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REVIEW ARTICLE

# Myeloid immunosuppression and immune checkpoints in the tumor microenvironment

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Tumor-promoting inflammation and the avoidance of immune destruction are hallmarks of cancer. While innate immune cells, such as neutrophils, monocytes, and macrophages, are critical mediators for sterile and nonsterile inflammation, persistent inflammation, such as that which occurs in cancer, is known to disturb normal myelopoiesis. This disturbance leads to the generation of immunosuppressive myeloid cells, such as myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). Due to their potent suppressive activities against effector lymphocytes and their abundance in the tumor microenvironment, immunosuppressive myeloid cells act as a major barrier to cancer immunotherapy. Indeed, various therapeutic approaches directed toward immunosuppressive myeloid cells are actively being tested in preclinical and clinical studies. These include anti-inflammatory agents, therapeutic blockade of the mobilization and survival of myeloid cells, and immunostimulatory adjuvants. More recently, immune checkpoint molecules expressed on tumor-infiltrating myeloid cells have emerged as potential therapeutic targets to redirect these cells to eliminate tumor cells. In this review, we discuss the complex crosstalk between cancer-related inflammation and immunosuppressive myeloid cells and possible therapeutic strategies to harness antitumor immune responses.

**Keywords:** myeloid; macrophage; innate immunity; immune checkpoint; inflammation

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## INTRODUCTION

Immunotherapy has emerged as a new pillar in cancer therapy. Although immune checkpoint blockade (ICB) therapies, such as anti-PD-1/anti-PD-L1, have dramatically improved outcomes in patients with melanoma, non-small-cell lung cancer, and other types of tumors with high tumor mutational burden, we have yet to find a way to achieve durable clinical responses in most cancer patients. In addition to tumor-intrinsic resistant phenotypes, the tumor microenvironment (TME) acts as a major barrier for the recruitment and activation of effector lymphocytes.[1] In 1986, Dvorak proposed that tumors behave as wounds that do not heal, based on phenotypic similarities between wound healing and tumor stroma formation, such as the infiltration of inflammatory cells, enhanced coagulation, and matrix remodeling.[2] In the past several decades, there have been significant advances in our understanding of the cellular and molecular mechanisms of cancer-related inflammation. It is now appreciated that inflammation not only directly fuels tumor growth but also critically contributes to the generation of TME with proangiogenic and immunosuppressive features. Notably, recent in-depth analyses of the TME have revealed heterogeneity across cancer types,[3,4] indicating that a wound-healing-like process might be differentially orchestrated in a tumor-type-specific manner. In this review, we will discuss the intricate interplay between cancer-related inflammation and immunosuppression and potential approaches to overcome therapeutic resistance to cancer immunotherapy.

## CANCER-RELATED INFLAMMATION AND IMMUNOSUPPRESSION IN THE TME

### Mechanisms of cancer-related inflammation

The link between inflammation and cancer has been extensively studied, both epidemiologically and experimentally, and it is now appreciated that inflammation is involved in essentially all stages of cancer progression: initiation, promotion, progression and metastasis.[5–7] Cancer-related inflammation is presumably initiated by the cell-autonomous secretion of proinflammatory cytokines from malignantly transformed cells. The activation of oncogenes is known to induce cellular senescence, which prevents tumor progression. Additionally, senescent cells also acquire the ability to produce proinflammatory cytokines, including IL-1, IL-6, and IL-8, namely, the senescence-associated secretory phenotype (SASP).[8] By using a proteomics analysis of senescent chromatin, Chien et al. identified the NF-κB p65 subunit as a key transcriptional factor for SASP in oncogenic H-Ras$^{V12}$-induced fibroblasts.[9] Aberrant production of cytokines and chemokines was observed in malignant transformed cells induced by K-Ras (in pancreatic and ovarian cells),[10,11] RET/PTC1 rearrangement (in thyroid follicular cells),[12] and HER2 (in mammary epithelial cells).[13] Thus, the generation of a proinflammatory milieu might concomitantly occur with malignant transformation, although host factors such as predisposing inflammatory conditions (e.g., obesity, inflammatory bowel diseases), environmental exposures (cigarette smoking), and microbial dysbiosis also have a strong impact on precancerous lesions.

