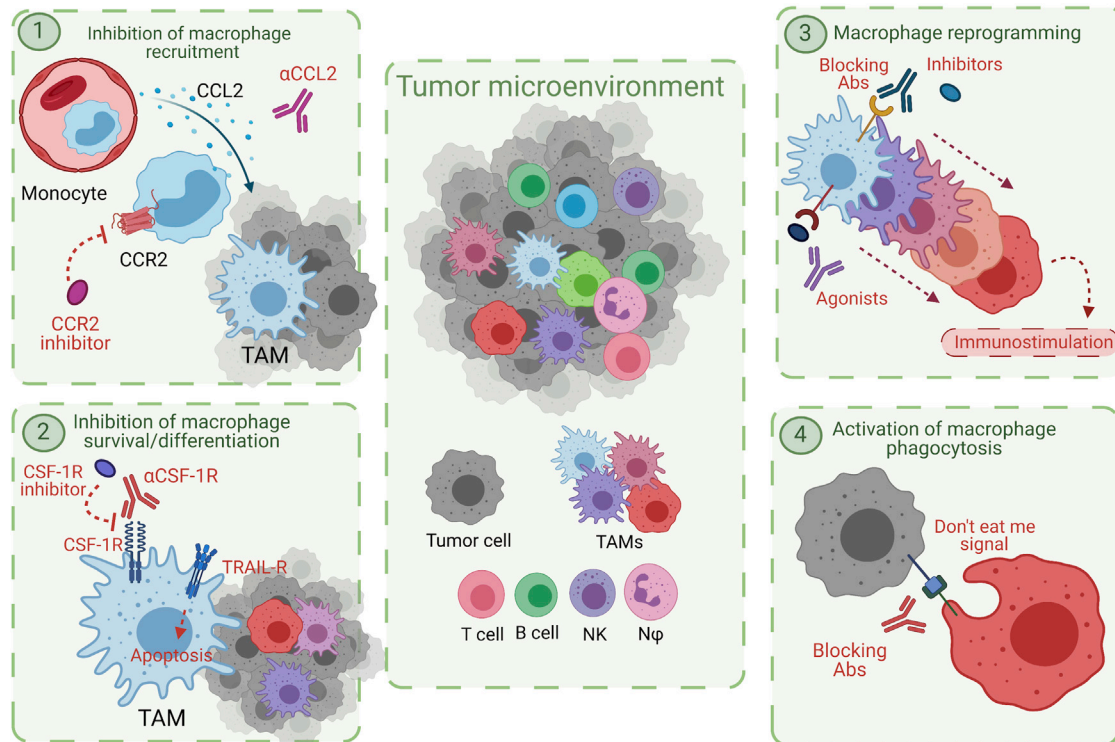


Figure 2. Strategies to target tumor-associated macrophages

(1) Inhibition of macrophage recruitment through blockade of CCL2-CCR2 axis; (2) inhibition of macrophage differentiation and survival through blockade of CSF1-CSF1R axis and induction of TRAIL-driven apoptosis; (3) macrophage reprogramming via blockade of inhibitory pathways or engagement of activation pathways; (4) induction of macrophage-mediated phagocytosis through blockade of “do not eat me” signals.

(CSF1R) and activation of the death receptor TRAIL-R impair maintenance and survival of TAMs within the tumor (Figure 2).^{23–25} Clinical trials based on these strategies are ongoing, although their efficacy so far seems limited (see for review Mantovani et al.,¹ Cassetta and Pollard,⁵ and Majety et al.²⁶). Despite a predominant pro-tumorigenic impact, in certain contexts, TAMs are capable of phagocytosing tumor cells, killing tumor cells through release of NO and ROS, secreting IL-12 and promoting anti-tumor CD8 T cell and Th1 responses, and ultimately restricting tumor growth.²⁶ Immunostimulatory and immunosuppressive TAMs can coexist within the same tumor. Thus, alternative therapeutic approaches have been designed that aim at reshaping the TAM landscape by converting the TAM profile from immunosuppressive into immunostimulatory. This goal can be achieved through various approaches: (1) blockade of cell surface receptors on immunosuppressive TAMs; (2) blockade of ligand-receptor pairs that inhibit phagocytosis; (3) blockade of signaling and epigenetic mechanisms of TAM immunosuppression; and (4) engagement of existing or artificially transduced activating receptors (Figure 2). In the following paragraphs, we discuss prototypic target molecules for each of these strategies.

Cell surface receptors of immunosuppressive TAMs

Cell surface phenotypes, transcriptional profiles, and functions of immunosuppressive TAMs are quite heterogeneous and change considerably, depending on the tumor and the microenvironment. Notwithstanding, several cell surface molecules have been frequently associated with TAM immunosuppressive functions, including CD206 (MRC1), CD204 (MSR1), CX3CR1, MARCO, SIGLEC1, TREM2, CD9, CD63, PD-1 ligand (PD-L1), CD73, and MERTK (Figure 3). While some of these molecules

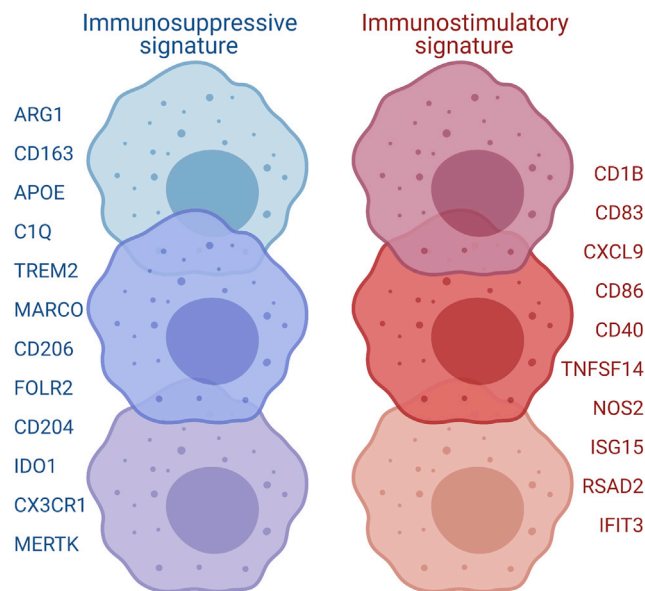


Figure 3. Macrophage distinct signatures

Molecules associated with immunosuppressive (left) or immunostimulatory (right) signatures of TAMs.

are simply markers so far, others directly contribute to immunosuppression and have been targeted to reprogram TAMs into immune-stimulating cells (Figure 4). These latter include TREM2, MARCO, SIGLEC1, PD-L1, and CD73.

TREM2

TREM2 is a cell surface receptor of the Ig superfamily expressed on many tissue macrophages that binds phospholipids, apoptotic cells, and lipoproteins. TREM2 transmits intracellular signals through the adaptor molecule DAP12, which recruits the protein tyrosine kinase SYK. This signaling pathway activates multiple downstream mediators, including phosphatidylinositol 3-kinase (PI3K), PLC γ 2, and mitogen-activated protein kinase (MAPK), and cooperates with the CSF1R pathway.^{27–29} Metalloproteases regulate TREM2 surface expression by cleaving the ectodomain, which is released as soluble TREM2.³⁰ TREM2 has been widely studied in Alzheimer's disease because of its important role in sustaining the microglia response to brain accumulation of β -amyloid plaques.³⁰ However, TREM2 is expressed in macrophages of various tissues in physiological and pathological conditions.³⁰ In the adipose tissue, TREM2 is required to sustain a population of lipid-associated macrophages that prevent dysmetabolism caused by high-fat diet.³¹ In cirrhotic patients and a mouse model of non-alcoholic fatty liver disease, TREM2 is highly expressed in liver CD9⁺ macrophages, which resemble lipid-associated macrophages and protect hepatocytes from lipid accumulation.^{32–34} In atherosclerosis, TREM2 contributes to lipid catabolism in macrophages of atherosclerotic plaques.³⁵ The expression of TREM2 has been recently reported in TAMs of cancer patients and mouse tumor models,^{36,37} and two studies showed that TREM2 sustains immunosuppressive TAMs.^{38,39} Using a novel technology that couples single-cell RNA sequencing (scRNA-seq) and intracellular protein measurements, one study identified a subset of TAMs in a model of methylcholanthrene (MCA)-induced sarcoma that coexpresses the immunosuppressive marker arginase 1 (Arg1) and TREM2.³⁸ This Arg1⁺ Trem2⁺ subset was associated with tumor progression, whereas TREM2 deletion restricted tumor growth. In another study, TREM2 deletion and TREM2 blockade

