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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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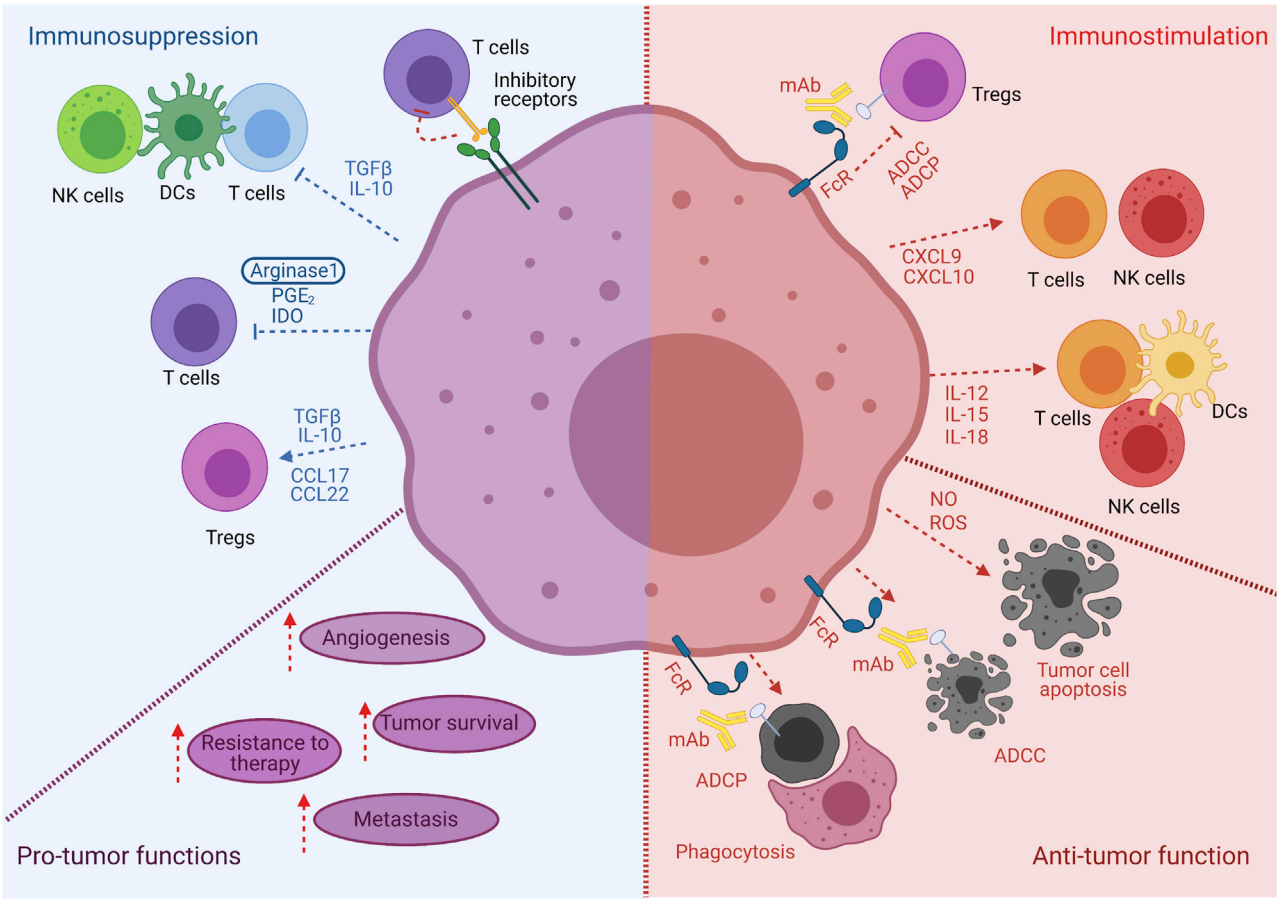

Figure 1. Pro-tumor and anti-tumor functions of TAMs

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 PGE_2. Moreover, TAMs contribute to differentiation and recruitment of regulatory T cells (Treg cells) via IL-10, TGFβ, 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