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According to the theory of cancer immunosurveillance and immunoediting, malignantly transformed cells are recognized and eliminated by immune cells. However, quantitative and qualitative changes in tumor cells lead to a cancer-immune equilibrium phase and a subsequent immune escape phase.[14] Intriguingly, it is reported that p53-induced senescent tumor cells recruit natural killer (NK) cells through the production of chemokine (C–C motif) ligand 2 (CCL2) and are eliminated by the activating receptor NKG2D,[15] indicating that malignantly transformed cells are controlled at early stages by both intrinsic (by cellular senescence) and extrinsic (by immunosurveillance) pathways. By contrast, mutant p53, but not wild-type p53, is known to repress IL-1 receptor antagonist expression in tumor cells, and thus, mutant p53 can render tumor cells in a ready-to-be-activated state in response to IL-1,[16] suggesting the differential roles of normal versus mutant p53. The cytosolic DNA sensor, the cGAS-STING (cyclic GMP-AMP synthase linked to stimulator of interferon genes) pathway, has also emerged as a key player at the crossroads of SASP and immunosurveillance.[17] Genotoxic stress triggers the translocation of chromatin fragments into the cytoplasm, which subsequently activates cGAS-STING, leading to the secretion of inflammatory cytokines. Whereas the SASP induced by the cGAS-STING pathway might at least temporarily enhance immunosurveillance through type 1 interferon secretion, persistent secretion of proinflammatory cytokines (IL-1, IL-6, and IL-8) can oppositely promote evasion from cellular senescence and immunosurveillance.[17,18] Thus, SASP-induced early inflammatory responses appear to regulate a fine balance between immunosurveillance and tumor progression. However, we are yet to understand how tumor-promoting inflammation eventually overwhelms immune-mediated control.

In addition to tumor-intrinsic inflammation, extrinsic pathways, namely, innate inflammatory responses, also critically fuel inflammation in established or clinically detectable tumors.[6] In the TME, various endogenous ligands called damage-associated molecular patterns (DAMPs) are released in response to hypoxia, cellular stress and tissue injury, which are subsequently recognized by pattern recognition receptors (PRRs) expressed by local environment host cells (such as macrophages) and certain types of tumor cells. These DAMPs include high-mobility group box-1 (HMGB1) (recognized by TLR-2/4 and RAGE), S100 proteins (by TLR-4 and RAGE),[19] versican (for TLR-2/6),[20] tumor exosomal RNAs (by TLR-3),[21] DNA (by STING and AIM2,[22] uric acid (by TLR-2/4 and NLRP3),[23] and ATP (by NLRP3).[24]

Despite the fact that the interaction between DAMPs and PRRs critically contributes to tumor progression, immunosuppression, and metastasis,[6,25,26] it remains largely unknown how tumor PRRs and host PRRs differentially recognize various DAMPs to orchestrate cancer-related inflammation in the TME. Moreover, it is appreciated that PRR signaling pathways are controlled by self-regulation (i.e., various posttranslational modifications, epigenetic modifications, and metabolic regulations)[27–29] and cross-regulation (synergy, enhancement, suppression, feedback enhancement or feedback suppression incorporated with other PRR signaling).[29] It is possible that PRR signaling in the TME might be reprogrammed to promote tumor progression rather than to stimulate an antitumor immune response. However, this aspect has been poorly addressed in cancer-related inflammation.

While persistent inflammation promotes tumor progression, acute inflammation elicited by therapeutic agents has been considered to be beneficial for antitumor immunity. Indeed, certain cytotoxic chemotherapies require host PRRs (such as TLRs and inflammasomes) for their optimal efficacy,[30,31] suggesting that therapy-induced inflammation can dynamically alter the immunosuppressive TME. However, several lines of evidence suggest that therapy-induced inflammation may also have detrimental potential to promote tumor growth. For instance, Sulciner et al. reported that chemotherapy-generated tumor cell debris stimulated proinflammatory cytokine release from macrophages, which allowed residual tumor cells to progressively grow.[32] Using mouse models of metastatic dormancy, Krall et al. showed that surgery-induced systemic inflammation can evoke dormant cells, leading to outgrowth of tumors.[33] Additionally, therapy-induced inflammation by adoptive T cell therapy or ICB therapies triggers the release of hepatic growth factor (HGF), which allows mobilization of c-MET$^+$ immunosuppressive neutrophils in the TME.[34] Moreover, small-molecule inhibitors against BRAF, ALK or EGFR kinase also dynamically alter the secretory phenotypes of tumor cells, which leads to metastatic dissemination and drug resistance.[35] Thus, the impact of therapy-induced inflammation on tumor cells and antitumor immunity will need to be carefully investigated.

Overall, we have yet to uncover the complex regulation of cancer-related inflammation, and at least four aspects will need to be better understood in the future: (1) discrete roles of tumor PRRs and host PRRs for cancer-related inflammation, (2) the characterization of tumor-type-specific DAMPs and their spatial-temporal distribution, (3) the regulation/reprograming of PRR signaling in the TME, and (4) the balance between cancer-promoting inflammation and cancer-inhibiting inflammation.