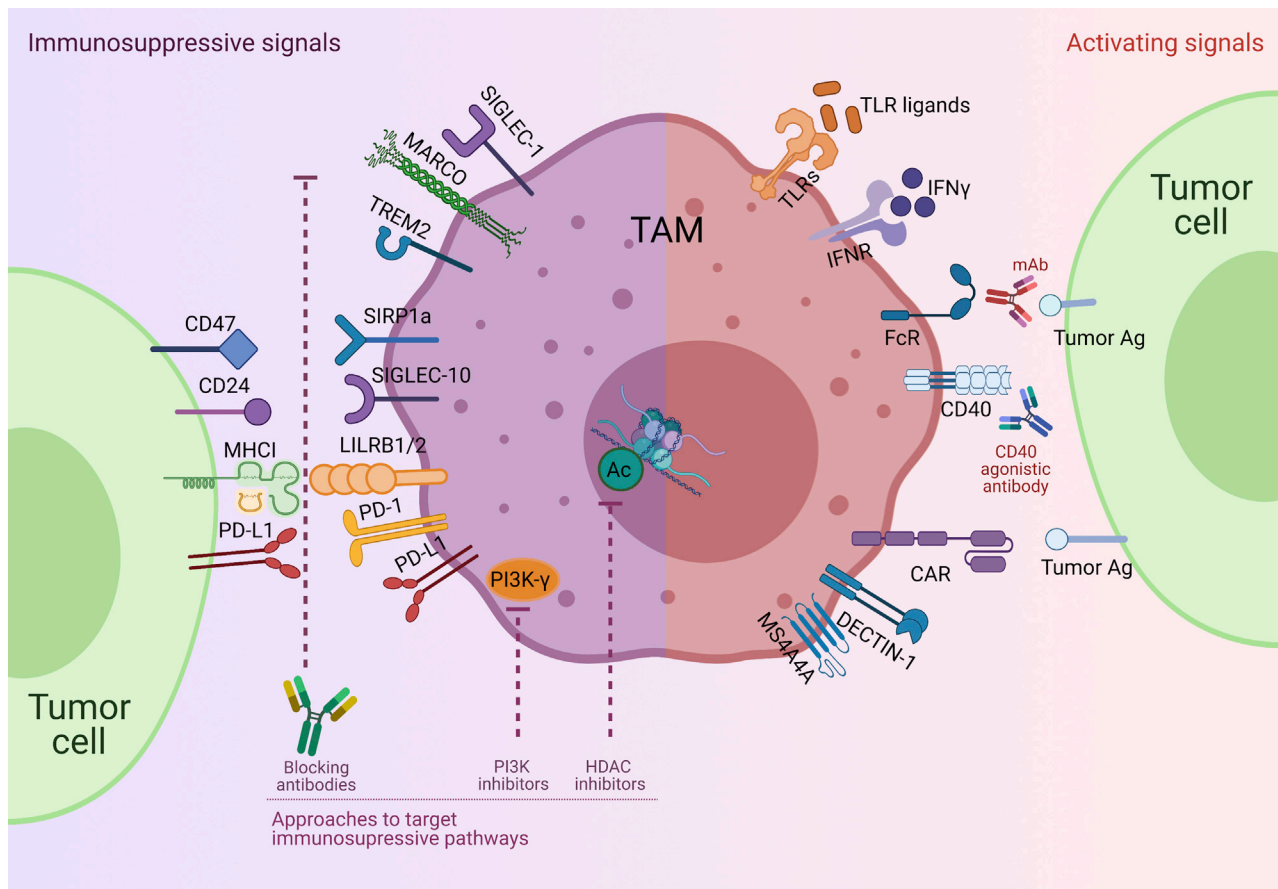


Figure 4. Strategies to promote macrophage anti-tumor activities

Left: approaches to block immunosuppressive signals. Blocking mAbs targeting macrophage receptors SIGLEC-1, MARCO, TREM2, SIRP1a, SIGLEC-10, LILRB1, LILRB2, PD-1, and PD-L1; PI3K inhibitors; and HDAC inhibitors. Right: approaches to enhance activating signals. TLR agonists; IFN γ ; FcR-mediated ADCC; agonistic anti-CD40 mAbs; CARs; and DECTIN1 signaling.

with a monoclonal antibody (mAb) reshaped the TAM landscape, facilitating T cell activation. The proportion of $Mrc1^+Cx3cr1^+$ TAMs declined, whereas the representation of $Cd83^+Cxl9^+$ and $Nos2^+$ TAMs increased. These changes in TAMs were associated with blunted tumor growth and an increased response to anti-PD-1 in models of sarcoma, colorectal cancer, and breast cancer. Immunohistochemistry analysis of specimens from cancer patients revealed that TREM2 is specifically expressed by TAMs of many human tumor types (skin, liver, lung, breast, colon, stomach, bladder, kidney cancer, pancreatic cancer, lymphoma, and glioma).³⁹ Moreover, high TREM2 expression in cohorts of patients with triple-negative breast carcinoma and colorectal cancer was associated with a negative prognosis. Together, these studies suggest that TREM2 blockade and, perhaps, TREM2 cleavage can be promising approaches for TAM reprogramming. Whether TREM2 signaling promotes macrophage immunosuppression directly, by cooperating with CSF1R signaling, or through other mechanisms is currently unknown.

MARCO

A member of the scavenger receptor family that is expressed by subsets of tissue macrophages, MARCO is upregulated upon bacterial infection and is expressed on TAMs in breast, colon cancer, endometrial cancer, melanoma, and non-small

cell lung cancer.^{40,41} TAMs expressing MARCO evinced a typical immunosuppressive signature in models of breast cancer, colon cancer, and melanoma. Treatment with an anti-MARCO mAb was associated with improved tumor control and limited metastases in mouse models of melanoma and breast cancer.⁴² Combined blockade of MARCO and CTLA-4 was more efficient than single blockade of CTLA-4 in models of colon cancer and melanoma and was dependent on skewing of macrophage phenotypes.⁴² The mechanism by which anti-MARCO mAb impacts TAM function remains to be determined. However, MARCO⁺ TAMs were found close to tumor cell nests in non-small cell lung cancer patients; co-expressed other TAM immunosuppressive markers, such as PD-L1 and CD163; and were associated with the expression of T cell inhibitory receptors and T cell inhibitory ligands, such as PD-1, PD-L1, CTLA-4, and VISTA.⁴⁰ Thus, combination of MARCO blockade with immunotherapy might be a promising approach in clinical settings.

SIGLEC1

SIGLEC1 (also known as CD169) is a phagocytic receptor of Ig-like lectin family that binds sialic acids and is expressed by normal macrophages and TAMs.⁴³ In humans, SIGLEC1 expression correlated with poor disease prognosis in several cohorts and was one of the top genes with expression enriched in TAMs within breast cancer compared to macrophages from healthy breast tissue.⁴⁴ To address the impact of SIGLEC1⁺ macrophages, a recombinant mouse line has been developed in which the diphtheria toxin receptor is expressed under the control of the *Siglec1* promoter, such that SIGLEC1⁺ macrophages can be eliminated *in vivo* by injection of diphtheria toxin. In a preclinical model of triple-negative breast cancer, selective depletion of SIGLEC1⁺ macrophages was associated with improved tumor control and limited metastases, likely due to more robust CD8 T cell expansion.⁴⁵ *In vitro*, co-culture of breast cancer cell lines with macrophages heightened SIGLEC1 expression and induced PD-L1 expression through the JAK2 signaling pathway, bolstering their immunosuppressive function. While these studies demonstrate the immunosuppressive impact of SIGLEC1⁺ TAMs, it is not yet clear that SIGLEC1 has a direct role in the induction of immunosuppression.

Adenosine receptors

Adenosine is mostly generated through the hydrolysis of adenosine triphosphate (ATP) released to the extracellular space by dying or stressed cells and immune cells.⁴⁶ The cell membrane enzyme CD39 sequentially hydrolyzes ATP to adenosine diphosphate (ADP) and monophosphate (AMP); the ectonucleotidase CD73 then hydrolyzes AMP to adenosine, which signals through G-protein-coupled receptors (A₁R, A_{2A}R, A_{2B}R, and A₃R). CD39, CD73, as well as adenosine receptors are widely expressed in both tumor cells and immune cells in the tumor microenvironment, including macrophages. Extracellular ATP promotes the inflammatory response in several conditions, whereas adenosine signaling has a broad anti-inflammatory effect.⁴⁶ It was reported that adenosine could induce an "M2-like" differentiation of macrophages in the absence of other type 2 cytokines, such as IL-4.⁴⁷ Moreover, A_{2B} receptor signaling inhibited interferon- γ (IFN γ) production in T cells, indirectly affecting macrophage polarization.⁴⁸ Adenosine was also reported to suppress phagocytic activity, NO production, and tumor necrosis factor (TNF) expression of differentiated macrophages *in vitro* and in murine models of bacterial infections.^{49,50} *In vitro* inhibition of CD39 in macrophages promoted IL-1 β production, possibly by preserving ATP and hindering adenosine accumulation.⁵¹ Along these lines, inhibition of CD73 with a small interfering RNA (siRNA) emulsion given intranasally was associated with reduced glioblastoma growth and proportionally fewer CD206⁺ macrophages in a preclinical model.⁵² Although the adenosine pathway

has a broad immunosuppressive effect in the tumor microenvironment,⁵³ specific and possibly inducible ablation of CD73, CD39, and adenosine receptor expression in macrophages will be essential to delineate the impact of adenosine on TAM function.

Ligand-receptor pairs that inhibit phagocytosis

Phagocytosis is triggered by “eat me” signals displayed on micro-organisms and target cells that engage phagocytic receptors on macrophages. Conversely, healthy cells display “do not eat me” signals that engage inhibitory receptors to prevent autoreactivity.⁵⁴ Tumor cells often exploit “do not eat me” signals to escape phagocytosis by macrophages. Thus, mAbs and other immunotherapeutic approaches have been developed to block “do not eat me” signaling pathways and facilitate phagocytosis of tumor cells (Figure 4).