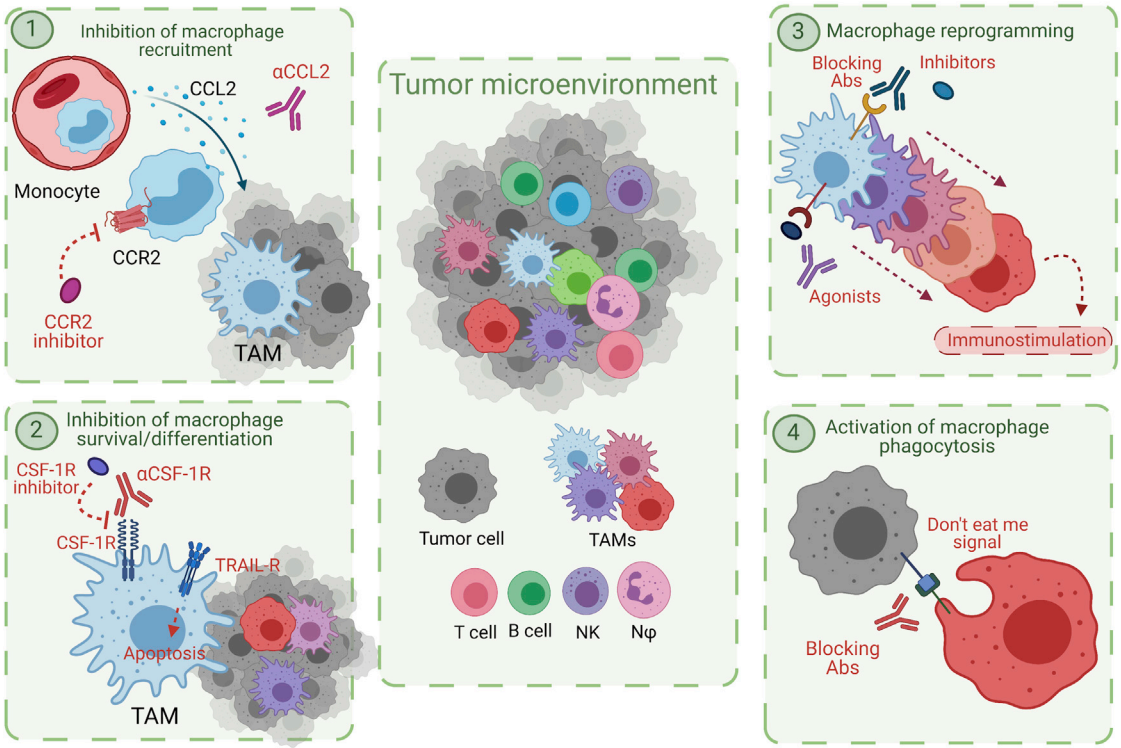

Figure 2. Strategies to target tumor-associated macrophages

(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

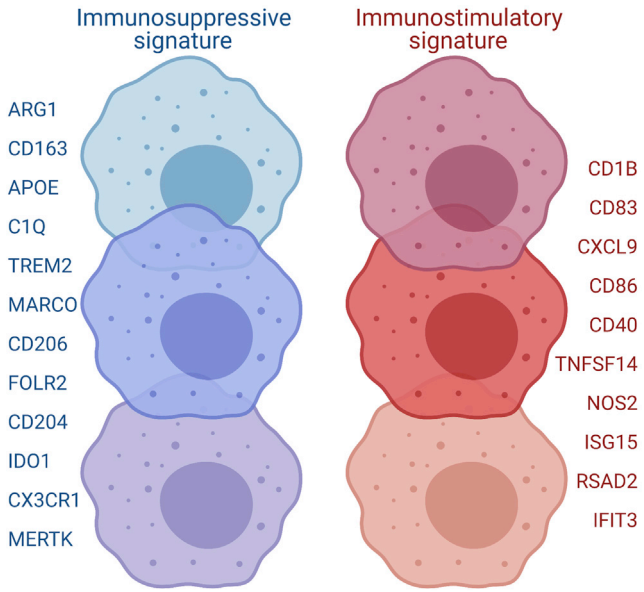

Figure 3. Macrophage distinct signatures

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), PLCγ2, 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