## Immunosuppressive cells induced by cancer-related inflammation

It is now appreciated that cancer-related inflammation triggers myeloid immunosuppression (Fig. 1). During acute systemic inflammation triggered by infection, the hematopoietic system needs to shift from steady-state hematopoiesis to emergency granulopoiesis to meet the high demand for granulocytes and monocytes.[36] Hematopoietic stem and progenitor cells (HSPCs) express TLRs and receptors for proinflammatory cytokines, and thus, a wide variety of pathogen-associated molecular patterns (PAMPs) and inflammatory stimuli are known to activate HSPCs either directly or indirectly, leading to granulopoiesis.[36,37] Among the various transcriptional factors that are activated in response to inflammation, CCAAT-enhancer-binding protein-β (C/EBPβ) acts as a key switch from steady-state hematopoiesis (controlled by C/EBPα) to expansion of granulocytes.[37] Mice deficient in C/EBPα

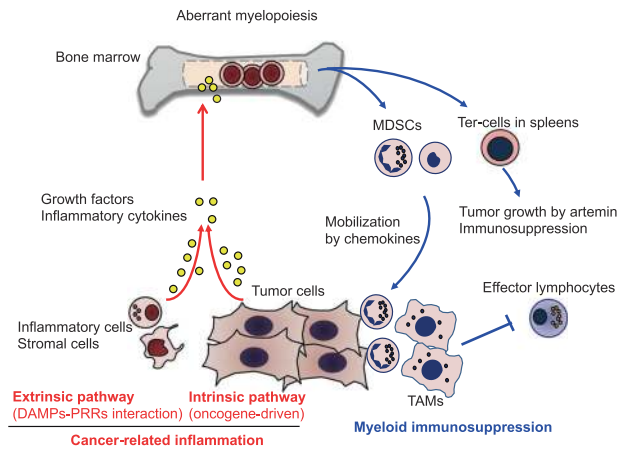


**Fig. 1** The link between cancer-related inflammation and myeloid immunosuppression. Cancer-related inflammation is orchestrated by intrinsic pathways (driven by oncogenes) and extrinsic pathways (by interaction between DAMPs and PRRs in environmental cells). A wide variety of growth factors and proinflammatory cytokines can alter myelopoiesis in the bone marrow, leading to the generation of immature myeloid cells with potent immunosuppressive activities (i.e., MDSCs). In response to chemokines, MDSCs are subsequently recruited into tumor tissues, where they further undergo dynamic alteration (e.g., differentiation into TAMs from monocytic MDSCs). MDSCs and TAMs act as a key barrier for antitumor immunity. In addition to monocytes and granulocytes, erythroblast-like cells (called Ter-cells) emerge in spleens under tumor-bearing conditions

show severe granulocytopenia under steady-state conditions due to impaired transition at the common myeloid progenitor (CMP) to granulocyte–monocyte progenitor (GMP) stage, but they can produce normal granulocytes in response to infection and cytokines.[38] On the other hand, mice deficient in C/EBPβ show impaired emergency granulopoiesis upon fungal infection, but they have normal levels of granulocytes under steady-state conditions.[39] Cancer-related inflammation can hijack this emergency granulopoiesis process and drives the generation and expansion of heterogeneous immature myeloid cells with immunosuppressive activities (i.e., myeloid-derived suppressor cells [MDSCs]), which consist of a polymorphonuclear subset (PMN-MDSCs) and a monocytic subset (M-MDSCs).[40]

As seen in acute inflammation, a broad range of inflammatory stimuli can trigger the generation of MDSCs. These factors can be classified into three groups: growth factors that are required for expansion (e.g., G-CSF, M-CSF, GM-CSF and S-CSF); cytokines for functional maturation (IL-1 family cytokines, IL-4, IL-6, IL-13, and TNF); and chemoattractants for mobilization into the TME (IL-8, CCL2, and CXCL12).[41–43] Not surprisingly, differential downstream signaling and transcriptional factors are intricately involved in each step. For instance, C/EBPβ, the aforementioned key transcriptional factor for emergency granulopoiesis, plays a crucial role in the expansion of MDSCs.[44] The C/EBPβ-driven expansion of MDSCs is positively regulated by retinoic acid-related orphan receptor C (RORC1) expressed on immature myeloid cells under cancer-related inflammation, though it should be noted that this positive regulation is independent of IL-17A, a key cytokine induced by RORC1/2.[45] By contrast, interferon regulatory factor–8 (IRF8) acts as a negative regulator of the generation of MDSCs, given that mice deficient in IRF8 harbored MDSC-like cells even under tumor-free conditions and that transgenic overexpression of IRF8 reduced the accumulation of MDSCs under tumor-bearing conditions.[46] NF-κB and JAK/STAT signaling are known to upregulate immunosuppressive mediators such as iNOS (in M-MDSCs), reactive oxygen species (ROS), and arginase (in PMN-MDSCs).[47] Upon recruitment into the TME, local metabolites (e.g., adenosine), hypoxia, and the unfolded protein response further augment immunosuppressive activities of MDSCs.[48–50] In addition, the imbalanced complement activation in the TME is known to cause recruitment and functional maturation of MDSCs via C3 and C5a components.[51,52] Key stimuli for the generation of MDSCs might also vary depending on tumor type, tumor site, clinical stage, gender, and therapy,[6,53–55] raising the possibility that therapeutic targeting of MDSCs might require a disease-specific approach and/or patient-specific approach. Phenotypically, PMN-MDSCs are characterized by $CD11b^{+}Ly6C^{lo}Ly6G^{+}$ (in mouse) and $CD14^{-}CD11b^{+}CD15^{+}$ (in human), while M-MDSCs are defined by $CD11b^{+}Ly6C^{hi}Ly6G^{-}$ (in mouse) and $CD11b^{+}CD14^{+}HLA\text{-}DR^{lo/-}CD15^{-}$ (in human).[40] Additionally, lectin-type oxidized LDL receptor-1 (LOX-1) was recently identified as a marker expressed on human PMN-MDSCs but not on circulating neutrophils.[56] However, it is very challenging to discriminate tumor-infiltrating PMN-MDSCs and M-MDSCs from neutrophils and inflammatory monocytes, respectively, given their similarities and phenotypic plasticity in the TME. Indeed, many studies characterizing either tumor-associated neutrophils (TANs) or PMN-MDSCs have used essentially the same markers to define these cells.[57] Additionally, it should be noted that, while their negative impacts on effector lymphocytes have been well demonstrated in in vitro expanded MDSCs and ex vivo isolated MDSCs, it is still technically difficult to examine the exact impact of MDSCs on tumor progression *in vivo*. Currently, conditional gene targeting approaches (utilizing mice carrying Cre recombinase under *Lyz2*, *Csf1r*, *Cx3cr1*, *CD11b*, or *Ly6g*)[58,59] or depletion approaches (anti-Gr-1, anti-Ly6G, anti-CCR2) are not strictly specific to MDSCs. Although these experimental limitations remain unsolved, a high abundance of MDSCs in cancer patients and their potent antigen-specific and nonspecific suppressive activities against T cells highlight that MDSCs act as key barriers for cancer immunotherapy.