CD47-SIRP1 α pathway

CD47 is a cell surface Ig-like molecule that is expressed in all cells. CD47 associates in *cis* with integrins, broadly contributing to migration, cell-cell fusion, activation, cytokine production, and phagocytosis. Moreover, CD47 acts as a “do not eat me” signal by engaging in *trans* SIRP1 α , a cell surface receptor that is constitutively expressed by macrophages, monocytes, neutrophils, and some dendritic cells.⁵⁵ The cytoplasmic domain of SIRP1 α contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Upon binding CD47, SIRP1 α ITIMs recruit the protein-tyrosine phosphatases SHP1 and SHP2, which transmit intracellular inhibitory signals.⁵⁶ Among various targets, SIRP1 α inhibits nonmuscle myosins that control cytoskeleton architecture, thereby blocking the formation of a phagocytic synapse between macrophages and tumor cells.⁵⁶ In steady-state, CD47-SIRP1 α interaction prevents phagocytosis of red blood cells by splenic macrophages.⁵⁵ However, CD47 overexpression is a mechanism of immune evasion for human solid tumors (e.g., bladder and breast cancer) and hematological malignancies (e.g., myeloid leukemia and non-Hodgkin’s lymphoma).^{57–60} Blockade of CD47 by mAbs *in vitro* restored macrophage phagocytosis of tumor cells.^{59,61,62} *In vivo* treatment of tumors with anti-CD47 mAbs in several humanized and syngeneic mouse models promoted phagocytosis of tumor cells along with immune activation and hence curbed tumor growth.^{61,63,64} mAbs and other molecules targeting the CD47-SIRP1 α axis tested in clinical trials include mAbs blocking CD47 (Hu5F9-G4, SRF231, IBI188, and AO-176), mAbs blocking SIRP1 α (BI-765063), fusion proteins targeting CD47 (TTI-621, TTI-622, and ALX148; NCT03530683; NCT02663518), and bispecific mAbs targeting both CD47 and PD-1. Most of these treatments were well tolerated, and some (alone or in combination) showed a certain degree of efficacy in patients with ovarian cancer, fallopian tube cancer, acute myeloid leukemia, myelodysplastic syndrome, B cell lymphomas, cutaneous T cell lymphoma, late-stage non-small cell lung cancer, and gastric cancer (NCT02367196).^{65–68} In addition to facilitating tumor cell phagocytosis, anti-CD47 mAb treatment promoted ADCP in a model of glioblastoma, as well as ADCC in models of ovarian and breast cancer.^{69–71} Accordingly, anti-CD47 mAbs with a functional Fc domain can be more efficacious than those with a mutated Fc lacking effector functions.⁶⁸ Although current literature and clinical trials support the beneficial effects of targeting the CD47-SIRP1 α pathway, there are also some limitations. First, the broad expression of CD47 by different cell types, including red and white blood cells, can cause off-target sequestration of anti-CD47 mAbs and adverse effects like anemia, neutropenia, and thrombocytopenia.⁶⁶ In this regard, targeting SIRP1 α instead of CD47 might be safer because of the restricted expression of SIRP1 α on myeloid cells.⁷² Second, CD47-SIRP1 α blockade is not always sufficient to impact phagocytosis of tumor cells because of other “do not eat

me" signals coexpressed on tumor cells.⁵⁴ Lastly, because it is ubiquitously expressed throughout the body, CD47 expression cannot be used to stratify patients and predict the response to anti-CD47 therapy.²⁶ Recently, effective induction of phagocytosis of hematological tumors by CD47 blockade was shown to require concomitant adhesion of macrophages to hematopoietic tumor cells through SLAMF7 and integrin α M β 2.⁷³ Thus, a better understanding of the complex mechanisms contributing to CD47-SIRP1 α blockade might help identify patients who would benefit from this therapy.

MHC class I-LILRB1/2 pathway

Major histocompatibility complex (MHC) class I (MHC-I) molecules can act as "do not eat me" signals in cancer by interacting with two members of the human leukocyte Ig-like receptor (LILR) family, LILRB1 (also known as ILT2) and LILRB2 (also known as ILT4). LILRB1 and LILRB2 broadly recognize MHC-I molecules through two binding sites: one site interacts with the α 3 domain of MHC-I heavy chain and another interacts with β 2M.^{74,75} Because the MHC-I α 3 domain and β 2M are highly conserved across species, LILRB1 and LILRB2 recognize both human classical (histocompatibility leukocyte antigen [HLA]-A, -B, and -C) and non-classical (e.g., HLA-E and -G) MHC-I.^{76,77} LILRB1 and LILRB2 contain cytoplasmic ITIMs that recruit SHP1 and SHP2, which mediate inhibition. LILRB1 is expressed by myeloid cells, B cells, subsets of CD8 T cells, and NK cells; LILRB2 is only expressed on myeloid cells.⁷⁸ *In vitro* studies demonstrated that deletion of MHC-I heavy chain or β 2M in tumor cells or LILRB1 deletion in macrophages promotes tumor cell phagocytosis.⁷⁹ In immunodeficient non-obese diabetic (NOD)-*scid* IL2R γ ^{null} mice, human MHC-I-deficient tumors were controlled more efficiently than MHC-I-expressing tumors. In C57BL/6 mice, macrophages more effectively controlled tumors deficient for both MHC-I and CD47 than did their CD47- and/or MHC-I-expressing counterparts.⁷⁹ Thus, concomitant blockade of CD47 and MHC-I can be a promising approach to promote macrophage phagocytosis of tumor cells. In contrast to LILRB1 blockade, LILRB2 blockade did not enhance phagocytosis of tumor cells induced by anti-CD47 treatment *in vitro*.⁷⁹ Perhaps LILRB2 blockade does not facilitate macrophage phagocytosis of tumor cells. However, anti-LILRB2 mAbs did enhance macrophage responses to LPS *in vitro*, inducing TNF upregulation and IL-10 downregulation.⁸⁰ In a mouse model of lung cancer, anti-LILRB2 mAb treatment enhanced the response to immune checkpoint therapy and skewed TAMs toward an immunostimulatory phenotype.⁸⁰ Thus, LILRB2 blockade may promote TAM reprogramming. The mouse counterparts of LILRs are known as paired Ig-like receptors (PIRs).^{81,82} However, PIRs include only one inhibitory receptor that recognizes MHC-I, PIRB, which is broadly expressed on hematopoietic cells. PIRB deficiency was associated with reduced tumor growth in a lung cancer model. Myeloid cells isolated from tumor bearing mice had an "M1-like" signature in the absence of PIRB, characterized by increased expression of *Nos2*, *Tnf*, *Il12*, and *Il1b* and reduced expression of *Arg1* and *Il10*. The discrepancy between the numbers of human and mouse genes encoding inhibitory LILRs and PIRs remains a challenge for translating mouse studies to humans; analysis of humanized mice may be necessary to establish the impact of LILRB1 and LILRB2 *in vivo*.⁸³

CD24-SIGLEC10 pathway

CD24 is a highly glycosylated surface protein expressed on several solid tumors. CD24 binds the inhibitory receptor SIGLEC10, a member of the Ig-like lectin family specific for sialic acids.⁸⁴ SIGLEC10 is expressed on B cells, macrophages, and dendritic cells and contains a cytoplasmic ITIM domain. The inhibitory effect of the CD24-SIGLEC10 pathway has been documented in inflammation, adaptive

immunity, autoimmune diseases, and cancer.^{85–88} Recently, it was observed that CD24 is more abundantly expressed in most tumor types than other “do not eat me” molecules (CD47 and β_2M) and is highly upregulated in ovarian cancer cells, triple-negative, estrogen-receptor-, and progesterone-receptor-positive breast cancer in comparison to the healthy tissue.⁸⁹ Moreover, CD24 expression negatively correlated with disease prognosis and TAMs expressed high levels of SIGLEC10.⁸⁹ CD24 deletion in tumor cell lines and mAb blockade of CD24 promoted phagocytosis of tumor cells *in vitro*; moreover, concomitant blockade of CD47 had a cooperative effect and enhanced phagocytosis. CD24 deficiency and CD24 targeting in tumor models *in vivo* facilitated control of tumor growth by TAMs.⁸⁹ A clinical trial to test the safety and efficacy of CD24 targeting has been developed based on the administration of a fusion protein (CD24-Fc) in combination with anti-CTLA4 and anti-PD-1 in patients with metastatic melanoma, colon cancer, and renal cell carcinoma (NCT04060407).

PD-1-PD-L1 pathway

PD-1 has been extensively characterized as a T cell inhibitory receptor induced by T cell activation that dampens effector functions.⁹⁰ PD-L1 expressed in tumor cells and immune cells in the tumor microenvironment has been shown to strongly inhibit T cells, thereby promoting tumor immune escape.⁹⁰ Accordingly, PD-1-PD-L1 blockade has proven very efficacious in activating T cell responses in different cohorts of cancer patients.^{91,92} Interestingly, it was recently reported that PD-1 is not only expressed by T cells but also by other immune cells, including human and murine TAMs, and hence may have T-cell-independent functions.⁹³ PD-1 expression in TAMs increased during tumor progression in murine models and cancer patients and was negatively associated with macrophage phagocytic activity.⁹³ Moreover, PD-L1 was also expressed by TAMs. PD-1-PD-L1 blockade *in vivo* led to improved macrophage-dependent tumor control and increased phagocytosis of tumor cells in the CT26 model of colorectal carcinoma and a model of osteosarcoma lung metastases.^{93,94} In murine models and patients with colorectal cancer, PD-1 expression in TAMs correlated with expression of markers of immune suppression (e.g., CD206) and weakened phagocytic potential.⁹³ *In vitro*, anti-PD-L1 treatment promoted survival, proliferation, and activation of both human and murine macrophages, corroborating that PD-1-PD-L1 blockade can have a direct effect on macrophages.⁹⁵ Further supporting this conclusion, anti-PD-1 treatment has been widely reported to affect macrophage function and polarization, mitigating their immunosuppressive phenotype.⁹⁶ However, it should be noted that the concomitant restoration of T cell function and IFN γ production by PD-1-PD-L1 blockade may have a considerable indirect effect on TAM phenotype, such that it is difficult to dissect macrophage-intrinsic from macrophage-extrinsic effects of anti-PD-1/PD-L1 blockade in many experimental settings.