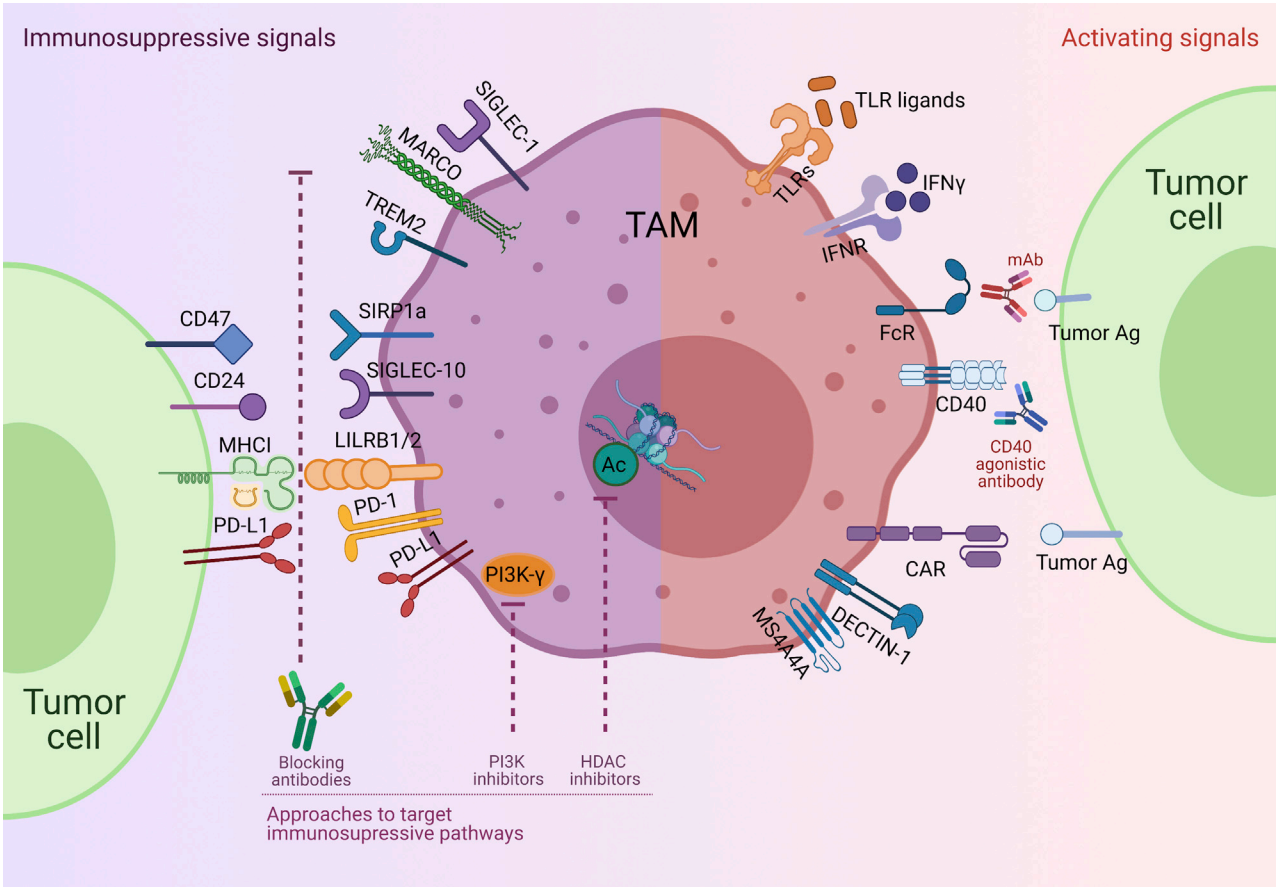

Figure 4. Strategies to promote macrophage anti-tumor activities

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; IFNγ; 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*$^{+}$*Cx*$_{3}$*cr1*$^{+}$ TAMs declined, whereas the representation of *Cd83*$^{+}$*Cxcl9*$^{+}$ 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 immunocheckpoint therapies 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 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 (A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R). 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 (IFNγ) 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-1β 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-SIRP1α 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* SIRP1α, a cell surface receptor that is constitutively expressed by macrophages, monocytes, neutrophils, and some dendritic cells.[55] The cytoplasmic domain of SIRP1α contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Upon binding CD47, SIRP1α ITIMs recruit the protein-tyrosine phosphatases SHP1 and SHP2, which transmit intracellular inhibitory signals.[56] Among various targets, SIRP1α 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-SIRP1α 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-SIRP1α axis tested in clinical trials include mAbs blocking CD47 (Hu5F9-G4, SRF231, IBI188, and AO-176), mAbs blocking SIRP1α (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-SIRP1α 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 SIRP1α instead of CD47 might be safer because of the restricted expression of SIRP1α on myeloid cells.[72] Second, CD47-SIRP1α 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 αMβ2.73 Thus, a better understanding of the complex mechanisms contributing to CD47-SIRP1α 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 a3 domain of MHC-I heavy chain and another interacts with β2M.[74,75] Because the MHC-I a3 domain and β2M are highly conserved across species, LILRB1 and LILB2 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 LILB2 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* IL2Rgamma^{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 IFNγ 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 (PI3Kγ), 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 PI3Kγ 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 PI3Kγ inhibitors in preclinical models, several strategies have been developed to inhibit PI3Kγ in the clinic. To date, ongoing clinical trials to target PI3Kγ have tested the PI3Kγ 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 PI3Kγ activation and immunosuppression in TAMs. Blockade of either BTK or PI3Kγ 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] MP195 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 E_2 (PGE_2)

PGE_2 is a product of arachidonic acid metabolism synthetized by the cyclo-oxygenase (COX) enzymes. PGE_2 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, PGE_2 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 PGE_2 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 PGE_2 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 PGE_2 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 PGE_2 led to the enrichment of p50-nuclear factor κB (NF-κB) in monocyte-related MDSCs within tumors in a sarcoma model; in turn, this engendered defective IFNγ responsiveness and an immunosuppressive signature in the monocyte-related MDSCs.[110] Furthermore, it was reported that PGE_2 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 PGE_2 acts on multiple cell types, it can have a broad effect in the tumor microenvironment; therefore, delineating the direct contribution of PGE_2 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, IFNγ, was directly used to reprogram macrophages. *In vivo* administration IFNγ was tested in tumor models, and IFNγ 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.

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