Tumor-associated macrophages (TAMs) also consist of key immunosuppressive myeloid cells in the TME,[60] and a meta-analysis showed that a high density of TAMs is associated with worse prognosis in gastric cancer, urogenital cancer and head and neck cancer.[61] TAMs exert pleiotropic protumor activities, including immunosuppression, angiogenesis, and supporting chemoresistance in tumor cells, while they also contribute to antitumor immunity under certain conditions by phagocytosis, antigen presentation, and direct tumoricidal activity.[60] In early work investigating the ontogeny of TAMs, by using de novo and transplantable breast cancer models, researchers considered circulating inflammatory monocytes (and M-MDSCs) to be the main cellular source of TAMs,[60,62,63] while tissue-resident macrophages (originally derived from myeloid progenitors in the yolk sac)[64,65] were functionally and phenotypically distinct from monocyte-derived TAMs.[63] However, several lines of evidence now suggest that tissue-resident macrophages are also indispensable regulators of the TME among TAMs. For instance, in malignant glioma, both microglia and monocyte-derived macrophages undergo dynamic education in the TME, and glioma-associated microglia contribute to tumor progression.[66–68] In pancreatic ductal adenocarcinoma (PDAC) models, Zhu et al. showed that a significant proportion of TAMs contain tissue-resident macrophages with in situ proliferation ability and that tissue-resident macrophages have a greater ability to promote fibrosis, a major barrier for PDAC therapy.[69] Additionally, in lung cancer models, Loyher et al. showed that monocyte-derived macrophages contribute to tumor spreading, while tissue-resident macrophages directly support tumor proliferation of lung cancer,[70] further providing evidence that TAMs harbor ontogenetically and functionally different macrophages. In addition to the intratumor heterogeneity of TAMs, the intertumor heterogeneity of TAMs might influence the diversity of the TME. Recently, Cassetta et al. performed a transcriptional comparison of circulating monocytes derived from healthy female subjects, breast cancer patients, and endometrial cancer patients, as well as the characterization of TAMs from breast cancer and endometrial cancer. While both breast and endometrial cancer conditions similarly altered transcriptomes in circulating monocytes compared to healthy subjects (referred to as "tumor-educated monocyte signature"), TAM transcriptomes were distinct between breast cancer and endometrial cancer. Moreover, TAM signatures of each tumor were distinct from transcriptomes of their respective tumor-educated circulating monocyte and tissue-resident macrophages,[71] indicating that the TAM signatures were created in a tumor-type-specific manner in the unique TME.