Signaling and epigenetic mechanisms that promote immunosuppressive TAMs

PI3K gamma

PI3K is a lipid kinase consisting of a regulatory and a catalytic subunit that phosphorylates phosphatidylinositol 4,5-bisphosphate to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 recruits PH-domain-containing proteins, such as AKT, to the membrane, which activates signaling cascades involved in cell growth, survival, proliferation, motility, and morphology. PI3K impacts macrophage differentiation and function.⁹⁷ The gamma isoform of PI3K catalytic subunit (PI3K γ), which is highly expressed in the tumor microenvironment, is a leukocyte-specific lipid kinase and promotes a macrophage immunosuppressive phenotype.⁹⁸ Genetic deletion or pharmacological inhibition of PI3K γ in mouse tumor models broadly affected TAM

number and phenotype: expression of MHCII and production of pro-inflammatory IL-12 increased, whereas expression of immunosuppressive markers (i.e., arginase, CD206, and PD-L1) and secretion of anti-inflammatory IL-10 declined,^{99,100} as did VEGF production and consequent neoangiogenesis.¹⁰⁰ TAM reprogramming was associated with enhanced T cell activation, T cell recruitment into the tumor, tumor control, and response to anti-PD-1 in several models, including breast and lung cancer, as well as head and neck squamous cell carcinoma.⁹⁹ Given the efficacy of PI3K γ inhibitors in preclinical models, several strategies have been developed to inhibit PI3K γ in the clinic. To date, ongoing clinical trials to target PI3K γ have tested the PI3K γ inhibitor IPI-549 alone or in combination with adenosine receptor antagonists, chemotherapy drugs (e.g., paclitaxel and doxorubicin), or anti-VEGF in triple-negative breast cancer, ovarian, and renal cancer patients. IPI-549 in combination with anti-PD-1 has been tested in non-small cell lung cancer, head and neck squamous cell carcinoma, triple-negative breast cancer, bladder cancer, and urothelial carcinoma (NCT03719326; NCT03961698; NCT02637531; NCT03980041). In a model of pancreatic cancer, it was observed that protein-tyrosine kinase BTK induced PI3K γ activation and immunosuppression in TAMs. Blockade of either BTK or PI3K γ was protective in the model, and BTK blockade is currently under evaluation in a clinical trial in pancreatic adenocarcinoma patients (NCT02052492).¹⁰¹

Histone deacetylases (HDACs)

HDACs are enzymes that mediate epigenetic regulation of gene expression by removal of acetyl groups from an ϵ -N-acetyl lysine on histones.¹⁰² Abnormal HDAC expression patterns occur in several types of cancer and correlate with survival disadvantage in patients. HDAC blockade has been shown to be a promising strategy to help control aberrant gene expression in tumors, and HDAC inhibitors have been tested in several clinical trials.¹⁰³ Some studies have specifically addressed the impact of HDAC inhibitors on TAMs. TMP195 is an HDAC inhibitor that was shown to target TAMs and inhibit primary tumor growth and lung metastatization in a breast cancer model. The administration of TMP195 promoted monocyte recruitment and skewed macrophages toward a highly phagocytic phenotype.¹⁰³ TMP195 treatment tempered TAM production of CCL2, a chemoattractant for monocytes, whereas CCL1 production was elevated.¹⁰⁴ TMP195 was also tested in preclinical models in combination with chemotherapy or anti-PD-1 and shown to enhance response to treatments.¹⁰³ Treatment with HDAC inhibitors was also associated with poor recruitment of immunosuppressive macrophage subsets to the metastatic niche in preclinical models,¹⁰⁵ suggesting that HDACs may have a beneficial effect in metastatic disease.

Prostaglandin E₂ (PGE₂)

PGE₂ is a product of arachidonic acid metabolism synthesized by the cyclo-oxygenase (COX) enzymes. PGE₂ is sensed by a family of G-coupled receptors (EP1–4) and was reported to promote tumor growth by directly impacting tumor cell proliferation, survival, and migration. Moreover, PGE₂ was shown to promote the establishment of an immunosuppressive microenvironment by inhibiting T and NK cell activation.^{106–108} TAMs express EP receptors,¹⁰⁹ and there is growing evidence that PGE₂ signaling in TAMs can contribute to skewing toward a pro-tumoral phenotype.¹⁰⁶ In a model of mammary carcinoma, EP-2-deficient mice controlled tumors better than wild-type (WT) controls, and this was associated with a reduction of immunosuppressive myeloid cells. *In vitro*, it was observed that PGE₂ can directly impact bone marrow myeloid progenitors, driving the differentiation of suppressor cells.¹⁰⁹ Given the protective effect of aspirin and other nonsteroidal anti-inflammatory drugs that target PGE₂ in cancer, macrophages may contribute to this effect. Indeed, the treatment of tumor-bearing mice with a COX-2 inhibitor resulted in a

lower tumor burden, and this was associated with proportionally fewer suppressive myeloid cells.¹⁰⁹ Recently, it was shown that PGE₂ led to the enrichment of p50-nuclear factor κ B (NF- κ B) in monocyte-related MDSCs within tumors in a sarcoma model; in turn, this engendered defective IFN γ responsiveness and an immunosuppressive signature in the monocyte-related MDSCs.¹¹⁰ Furthermore, it was reported that PGE₂ might also contribute to macrophage recruitment into the tumor in a mouse model of glioma; COX-2 inhibition was associated with lower CCL2 levels in the tumor and consequent ineffectual accumulation of myeloid cells.¹¹¹ Because PGE₂ acts on multiple cell types, it can have a broad effect in the tumor microenvironment; therefore, delineating the direct contribution of PGE₂ sensing in TAMs *in vivo* requires further investigation.

Reprogramming immunosuppressive TAMs through activating receptors

Certain cell surface receptors expressed on immunosuppressive TAMs can deliver intracellular signals that override immunosuppression and skew TAMs toward immunostimulation (Figure 4). Most of these receptors are not unique to macrophages but are also expressed in other immune and non-immune cells. One prototypic example is the TNF receptor family member CD40. Engagement of CD40 with mAbs has been extensively tested and shown to induce activation and cytokine production in dendritic cells (DCs), as well as enhancing their ability to sustain T cell activation. In a pancreatic ductal adenocarcinoma model, a CD40 agonistic mAb stimulated TAMs to secrete high amounts of MMP13, which degraded fibrotic tissue and facilitated control of tumor growth.^{112,113} Among several mAbs targeting CD40 tested in clinical trials with promising outcomes, some were shown to have a direct effect on TAMs. Activation of toll-like receptors (TLRs) can also overcome TAM immunosuppression. Numerous TLR synthetic ligands have been tested in tumor models and clinical studies, and some were shown to skew TAMs toward an anti-tumoral phenotype.^{114–120} In a melanoma model, local treatment with a TLR7/TLR8 agonist was associated with a modulation of macrophage phenotype that promoted tumor regression.^{121,122} Finally, the prototypic cytokine that drives M1-like macrophage polarization, IFN γ , was directly used to reprogram macrophages. *In vivo* administration IFN γ was tested in tumor models, and IFN γ was injected intraperitoneally in patients with ovarian cancer, leading to favorable responses associated with increased immune activation.¹²³ One important limitation of all described agonists of activating pathways is that they act on multiple cells in addition to TAMs, causing non-specific side effects. Thus, it is likely that these therapeutic agents may be used as adjuvants in combination with other approaches rather than stand-alone therapies.

One recently developed avenue to induce specific activation of TAMs is the transduction of chimeric antigen receptors (CARs). CARs consist of a single-chain variable fragment antibody specific for a tumor antigen, which is fused to a transmembrane domain that anchors the antibody to the cell membrane, followed by intracellular domains that transmit activation and costimulatory signals.¹²⁴ Autologous T cells transduced with CARs (CAR-Ts) acquire tumor specificity as well as signals that drive full T cell activation. CAR-T cell therapy has been very effective in hematological malignancies, whereas success in solid tumors has been limited.¹²⁵ Macrophages may be a valid alternative to T cells as recipient of CARs, because of their capacity to infiltrate tumors. Recent preclinical studies have addressed the potential anti-tumor impact of macrophages engineered to express a CAR targeting a tumor cell antigen (CAR-Ms).¹²⁶ The human macrophage cell line THP-1 was transduced with a first-generation CAR targeting CD19 and was shown to acquire high phagocytic potential against CD19⁺ K562 tumor cells. Primary monocyte-derived macrophages were also engineered to express an anti-HER2 CAR, which promoted phagocytosis of an HER2-expressing tumor cell line.¹²⁶

In vivo, treatment with CAR-Ms was protective in a HER2⁺ ovarian cancer model.^{126,127} Interestingly, it was observed that, regardless of CAR expression, transduced macrophages displayed a pro-inflammatory phenotype that facilitated anti-tumor response, perhaps reflecting an adjuvant effect.¹²⁶ Given the promising results using CAR-Ms, it will be important to test different tumor antigens.

Conclusions

As macrophage-targeting therapies are emerging as potential strategies to reprogram immunosuppressive TAMs, some important questions must be addressed. Which molecules should be chosen as targets? Which patients might benefit from TAM reprogramming? Should TAM-based therapies be used alone or in combination with either checkpoint blockade therapies or chemotherapies? Answering these questions requires a detailed knowledge of the TAM landscape in the tumor of interest. Single-cell technologies developed in the last decade are providing a granular view of the macrophage phenotypic profiles in many human tumors and mouse models. One important result of these studies is the demonstration of the great heterogeneity of TAMs. Multiple factors can imprint TAMs, including the tumor type, the tissue in which the tumor develops, and the presence of immune and stromal cells within the tumor. A detailed knowledge of TAM phenotype and function will help to stratify patients and choose the most appropriate TAM-oriented therapies. Single-cell technologies can further boost the discovery of novel targets for immunotherapy and the identification of TAM markers predictive of treatment outcome and prognosis. Various studies have suggested that TAMs may have different origins: some may originate from resident macrophages that seed the tissue during fetal hematopoiesis, whereas others may originate from bone-marrow-derived blood monocytes.¹²⁸ The recent development of fate map mice that can trace macrophages of bone marrow origin based on the expression of membrane-spanning 4-domains subfamily A member 3 (Ms4A3) will help delineate how the developmental origin of TAMs influences their response to tumor growth.¹²⁹ Given the burgeoning availability of multi-omics data on the heterogeneous origins, phenotypes, and functions of TAMs in human tumors, together with the data from clinical trials of TAM therapies in combination with checkpoint blockade therapies, artificial intelligence/machine learning analyses will be required to identify TAM pathways that can be successfully targeted in human cancer.