The exact process for the functional maturation of TAMs remains to be elucidated; however, several candidate signaling pathways have been identified. Notch signaling is an evolutionarily conserved pathway that plays a crucial role in development, homeostasis, and hematopoiesis.[72] The activation of Notch signaling was initially shown to drive the polarization of TAMs into a classically activated phenotype (M1-like).[73] Indeed, the activation of Notch1 enhanced glycolysis and mitochondrial ROS, which allowed macrophages to upregulate M1-associated genes.[74] These results suggest that the activation of Notch1 might be able to convert TAMs into tumoricidal macrophages. However, Notch1 signaling is also critically involved in the differentiation of monocytes into TAMs, which was supported by the fact that myeloid-specific ablation of RBPJ, a key nuclear effector of Notch, inhibited the differentiation of inflammatory monocytes into TAMs in preclinical breast cancer models.[63] Moreover, Sierra et al. showed that therapeutic blockade of ligands for Notch receptors by anti-Jagged1/2 mAb (CTX014) attenuated the suppressive activities of tumor-infiltrating myeloid cells.[75] It should also be noted that the tumor-intrinsic Notch

signaling and differential distribution of ligands further modulated phenotypes of TAMs in the TME.[76,77] Thus, despite its strong impact on both the differentiation and functional polarization of TAMs, the temporal regulatory mechanisms of Notch signaling in TAMs are yet to be fully understood. Recently, phosphatidylinositol 3-kinase γ (PI3Kγ) in myeloid cells has been recognized as a common downstream effector in cancer-related inflammation, including receptor tyrosine kinase (activated by growth factors), G protein-coupled receptors (by chemoattractants), and TLR/IL1R (by DAMPs and IL-1).[78] PI3Kγ signaling activates C/EBPβ in response to inflammatory stimuli, but at the same time, it negatively regulates NF-κB activities, indicating that PI3Kγ signaling is a key driver for immunosuppression in myeloid cells.[79] Indeed, pharmacological inhibition of PI3Kγ signaling shows promising results either as a monotherapy or in combination with ICB in various preclinical tumor models.[79–81] Additional studies are warranted to understand whether blockade of PI3Kγ can effectively attenuate immunosuppressive activities of heterogeneous TAMs in human cancers.

In addition to MDSCs/TAMs, two independent groups recently demonstrated that cancer-related inflammation also triggered aberrant erythropoiesis, leading to the generation of protumor erythroid progenitor cells in spleens.[82–84] The erythroid subset, termed Ter-cells (lineage-negative Ter-119$^+$CD71$^+$CD41$^+$ cells), abundantly populated the spleens of tumor-bearing mice (up to 30% in total splenocytes) as well as the spleens of patients with hepatocellular carcinoma (HCC).[82] Phenotypic and transcriptional characterization showed that Ter-cells originate from megakaryocyte/erythroid progenitors (MEPs) and are induced by TGF-β/SMAD3 signaling.[82] Functionally, Han et al. concluded that Ter-cells do not have direct immunosuppressive activities against T cells but rather abundantly secrete a neurotrophic growth factor, artemin, that directly fuels HCC growth.[82,84] By contrast, Zhao et al. found that CD45$^+$CD71$^+$Ter-119$^+$ erythroid progenitor cells with transcriptional similarity to MDSCs accumulate in tumor-bearing mice and contribute to direct immunosuppression against T cells via ROS production.[83] While further ontogeny and functional characterization are warranted to understand the role of protumor erythroid progenitors, these studies highlight that cancer-related inflammation has a strong impact on a wide spectrum of hematopoietic progenitor cells.

## CURRENT APPROACH TARGETING INNATE IMMUNITY

There are several approaches to target the vicious cycle of inflammation and immunosuppression in the TME: controlling cancer-promoting inflammation; blocking the mobilization or survival of myeloid cells; and activating myeloid cells by acute inflammatory stimuli. Another potential strategy, the reprogramming of a protumor phenotype by targeting checkpoint molecules on myeloid cells, will be described in the next section.

### Targeting cancer-promoting inflammation

The beneficial effects of anti-inflammatory agents have been well studied in cancer prevention, as demonstrated by NSAIDs (especially low-dose aspirin).[85] A large randomized clinical trial, called the "Add-Aspirin trial", is currently ongoing (NCT02804815) to address whether regular use of aspirin is beneficial to prolong survival in various cancer patients after standard therapy.[86] Although it has been demonstrated that the inhibition of cyclooxygenase 2 (COX-2) in combination with anti-PD-1 synergistically improves tumor control in preclinical melanoma models,[87] the clinical efficacy of the combination of immune checkpoint blockade and NSAIDs has yet to be determined in clinical trials (NCT03396952 and NCT02659384). Intriguingly, in colorectal cancer patients, postdiagnostic aspirin use improved survival in patients with PD-L1-low tumors but not in PDL-1-high tumors,[88] implying that PD-L1-mediated T cell suppression might limit the beneficial effects of aspirin. In this context, it will be rational to test the combination of aspirin with anti-PD-1 blockade in colorectal cancer with DNA mismatch repair deficiency/microsatellite instability, given that clinical efficacies of anti-PD-1 therapy have been well demonstrated in patients with this subtype.[89,90]

Targeting IL-1β, a key initiator of inflammation, has also shown promising results in preventing carcinogenesis. In patients with smoldering/indolent multiple myeloma, anakinra, an IL-1R antagonist, delayed the progression from asymptomatic phases into active myeloma possibly by inhibiting the production of IL-6, a key survival factor for plasma cells.[91] In a large randomized clinical trial called CANTOS (canakinumab anti-inflammatory thrombosis outcomes study) in over 10,000 patients with prior myocardial infarction, anti-IL-1β-neutralizing mAb (canakinumab) treatment dramatically reduced the incidence and mortality in patients with prior myocardial infarction.[92] It should be noted that a cell-type-specific role of IL-1R might need to be considered in the future to maximize the preventive effect of IL-1 blockade. In colorectal carcinogenesis models, genetic ablation of IL-1R in epithelial cells or T cells can reduce the incidence of tumor development by reducing tumor proliferation and the Th17 response, respectively, whereas the ablation of IL-1R in neutrophils exacerbates tumor progression by triggering dysbiosis-induced inflammation.[93] There are several clinical trials using IL-1 blockade in patients with advanced colon cancer (NCT02090101), pancreatic cancer (NCT02021422), and breast cancer (NCT01802970)[6]; however, the therapeutic efficacies of IL-1 blockade are yet to be understood. An alarmin cytokine, IL-1α, might play a unique role in cancer patients, as anti-IL-1α mAb (MABp1) improved cancer cachexia in a phase I clinical trial in patients with metastatic cancers.[94] As cancer cachexia is known to be associated with a higher clearance of pembrolizumab (anti-PD-1), leading to poor clinical response in melanoma and non-small-cell lung cancer patients,[95] controlling cancer cachexia will have a significant benefit in patients. Additional studies are warranted to understand whether IL-1 blockade should be considered for subjects at high risk for cancer or can be utilized as an adjuvant for immunotherapy in advanced cancer patients.[96]

TNF-α was originally characterized as a tumoricidal cytokine; however, therapeutic doses of TNF-α are intolerable in patients.[97] Since TNF-α has multifaceted protumor functions, such as prosurvival signaling, the invasion of tumor cells, and the enhancement of MDSC activities, TNF-α blockade therapy has been tested in various solid malignancies, including renal, pancreatic, breast, and ovarian cancer.[6,98–101] However, it has not demonstrated significant clinical responses. Blockade of TNF-α by infliximab is commonly used to control steroid-refractory immune-related adverse events (irAEs) in patients who are treated with immune checkpoint blockade (ICB) (especially in patients with ipilimumab-induced colitis). These patients generally require a single dose of infliximab,[102] and it remains unclear whether blockade of TNF-α alters antitumor immune responses. Recently, two preclinical studies suggested that the blockade of TNF-α augmented the efficacies of ICB by preventing TNF-driven activation-induced cell death in T cells.[103,104] On the other hand, Vredevoogd et al. showed that T cell-derived TNF-α still played an important role in eliminating tumor cells under ICB therapy when tumor cells were pharmacologically sensitized to TNF-mediated apoptosis.[105] Therefore, TNF sensitivity in tumor cells might be an important factor to be considered for therapeutic blockade of TNF-α.

One of the key limitations of single cytokine blockade therapy is that functional redundancy among cytokines or compensatory effects by other cytokines might attenuate its therapeutic efficacy.[6] Thus, targeting downstream signaling by a small-molecule inhibitor might be an alternative approach. For instance, ibrutinib is clinically used for the treatment of B cell

malignancies due to its potent and irreversible inhibition of Bruton's tyrosine kinase, a key downstream signaling pathway of B cell receptor (BCR).[106] Intriguingly, ibrutinib, in combination with anti-PD-L1, synergistically improved tumor control, not only in a preclinical B cell lymphoma model but also in solid tumor models.[107] These effects can be explained by the broad impact of ibrutinib on immune responses, such as the induction of Th1 responses,[108] the inhibition of TLR signaling,[109] and the inhibition of NLRP3 inflammasome activation.[110] Despite promising preclinical results, a recent clinical trial using anti-PD-L1 in combination with ibrutinib showed disappointing results in patients with advanced pancreatic, breast and lung cancer.[111] There are several ongoing clinical trials using ibrutinib against solid malignancies,[112] and detailed analyses of the TME in patients treated with ibrutinib will provide more information and future therapeutic strategies.