It is predictable that future studies will also delve into investigation of the complex and different mechanisms by which TAMs control immune responses, which remain only partially understood. A recent study identified the tetraspanin MS4A4A as an M2-like pro-tumoral marker that is induced in macrophages by IL-4 or glucocorticoid hormones.¹³⁰ In mouse metastasis models, MS4A4A deficiency in macrophages impaired signaling of the surface receptor DECTIN1, which promotes the ability of macrophages to activate NK cells. Thus, MS4A4A deficiency ultimately undermined NK-cell-mediated resistance to metastases.¹³⁰ In another study, APOE emerged as a biomarker of prognosis and response to therapy in melanoma patients.¹³¹ Of the three allelic APOE variants (APOE2, APOE3, and APOE4), APOE4 was associated with attenuated disease progression and an improved response to anti-PD1. The beneficial effect of APOE4 on tumor control was recapitulated in a mouse model expressing the same human variant and was associated with fewer MDSCs in the tumor. Remarkably, APOE is a TREM2 ligand, and both APOE and TREM2 variants are genetically linked to Alzheimer's disease.³⁰ Thus, the role of APOE4 in tumors may be linked to that of TREM2 in TAMs.¹³¹ We envision that the complexity of mechanisms through which TAMs control anti-tumor immune responses will reflect the diversity of TAMs infiltrating the tumors.

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DECLARATION OF INTERESTS

M.C. receives research support from Pfizer and NGM Biotechnology, is a scientific advisory board member of NGM Biotechnology and Vigil, is a consultant for Cell Signaling Technologies, and has a patent for TREM2 pending.

REFERENCES

- Mantovani, A., Marchesi, F., Malesci, A., Laghi, L., and Allavena, P. (2017). Tumour-associated macrophages as treatment targets in oncology. *Nat. Rev. Clin. Oncol.* 14, 399–416.
- Weiskopf, K., and Weissman, I.L. (2015). Macrophages are critical effectors of antibody therapies for cancer. *MAbs* 7, 303–310.
- O'Neill, L.A., and Pearce, E.J. (2016). Immunometabolism governs dendritic cell and macrophage function. *J. Exp. Med.* 213, 15–23.
- Murray, P.J., Allen, J.E., Biswas, S.K., Fisher, E.A., Gilroy, D.W., Goerdt, S., Gordon, S., Hamilton, J.A., Ivashkiv, L.B., Lawrence, T., et al. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 41, 14–20.
- Cassetta, L., and Pollard, J.W. (2018). Targeting macrophages: therapeutic approaches in cancer. *Nat. Rev. Drug Discov.* 17, 887–904.
- Bottazzi, B., Polentarutti, N., Acero, R., Balsari, A., Boraschi, D., Ghezzi, P., Salmons, M., and Mantovani, A. (1983). Regulation of the macrophage content of neoplasms by chemoattractants. *Science* 220, 210–212.
- Müller, S., Kohanbash, G., Liu, S.J., Alvarado, B., Carrera, D., Bhaduri, A., Watchmaker, P.B., Yagnik, G., Di Lullo, E., Malatesta, M., et al. (2017). Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis for regional differences in macrophage activation in the tumor microenvironment. *Genome Biol.* 18, 234.
- Franklin, R.A., Liao, W., Sarkar, A., Kim, M.V., Bivona, M.R., Liu, K., Pamer, E.G., and Li, M.O. (2014). The cellular and molecular origin of tumor-associated macrophages. *Science* 344, 921–925.
- Qian, B.Z., Li, J., Zhang, H., Kitamura, T., Zhang, J., Campion, L.R., Kaiser, E.A., Snyder, L.A., and Pollard, J.W. (2011). CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. *Nature* 475, 222–225.
- Loyher, P.L., Hamon, P., Laviron, M., Meghraoui-Kheddar, A., Goncalves, E., Deng, Z., Torstensson, S., Bercovici, N., Baudesson de Chanville, C., Combadière, B., et al. (2018). Macrophages of distinct origins contribute to tumor development in the lung. *J. Exp. Med.* 215, 2536–2553.
- Zhu, Y., Herndon, J.M., Sojka, D.K., Kim, K.W., Knolhoff, B.L., Zuo, C., Cullinan, D.R., Luo, J., Bearden, A.R., Lavine, K.J., et al. (2017). Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* 47, 597.
- Kwak, T., Wang, F., Deng, H., Condamine, T., Kumar, V., Perego, M., Kossenkova, A., Montaner, L.J., Xu, X., Xu, W., et al. (2020). Distinct populations of immune-suppressive macrophages differentiate from monocytic myeloid-derived suppressor cells in cancer. *Cell Rep.* 33, 108571.
- Sharma, A., Seow, J.J.W., Dutertre, C.A., Pai, R., Blériot, C., Mishra, A., Wong, R.M.M., Singh, G.S.N., Sudhagar, S., Khalilnezhad, S., et al. (2020). Onco-fetal reprogramming of endothelial cells drives immunosuppressive macrophages in hepatocellular carcinoma. *Cell* 183, 377–394.e21.
- Haldar, M., and Murphy, K.M. (2014). Origin, development, and homeostasis of tissue-resident macrophages. *Immunol. Rev.* 262, 25–35.
- Bronte, V., Brandau, S., Chen, S.H., Colombo, M.P., Frey, A.B., Greten, T.F., Mandruzzato, S., Murray, P.J., Ochoa, A., Ostrand-Rosenberg, S., et al. (2016). Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat. Commun.* 7, 12150.
- Zhao, X., Qu, J., Sun, Y., Wang, J., Liu, X., Wang, F., Zhang, H., Wang, W., Ma, X., Gao, X., and Zhang, S. (2017). Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. *Oncotarget* 8, 30576–30586.
- Zhang, J., Chang, L., Zhang, X., Zhou, Z., and Gao, Y. (2021). Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in hepatocellular carcinoma. *J. Invest. Surg.* 34, 297–306.
- Yuan, X., Zhang, J., Li, D., Mao, Y., Mo, F., Du, W., and Ma, X. (2017). Prognostic significance of tumor-associated macrophages in ovarian cancer: a meta-analysis. *Gynecol. Oncol.* 147, 181–187.
- Yin, S., Huang, J., Li, Z., Zhang, J., Luo, J., Lu, C., Xu, H., and Xu, H. (2017). The prognostic and clinicopathological significance of tumor-associated macrophages in patients with gastric cancer: a meta-analysis. *PLoS ONE* 12, e0170042.
- Yang, Z., Zhang, M., Peng, R., Liu, J., Wang, F., Li, Y., Zhao, Q., and Liu, J. (2020). The prognostic and clinicopathological value of tumor-associated macrophages in patients with colorectal cancer: a systematic review and meta-analysis. *Int. J. Colorectal Dis.* 35, 1651–1661.
- Bonapace, L., Coissieux, M.M., Wyckoff, J., Mertz, K.D., Varga, Z., Junt, T., and Bentières-Alj, M. (2014). Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. *Nature* 515, 130–133.
- Loberg, R.D., Ying, C., Craig, M., Day, L.L., Sargent, E., Neeley, C., Wojno, K., Snyder, L.A., Yan, L., and Pienta, K.J. (2007). Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. *Cancer Res.* 67, 9417–9424.
- DeNardo, D.G., Brennan, D.J., Rexhepaj, E., Ruffell, B., Shiao, S.L., Madden, S.F., Gallagher, W.M., Wadhwani, N., Keil, S.D., Junaid, S.A., et al. (2011). Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov.* 1, 54–67.
- Pyonteck, S.M., Akkari, L., Schuhmacher, A.J., Bowman, R.L., Sevenich, L., Quail, D.F., Olson, O.C., Quick, M.L., Huse, J.T., Teijeiro, V., et al. (2013). CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat. Med.* 19, 1264–1272.
- Banerjee, P., Zhang, R., Ivan, C., Galletti, G., Clise-Dwyer, K., Barbaglio, F., Scarfò, L., Aracil, M., Klein, C., Wierda, W., et al. (2019). Trabectedin reveals a strategy of immunomodulation in chronic lymphocytic leukemia. *Cancer Immunol. Res.* 7, 2036–2051.
- Majety, M., Runza, V., Lehmann, C., Hoves, S., and Ries, C.H. (2018). A drug development perspective on targeting tumor-associated myeloid cells. *FEBS J.* 285, 763–776.
- Ulland, T.K., Song, W.M., Huang, S.C., Ulrich, J.D., Sergushichev, A., Beatty, W.L., Loboda, A.A., Zhou, Y., Cairns, N.J., Kambal, A., et al. (2017). TREM2 maintains microglial metabolic