Inhibitors for JAK kinase have been clinically approved in patients with rheumatoid arthritis and myeloproliferative neoplasms (MPNs) to target the IL-6/JAK1 pathway and JAK2 V617F mutations, respectively. Since constitutively active STAT3 is observed in a range of malignancies, possibly driven by various factors, including oncogenes, growth factors, and cytokines (IL-6, IL-10, and IL-23), JAK/STAT3 has been recognized as a possible target in cancers.[113] Recently, Simon et al. showed that tofacitinib (an inhibitor for JAK1/3) treatment reduced inflammatory myeloid cells in the TME and augmented delivery of antibody-based therapeutics into tumor tissues,[114] providing evidence that targeting JAK signaling alters the proinflammatory TME. However, the inhibition of the JAK signaling pathway might also have detrimental effects, given that immunostimulatory cytokines such as IL-12, IL-15 and interferons also share this pathway. Indeed, ruxolitinib (an inhibitor of JAK1/3) treatment functionally and numerically dampened NK cells in patients with MPNs,[115] as well as NK cell-mediated metastatic control of breast cancer in preclinical models.[116] Targeting STAT3 might be an alternative approach, given that the activation of STAT3 is a key driver for arginase expression in MDSCs.[117] Various approaches for STAT3 inhibition, such as inhibiting the (1) SH2 domain or dimerization, (2) antisense oligonucleotides, and (3) peptide mimetics, have been tested in clinical trials; however, they have yet to be clinically approved due to limited efficacies.[118] Notably, while STAT3 is frequently activated in circulating MDSCs, hypoxia-induced downregulation of STAT3 is known to trigger differentiation of M-MDSCs into TAMs in the TME.[119] Therefore, in addition to the requirement for a selective and potent inhibitor, a treatment strategy needs to be carefully designed.

### Blocking mobilization and survival

The CCL2-CCR2 pathway plays a crucial role in the recruitment of monocytes (including M-MDSCs), and blocking this pathway is a rational approach to inhibit the accumulation of TAMs in the TME. Therapeutic blockade of CCL2-CCR2 interactions has demonstrated promising antitumor efficacies in several preclinical cancer models;[120–122] however, the durability of this effect remains a concern. For instance, immunogenic cell death inducers such as doxorubicin require the CCL2-CCR2 pathway for the recruitment of functional DCs after chemotherapy, suggesting that this pathway contributes to antitumor immunity.[123] Additionally, the withdrawal of anti-CCL2 therapy triggers a rebound of CCL2 in the lungs and subsequent mobilization of BM monocytes, leading to the exacerbation of metastasis.[124] Indeed, in a phase II clinical trial of carlumab (anti-CCL2 mAb) in patients with metastatic castration-resistant prostate cancer, it was reported that the circulating concentration of free CCL2 rapidly rebounded and exceeded the pretreatment serum levels.[125] Moreover, it remains unknown whether blockade of CCL2-CCR2 signaling might be compensated by increasing PMN-MDSCs or TAMs derived from tissue-resident macrophages in the TME.

Another well-characterized target is the colony-stimulating factor 1 (CSF1)/colony-stimulating factor 1 receptor (CSF1R) axis.[126] The expression of CSF1R is restricted to monocytes and macrophages, and the inhibition of the CSF1/CSF1R axis has shown promising antitumor efficacies by inhibiting the survival of M-MDSCs and TAMs. Indeed, the depletion of TAMs by CSF1R inhibitors augmented the efficacies of cytotoxic chemotherapeutic agents,[120] antiangiogenic therapy (anti-VEGF),[127] and immunotherapies including anti-PD-1 and anti-CTLA-4.[128,129] Based on promising efficacies in preclinical models, various inhibitors against CSF1R (such as IMC-CS4, GW2580, PLX3397, AMG820, and emactuzumab) are being tested in combination with chemotherapy or ICB (reviewed in[126]). Notably, while anti-CSF1R has not shown remarkable clinical responses in many types of tumors as a single agent, it has shown promising efficacies in patients with tenosynovial giant-cell tumors, which are characterized by the overproduction of CSF1 due to the translocation of the *CSF1* gene.[130] Given that higher expression levels of CSF-1 in the tumor epithelium and surrounding stroma predict poor prognosis in breast cancer,[131] anti-CSF1R blockade might be particularly beneficial in patients with a $CSF1^{hi}$ tumor subtype. However, the inhibition of monocytes and TAMs by CSFR1 antibody resulted in increased granulocyte progenitors, suggesting that reciprocal regulation might limit efficacy.[45] The long-term safety and durability of CSF1R inhibitors require further investigation.

### The activation of myeloid cells

While persistent cancer-related inflammation triggers the generation of MDSCs and TAMs, acute inflammation induced by certain therapeutic agents dynamically directs these cells to antitumor function. Historically, Bacillus Calmette-Guerin (BCG) immunotherapy has been the standard treatment in patients with high-risk noninvasive bladder cancer. Upon BCG injection, the activation of innate proinflammatory responses (including the induction of proinflammatory cytokines) and subsequent infiltration of effector lymphocytes are observed in patients.[132] The therapeutic efficacy of BCG in combination with anti-PD-1 is being tested in patients with high-risk superficial bladder cancer (NCT02324582). TLR agonists (such as poly I:C, imiquimod, and CpG oligodeoxynucleotide)[133] and STING agonists (3′3′-cGAMP) also have the ability to stimulate antitumor immune responses mainly through type1 interferon secretion. While these adjuvants are well known to significantly improve the therapeutic efficacies of ICB in preclinical models, they need to be delivered safely to the TME to achieve optimal antitumor efficacy as well as to avoid systemic inflammation. To this end, several new TLR3 agonists and STING agonists are being developed and have shown good preclinical antitumor efficacies.[134,135] CD40 agonists have also been well studied in preclinical models and clinical trials by their potent ability to stimulate DCs.[136] However, due to dose-limiting toxicities, anti-CD40 agonists have not been successfully translated into standard care. Ravetch's group recently generated an Fc-engineered anti-CD40 agonist with binding affinity to FcγRIIB for optimal therapeutic efficacy and showed that intratumor injection of this new mAb minimizes deleterious off-target effects.[137,138] Oncolytic viruses have gained prominence based on promising preclinical results and are actively being tested in combination with ICB in clinical trials (reviewed in ref. [139]). Multiple mechanisms are considered to be involved in their efficacies, including the induction of proinflammatory cytokines and type 1 interferons, the direct lysis of tumor cells and the subsequent release of DAMPs, and stimulation of tumor-antigen presentation. The role of TAMs under oncolytic therapy remains controversial, and TAMs may either contribute to antitumor immunity or limit efficacy depending on the context.[140] Overall, several agents that activate myeloid cells have the ability to potently augment antitumor immune responses in preclinical models; however, treatment-related toxicity remains a major concern for clinical translation.