- fitness in Alzheimer's disease. *Cell* 170, 649–663.e13.
28. Peng, Q., Malhotra, S., Torchia, J.A., Kerr, W.G., Coggeshall, K.M., and Humphrey, M.B. (2010). TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1. *Sci. Signal.* 3, ra38.
29. Otero, K., Shinohara, M., Zhao, H., Cella, M., Gilfillan, S., Colucci, A., Faccio, R., Ross, F.P., Teitelbaum, S.L., Takayanagi, H., and Colonna, M. (2012). TREM2 and β -catenin regulate bone homeostasis by controlling the rate of osteoclastogenesis. *J. Immunol.* 188, 2612–2621.
30. Ulland, T.K., and Colonna, M. (2018). TREM2 - a key player in microglial biology and Alzheimer disease. *Nat. Rev. Neurol.* 14, 667–675.
31. Jaitin, D.A., Adlung, L., Thaiss, C.A., Weiner, A., Li, B., Descamps, H., Lundgren, P., Blieriot, C., Liu, Z., Deczkowska, A., et al. (2019). Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell* 178, 686–698.e14.
32. Daemen, S., Gainullina, A., Kalugotla, G., He, L., Chan, M.M., Beals, J.W., Liss, K.H., Klein, S., Feldstein, A.E., Finck, B.N., et al. (2021). Dynamic shifts in the composition of resident and recruited macrophages influence tissue remodeling in NASH. *Cell Rep.* 34, 108626.
33. Ramachandran, P., Dobie, R., Wilson-Kanamori, J.R., Dora, E.F., Henderson, B.E.P., Luu, N.T., Portman, J.R., Matchett, K.P., Brice, M., Marwick, J.A., et al. (2019). Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature* 575, 512–518.
34. Hou, J., Zhang, J., Cui, P., Zhou, Y., Liu, C., Wu, X., Ji, Y., Wang, S., Cheng, B., Ye, H., et al. (2021). TREM2 sustains macrophage-hepatocyte metabolic coordination in nonalcoholic fatty liver disease and sepsis. *J. Clin. Invest.* 131, 135197.
35. Cochain, C., Vafadarnejad, E., Arampatzis, P., Pelisek, J., Winkels, H., Ley, K., Wolf, D., Saliba, A.E., and Zernecke, A. (2018). Single-cell RNA-seq reveals the transcriptional landscape and heterogeneity of aortic macrophages in murine atherosclerosis. *Circ. Res.* 122, 1661–1674.
36. Song, Q., Hawkins, G.A., Wudel, L., Chou, P.C., Forbes, E., Pullikuth, A.K., Liu, L., Jin, G., Craddock, L., Topaloglu, U., et al. (2019). Dissecting intratumoral myeloid cell plasticity by single cell RNA-seq. *Cancer Med.* 8, 3072–3085.
37. Lavin, Y., Kobayashi, S., Leader, A., Amir, E.D., Elefant, N., Bigenwald, C., Remark, R., Sweeney, R., Becker, C.D., Levine, J.H., et al. (2017). Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. *Cell* 169, 750–765.e17.
38. Katzenelenbogen, Y., Sheban, F., Yalin, A., Yofe, I., Svetlichnyy, D., Jaitin, D.A., Bornstein, C., Moshe, A., Keren-Shaul, H., Cohen, M., et al. (2020). Coupled scRNA-seq and intracellular protein activity reveal an immunosuppressive role of TREM2 in cancer. *Cell* 182, 872–885.e19.
39. Molgora, M., Esaulova, E., Vermi, W., Hou, J., Chen, Y., Luo, J., Brioschi, S., Bugatti, M., Omodei, A.S., Ricci, B., et al. (2020). TREM2 modulation remodels the tumor myeloid landscape enhancing anti-PD-1 immunotherapy. *Cell* 182, 886–900.e17.
40. La Fleur, L., Boura, V.F., Alexeyenko, A., Berglund, A., Pontén, V., Mattsson, J.S.M., Djureinovic, D., Persson, J., Brunnström, H., Isaksson, J., et al. (2018). Expression of scavenger receptor MARCO defines a targetable tumor-associated macrophage subset in non-small cell lung cancer. *Int. J. Cancer* 143, 1741–1752.
41. Kraal, G., van der Laan, L.J., Elomaa, O., and Tryggvason, K. (2000). The macrophage receptor MARCO. *Microbes Infect.* 2, 313–316.
42. Georgoudaki, A.M., Prokopec, K.E., Boura, V.F., Hellqvist, E., Sohn, S., Östling, J., Dahan, R., Harris, R.A., Rantalainen, M., Klevebring, D., et al. (2016). Reprogramming tumor-associated macrophages by antibody targeting inhibits cancer progression and metastasis. *Cell Rep.* 15, 2000–2011.
43. O'Neill, A.S., van den Berg, T.K., and Mullen, G.E. (2013). Sialoadhesin - a macrophage-restricted marker of immunoregulation and inflammation. *Immunology* 138, 198–207.
44. Cassetta, L., Fragkogianni, S., Sims, A.H., Swierczak, A., Forrester, L.M., Zhang, H., Soong, D.Y.H., Cotecchini, T., Anur, P., Lin, E.Y., et al. (2019). Human tumor-associated macrophage and monocyte transcriptional landscapes reveal cancer-specific reprogramming, biomarkers, and therapeutic targets. *Cancer Cell* 35, 588–602.e10.
45. Jing, W., Guo, X., Wang, G., Bi, Y., Han, L., Zhu, Q., Qiu, C., Tanaka, M., and Zhao, Y. (2020). Breast cancer cells promote CD169⁺ macrophage-associated immunosuppression through JAK2-mediated PD-L1 upregulation on macrophages. *Int. Immunopharmacol.* 78, 106012.
46. Strakhova, R., Cadassou, O., Cros-Perrial, E., and Jordheim, L.P. (2020). Regulation of tumor infiltrated innate immune cells by adenosine. *Purinergic Signal.* 16, 289–295.
47. Ferrante, C.J., Pinhal-Enfield, G., Elson, G., Cronstein, B.N., Hasko, G., Outram, S., and Leibovich, S.J. (2013). The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4R α) signaling. *Inflammation* 36, 921–931.
48. Xaus, J., Mirabet, M., Lloberas, J., Soler, C., Lluís, C., Franco, R., and Celada, A. (1999). IFN-gamma up-regulates the A2B adenosine receptor expression in macrophages: a mechanism of macrophage deactivation. *J. Immunol.* 162, 3607–3614.
49. Haskó, G., Szabó, C., Németh, Z.H., Kvetan, V., Pastores, S.M., and Vizi, E.S. (1996). Adenosine receptor agonists differentially regulate IL-10, TNF- α , and nitric oxide production in RAW 264.7 macrophages and in endotoxemic mice. *J. Immunol.* 157, 4634–4640.
50. Belikoff, B.G., Hatfield, S., Georgiev, P., Ohta, A., Lukashev, D., Buras, J.A., Remick, D.G., and Sitkovsky, M. (2011). A2B adenosine receptor blockade enhances macrophage-mediated bacterial phagocytosis and improves polymicrobial sepsis survival in mice. *J. Immunol.* 186, 2444–2453.
51. Perrot, I., Michaud, H.A., Giraudon-Paoli, M., Augier, S., Docquier, A., Gros, L., Courtois, R., Déjou, C., Jecko, D., Becquart, O., et al. (2019). Blocking antibodies targeting the CD39/CD73 immunosuppressive pathway unleash immune responses in combination cancer therapies. *Cell Rep.* 27, 2411–2425.e9.
52. Azambuja, J.H., Schuh, R.S., Michels, L.R., Iser, I.C., Beckenkamp, L.R., Roliano, G.G., Lenz, G.S., Scholl, J.N., Sévigny, J., Wink, M.R., et al. (2020). Blockade of CD73 delays glioblastoma growth by modulating the immune environment. *Cancer Immunol. Immunother.* 69, 1801–1812.
53. Antonoli, L., Pacher, P., Vizi, E.S., and Haskó, G. (2013). CD39 and CD73 in immunity and inflammation. *Trends Mol. Med.* 19, 355–367.
54. Hochreiter-Hufford, A., and Ravichandran, K.S. (2013). Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. *Cold Spring Harb. Perspect. Biol.* 5, a008748.
55. Oldenborg, P.A., Zheleznyak, A., Fang, Y.F., Lagenaur, C.F., Gresham, H.D., and Lindberg, F.P. (2000). Role of CD47 as a marker of self on red blood cells. *Science* 288, 2051–2054.
56. Barclay, A.N., and Van den Berg, T.K. (2014). The interaction between signal regulatory protein alpha (SIRP α) and CD47: structure, function, and therapeutic target. *Annu. Rev. Immunol.* 32, 25–50.
57. Manna, P.P., and Frazier, W.A. (2004). CD47 mediates killing of breast tumor cells via Gi-dependent inhibition of protein kinase A. *Cancer Res.* 64, 1026–1036.
58. Kim, M.J., Lee, J.C., Lee, J.J., Kim, S., Lee, S.G., Park, S.W., Sung, M.W., and Heo, D.S. (2008). Association of CD47 with natural killer cell-mediated cytotoxicity of head-and-neck squamous cell carcinoma lines. *Tumour Biol.* 29, 28–34.
59. Jaiswal, S., Jamieson, C.H., Pang, W.W., Park, C.Y., Chao, M.P., Majeti, R., Traver, D., van Rooijen, N., and Weissman, I.L. (2009). CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. *Cell* 138, 271–285.
60. Chan, K.S., Espinosa, I., Chao, M., Wong, D., Ailles, L., Diehn, M., Gill, H., Presti, J., Jr., Chang, H.Y., van de Rijn, M., et al. (2009). Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc. Natl. Acad. Sci. USA* 106, 14016–14021.
61. Chao, M.P., Alizadeh, A.A., Tang, C., Myklebust, J.H., Varghese, B., Gill, S., Jan, M., Cha, A.C., Chan, C.K., Tan, B.T., et al. (2010). Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma. *Cell* 142, 699–713.
62. Majeti, R., Chao, M.P., Alizadeh, A.A., Pang, W.W., Jaiswal, S., Gibbs, K.D., Jr., van Rooijen, N., and Weissman, I.L. (2009). CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 138, 286–299.

63. Xiao, Z., Chung, H., Banan, B., Manning, P.T., Ott, K.C., Lin, S., Capoccia, B.J., Subramanian, V., Hiebsch, R.R., Upadhy, G.A., et al. (2015). Antibody mediated therapy targeting CD47 inhibits tumor progression of hepatocellular carcinoma. *Cancer Lett.* 360, 302–309.
64. Willingham, S.B., Volkmer, J.P., Gentles, A.J., Sahoo, D., Dalerba, P., Mitra, S.S., Wang, J., Contreras-Trujillo, H., Martin, R., Cohen, J.D., et al. (2012). The CD47-signal regulatory protein alpha (SIRPα) interaction is a therapeutic target for human solid tumors. *Proc. Natl. Acad. Sci. USA* 109, 6662–6667.
65. Swoboda, D.M., and Sallman, D.A. (2020). The promise of macrophage directed checkpoint inhibitors in myeloid malignancies. *Best Pract. Res. Clin. Haematol.* 33, 101221.
66. Sikic, B.I., Lakhani, N., Patnaik, A., Shah, S.A., Chandana, S.R., Rasco, D., Colevas, A.D., O'Rourke, T., Narayanan, S., Papadopoulos, K., et al. (2019). First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. *J. Clin. Oncol.* 37, 946–953.
67. Lopez-Yrigoyen, M., Cassetta, L., and Pollard, J.W. (2020). Macrophage targeting in cancer. *Ann. N Y Acad. Sci.* Published online May 22, 2020. <https://doi.org/10.1111/nyas.14377>.
68. Huang, Y., Ma, Y., Gao, P., and Yao, Z. (2017). Targeting CD47: the achievements and concerns of current studies on cancer immunotherapy. *J. Thorac. Dis.* 9, E168–E174.
69. Zhao, X.W., van Beek, E.M., Schornagel, K., Van der Maaden, H., Van Houdt, M., Otten, M.A., Finetti, P., Van Egmond, M., Matozaki, T., Kraal, G., et al. (2011). CD47-signal regulatory protein-α (SIRPα) interactions form a barrier for antibody-mediated tumor cell destruction. *Proc. Natl. Acad. Sci. USA* 108, 18342–18347.
70. Liu, R., Wei, H., Gao, P., Yu, H., Wang, K., Fu, Z., Ju, B., Zhao, M., Dong, S., Li, Z., et al. (2017). CD47 promotes ovarian cancer progression by inhibiting macrophage phagocytosis. *Oncotarget* 8, 39021–39032.
71. Cheung, A., Opzomer, J., Ilieva, K.M., Gazinska, P., Hoffmann, R.M., Mirza, H., Marlow, R., Francesch-Domenech, E., Fittall, M., Dominguez Rodriguez, D., et al. (2018). Anti-folate receptor alpha-directed antibody therapies restrict the growth of triple-negative breast cancer. *Clin. Cancer Res.* 24, 5098–5111.
72. Yanagita, T., Murata, Y., Tanaka, D., Motegi, S.I., Arai, E., Daniwijaya, E.W., Hazama, D., Washio, K., Saito, Y., Kotani, T., et al. (2017). Anti-SIRPα antibodies as a potential new tool for cancer immunotherapy. *JCI Insight* 2, e89140.
73. Chen, J., Zhong, M.C., Guo, H., Davidson, D., Mishel, S., Lu, Y., Rhee, I., Pérez-Quintero, L.A., Zhang, S., Cruz-Munoz, M.E., et al. (2017). SLAMF7 is critical for phagocytosis of haematopoietic tumour cells via Mac-1 integrin. *Nature* 544, 493–497.
74. Kuroki, K., Matsubara, H., Kanda, R., Miyashita, N., Shiroishi, M., Fukunaga, Y., Kamishikiri, J., Fukunaga, A., Fukuhara, H., Hirose, K., et al. (2019). Structural and functional basis for LILRB immune checkpoint receptor recognition of HLA-G isoforms. *J. Immunol.* 203, 3386–3394.
75. Willcox, B.E., Thomas, L.M., and Bjorkman, P.J. (2003). Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor. *Nat. Immunol.* 4, 913–919.
76. Colonna, M., Navarro, F., Bellón, T., Llano, M., García, P., Samaridis, J., Angman, L., Cella, M., and López-Botet, M. (1997). A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *J. Exp. Med.* 186, 1809–1818.
77. Allan, D.S., McMichael, A.J., and Braud, V.M. (2000). The ILT family of leukocyte receptors. *Immunobiology* 202, 34–41.
78. Cella, M., Nakajima, H., Facchetti, F., Hoffmann, T., and Colonna, M. (2000). ILT receptors at the interface between lymphoid and myeloid cells. *Curr. Top. Microbiol. Immunol.* 251, 161–166.
79. Barkal, A.A., Weiskopf, K., Kao, K.S., Gordon, S.R., Rosental, B., Yiu, Y.Y., George, B.M., Markovic, M., Ring, N.G., Tsai, J.M., et al. (2018). Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. *Nat. Immunol.* 19, 76–84.
80. Chen, H.M., van der Touw, W., Wang, Y.S., Kang, K., Mai, S., Zhang, J., Alsina-Beauchamp, D., Duty, J.A., Mungamuri, S.K., Zhang, B., et al. (2018). Blocking immunoinhibitory receptor LILRB2 reprograms tumor-associated myeloid cells and promotes antitumor immunity. *J. Clin. Invest.* 128, 5647–5662.
81. Takai, T. (2005). Paired immunoglobulin-like receptors and their MHC class I recognition. *Immunology* 115, 433–440.
82. Kubagawa, H., Burrows, P.D., and Cooper, M.D. (1997). A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. *Proc. Natl. Acad. Sci. USA* 94, 5261–5266.
83. Ma, G., Pan, P.Y., Eisenstein, S., Divino, C.M., Lowell, C.A., Takai, T., and Chen, S.H. (2011). Paired immunoglobulin-like receptor-B regulates the suppressive function and fate of myeloid-derived suppressor cells. *Immunity* 34, 385–395.
84. Crocker, P.R., Paulson, J.C., and Varki, A. (2007). Siglecs and their roles in the immune system. *Nat. Rev. Immunol.* 7, 255–266.
85. Chen, W., Han, C., Xie, B., Hu, X., Yu, Q., Shi, L., Wang, Q., Li, D., Wang, J., Zheng, P., et al. (2013). Induction of Siglec-G by RNA viruses inhibits the innate immune response by promoting RIG-I degradation. *Cell* 152, 467–478.
86. Chen, G.Y., Chen, X., King, S., Cavassani, K.A., Cheng, J., Zheng, X., Cao, H., Yu, H., Qu, J., Fang, D., et al. (2011). Amelioration of sepsis by inhibiting sialidase-mediated disruption of the CD24-SiglecG interaction. *Nat. Biotechnol.* 29, 428–435.
87. Chen, G.Y., Tang, J., Zheng, P., and Liu, Y. (2009). CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. *Science* 323, 1722–1725.
88. Toubai, T., Hou, G., Mathewson, N., Liu, C., Wang, Y., Oravecz-Wilson, K., Cummings, E., Rossi, C., Evers, R., Sun, Y., et al. (2014). Siglec-G-CD24 axis controls the severity of graft-versus-host disease in mice. *Blood* 123, 3512–3523.
89. Barkal, A.A., Brewer, R.E., Markovic, M., Kowarsky, M., Barkal, S.A., Zaro, B.W., Krishnan, V., Hatakeyama, J., Dorigo, O., Barkal, L.J., and Weissman, I.L. (2019). CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* 572, 392–396.
90. Baumeister, S.H., Freeman, G.J., Dranoff, G., and Sharpe, A.H. (2016). Coinhibitory pathways in immunotherapy for cancer. *Annu. Rev. Immunol.* 34, 539–573.
91. Zou, W., Wolchok, J.D., and Chen, L. (2016). PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci. Transl. Med.* 8, 328rv4.
92. Topalian, S.L., Hodi, F.S., Brahmer, J.R., Gettinger, S.N., Smith, D.C., McDermott, D.F., Powderly, J.D., Sosman, J.A., Atkins, M.B., Leming, P.D., et al. (2019). Five-year survival and correlates among patients with advanced melanoma, renal cell carcinoma, or non-small cell lung cancer treated with nivolumab. *JAMA Oncol.* 5, 1411–1420.
93. Gordon, S.R., Maute, R.L., Dulken, B.W., Hutter, G., George, B.M., McCracken, M.N., Gupta, R., Tsai, J.M., Sinha, R., Corey, D., et al. (2017). PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 545, 495–499.
94. Dhupkar, P., Gordon, N., Stewart, J., and Kleinerman, E.S. (2018). Anti-PD-1 therapy redirects macrophages from an M2 to an M1 phenotype inducing regression of OS lung metastases. *Cancer Med.* 7, 2654–2664.
95. Hartley, G.P., Chow, L., Ammons, D.T., Wheat, W.H., and Dow, S.W. (2018). Programmed cell death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation. *Cancer Immunol. Res.* 6, 1260–1273.
96. Gubin, M.M., Esaulova, E., Ward, J.P., Malkova, O.N., Runci, D., Wong, P., Noguchi, T., Arthur, C.D., Meng, W., Alspach, E., et al. (2018). High-dimensional analysis delineates myeloid and lymphoid compartment remodeling during successful immune-checkpoint cancer therapy. *Cell* 175, 1014–1030.e19.
97. Vergadi, E., Ieronymaki, E., Lyrioni, K., Vaporidi, K., and Tsatsanis, C. (2017). Akt signaling pathway in macrophage activation and M1/M2 polarization. *J. Immunol.* 198, 1006–1014.
98. Schmid, M.C., Avraamides, C.J., Dippold, H.C., Franco, I., Foubert, P., Ellies, L.G., Acevedo, L.M., Manglicmot, J.R., Song, X., Wrasidlo, W., et al. (2011). Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3ky, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* 19, 715–727.

99. Kaneda, M.M., Messer, K.S., Ralainirina, N., Li, H., Leem, C.J., Gorjestani, S., Woo, G., Nguyen, A.V., Figueiredo, C.C., Foubert, P., et al. (2016). PI3K γ is a molecular switch that controls immune suppression. *Nature* 539, 437–442.
100. Joshi, S., Singh, A.R., Zulcic, M., and Durden, D.L. (2014). A macrophage-dominant PI3K isoform controls hypoxia-induced HIF1 α and HIF2 α stability and tumor growth, angiogenesis, and metastasis. *Mol. Cancer Res.* 12, 1520–1531.
101. Gunderson, A.J., Kaneda, M.M., Tsujikawa, T., Nguyen, A.V., Affara, N.I., Ruffell, B., Gorjestani, S., Liudahl, S.M., Truitt, M., Olson, P., et al. (2016). Bruton tyrosine kinase-dependent immune cell cross-talk drives pancreas cancer. *Cancer Discov.* 6, 270–285.
102. Højfeldt, J.W., Agger, K., and Helin, K. (2013). Histone lysine demethylases as targets for anticancer therapy. *Nat. Rev. Drug Discov.* 12, 917–930.
103. Guerriero, J.L., Sotayo, A., Ponichtera, H.E., Castrillon, J.A., Pourzia, A.L., Schad, S., Johnson, S.F., Carrasco, R.D., Lazo, S., Bronson, R.T., et al. (2017). Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. *Nature* 543, 428–432.
104. Lobera, M., Madauss, K.P., Pohlhaus, D.T., Wright, Q.G., Trocha, M., Schmidt, D.R., Baloglu, E., Trump, R.P., Head, M.S., Hofmann, G.A., et al. (2013). Selective class IIa histone deacetylase inhibition via a nonchelating zinc-binding group. *Nat. Chem. Biol.* 9, 319–325.
105. Lu, Z., Zou, J., Li, S., Topper, M.J., Tao, Y., Zhang, H., Jiao, X., Xie, W., Kong, X., Vaz, M., et al. (2020). Epigenetic therapy inhibits metastases by disrupting premetastatic niches. *Nature* 579, 284–290.
106. Wang, D., and Dubois, R.N. (2010). Eicosanoids and cancer. *Nat. Rev. Cancer* 10, 181–193.
107. Zelenay, S., van der Veen, A.G., Böttcher, J.P., Snelgrove, K.J., Rogers, N., Acton, S.E., Chakravarty, P., Girotti, M.R., Marais, R., Quezada, S.A., et al. (2015). Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* 162, 1257–1270.
108. Bonavita, E., Bromley, C.P., Jonsson, G., Pelly, V.S., Sahoo, S., Walwyn-Brown, K., Mensurado, S., Moeini, A., Flanagan, E., Bell, C.R., et al. (2020). Antagonistic inflammatory phenotypes dictate tumor fate and response to immune checkpoint blockade. *Immunity* 53, 1215–1229.e8.
109. Sinha, P., Clements, V.K., Fulton, A.M., and Ostrand-Rosenberg, S. (2007). Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* 67, 4507–4513.
110. Porta, C., Consonni, F.M., Morlacchi, S., Sangaletti, S., Bleva, A., Totaro, M.G., Larghi, P., Rimoldi, M., Tripodo, C., Strauss, L., et al. (2020). Tumor-derived prostaglandin E2 promotes p50 NF- κ B-dependent differentiation of monocytic MDSCs. *Cancer Res.* 80, 2874–2888.
111. Fujita, M., Kohanbash, G., Fellows-Mayle, W., Hamilton, R.L., Komohara, Y., Decker, S.A., Ohlfest, J.R., and Okada, H. (2011). COX-2 blockade suppresses gliomagenesis by inhibiting myeloid-derived suppressor cells. *Cancer Res.* 71, 2664–2674.
112. Long, K.B., Gladney, W.L., Tooker, G.M., Graham, K., Fraietta, J.A., and Beatty, G.L. (2016). IFN γ and CCL2 cooperate to redirect tumor-infiltrating monocytes to degrade fibrosis and enhance chemotherapy efficacy in pancreatic carcinoma. *Cancer Discov.* 6, 400–413.
113. Beatty, G.L., Chiorean, E.G., Fishman, M.P., Saboury, B., Teitelbaum, U.R., Sun, W., Huhn, R.D., Song, W., Li, D., Sharp, L.L., et al. (2011). CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 331, 1612–1616.
114. Monk, B.J., Brady, M.F., Aghajanian, C., Lankes, H.A., Rizack, T., Leach, J., Fowler, J.M., Higgins, R., Hanjani, P., Morgan, M., et al. (2017). A phase 2, randomized, double-blind, placebo-controlled study of chemo-immunotherapy combination using motolimod with pegylated liposomal doxorubicin in recurrent or persistent ovarian cancer: a Gynecologic Oncology Group partners study. *Ann. Oncol.* 28, 996–1004.
115. Chow, L.Q.M., Morishima, C., Eaton, K.D., Baik, C.S., Goulart, B.H., Anderson, L.N., Manjarrez, K.L., Dietsch, G.N., Bryan, J.K., Hershberg, R.M., et al. (2017). Phase Ib Trial of the Toll-like receptor 8 agonist, motolimod (VTX-2337), combined with cetuximab in patients with recurrent or metastatic SCCHN. *Clin. Cancer Res.* 23, 2442–2450.
116. Ribas, A., Medina, T., Kummer, S., Amin, A., Kalbasi, A., Drabick, J.J., Barve, M., Daniels, G.A., Wong, D.J., Schmidt, E.V., et al. (2018). SD-101 in combination with pembrolizumab in advanced melanoma: results of a phase Ib, multicenter study. *Cancer Discov.* 8, 1250–1257.
117. Kyi, C., Roudko, V., Sabado, R., Saenger, Y., Loging, W., Mandeli, J., Thin, T.H., Lehrer, D., Donovan, M., Posner, M., et al. (2018). Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. *Clin. Cancer Res.* 24, 4937–4948.
118. Kell, S.A., Kachura, M.A., Renn, A., Traquina, P., Coffman, R.L., and Campbell, J.D. (2019). Preclinical development of the TLR9 agonist DV281 as an inhaled aerosolized immunotherapeutic for lung cancer: Pharmacological profile in mice, non-human primates, and human primary cells. *Int. Immunopharmacol.* 66, 296–308.
119. Adams, S., Kozhaya, L., Martiniuk, F., Meng, T.C., Chiriboga, L., Liebes, L., Hochman, T., Shuman, N., Axelrod, D., Speyer, J., et al. (2012). Topical TLR7 agonist imiquimod can induce immune-mediated rejection of skin metastases in patients with breast cancer. *Clin. Cancer Res.* 18, 6748–6757.
120. Camargo, J.A., Passos, G.R., Ferrari, K.L., Billis, A., Saad, M.J.A., and Reis, L.O. (2018). Intravesical immunomodulatory imiquimod enhances *Bacillus Calmette-Guérin* downregulation of nonmuscle-invasive bladder cancer. *Clin. Genitourin. Cancer* 16, e587–e593.
121. Rodell, C.B., Arlauckas, S.P., Cuccarese, M.F., Garra, C.S., Li, R., Ahmed, M.S., Kohler, R.H., Pittet, M.J., and Weissleder, R. (2018). TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. *Nat. Biomed. Eng.* 2, 578–588.
122. Singh, M., Khong, H., Dai, Z., Huang, X.F., Wargo, J.A., Cooper, Z.A., Vasilakos, J.P., Hwu, P., and Overwijk, W.W. (2014). Effective innate and adaptive antitumor immunity through localized TLR7/8 activation. *J. Immunol.* 193, 4722–4731.
123. Pujade-Lauraine, E., Guastalla, J.P., Colombo, N., Devillier, P., François, E., Fumoleau, P., Monnier, A., Nooy, M., Mignot, L., Bugat, R., et al. (1996). Intraperitoneal recombinant interferon gamma in ovarian cancer patients with residual disease at second-look laparotomy. *J. Clin. Oncol.* 14, 343–350.
124. Larson, R.C., and Maus, M.V. (2021). Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat. Rev. Cancer* 21, 145–161.
125. Kingwell, K. (2017). CAR T therapies drive into new terrain. *Nat. Rev. Drug Discov.* 16, 301–304.
126. Klichinsky, M., Ruella, M., Shestova, O., Lu, X.M., Best, A., Zeeman, M., Schmierer, M., Gabrusiewicz, K., Anderson, N.R., Petty, N.E., et al. (2020). Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat. Biotechnol.* 38, 947–953.
127. Zhang, W., Liu, L., Su, H., Liu, Q., Shen, J., Dai, H., Zheng, W., Lu, Y., Zhang, W., Bei, Y., and Shen, P. (2019). Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix. *Br. J. Cancer* 121, 837–845.
128. Franklin, R.A., and Li, M.O. (2016). Ontogeny of tumor-associated macrophages and its implication in cancer regulation. *Trends Cancer* 2, 20–34.
129. Liu, Z., Gu, Y., Chakarov, S., Blierot, C., Kwok, I., Chen, X., Shin, A., Huang, W., Dress, R.J., Dutertre, C.-A., et al. (2019). Fate mapping via Ms4a3-expression history traces monocyte-derived cells. *Cell* 178, 1509–1525.e19.
130. Mattioli, I., Tomay, F., De Pizzol, M., Silva-Gomes, R., Savino, B., Gulic, T., Doni, A., Lonardi, S., Astrid Boutet, M., Nerviani, A., et al. (2019). The macrophage tetraspan MS4A4A enhances dectin-1-dependent NK cell-mediated resistance to metastasis. *Nat. Immunol.* 20, 1012–1022.
131. Ostendorf, B.N., Bilanovic, J., Adaku, N., Tafreshian, K.N., Tavora, B., Vaughan, R.D., and Tavazoie, S.F. (2020). Common germline variants of the human APOE gene modulate melanoma progression and survival. *Nat. Med.* 26, 1048–1053.