Additionally, it is important to carefully optimize the treatment schedule, given that persistent inflammatory stimuli could augment immunosuppressive activities.

## TARGETING IMMUNE CHECKPOINT MOLECULES ON MYELOID CELLS

Immune checkpoint molecules such as PD-1 and Tim-3 have been intensively studied in adaptive immune responses; however, a subset of TAMs and DCs also express these immune checkpoint molecules (Fig. 2a). Additionally, there are several myeloid-specific immunoregulatory receptors (Fig. 2b, c), which provide potential therapeutic targets to augment innate immune responses against cancer.

### PD-1 and PD-L1 on macrophages and dendritic cells

PD-L1 expressed on antigen-presenting cells (APCs) has been recognized as a key regulatory molecule that interacts with PD-1

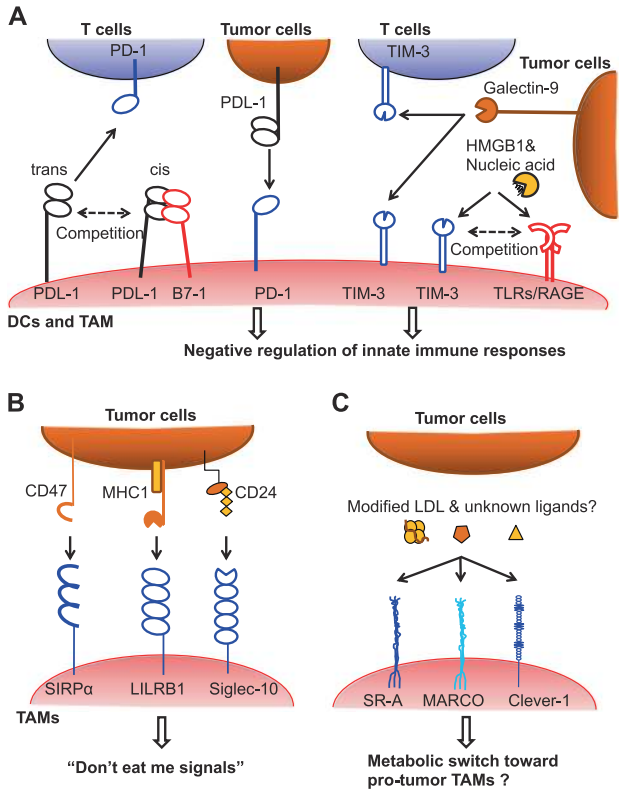


**Fig. 2** Potential immune checkpoint molecules on myeloid cells. **a** Tumor-associated macrophages and dendritic cells (DCs) express several T cell immune checkpoints and their ligands. While the trans interaction between PD-L1 on myeloid cells and PD-1 on T cells critically regulates T cell activation, the cis interaction between PD-L1 and B7-1 on antigen-presenting cells competitively inhibits the trans interaction. TAMs and DCs also express PD-1, which negatively regulates innate immune responses. TIM-3 on DCs also negatively regulates cytokine production, either directly by interacting with galectin-9 on tumor cells or indirectly by the sequestration of the HMGB1-nucleic acid complex (ligands for RAGE and TLRs). **b** ITIM-containing receptors SIRPα, LILRB1, and Siglec-10 have emerged as key receptors that negatively regulate cellular phagocytosis through the recognition of CD47, MHC class 1, and CD24, respectively. Note that PD-1 on macrophages is also known to regulate phagocytosis. **c** Through the recognition of certain ligands in the TME, several scavenger receptors might contribute to metabolic switching toward protumor TAMs, given that genetic ablation or pharmacological inhibition of these receptors can direct TAMs into antitumor phenotypes

on T cells at the immunological synapse.[141] Lin et al. recently showed that the therapeutic efficacies of anti-PDL-1 blockade against PDL-1$^{hi}$ tumors are completely abrogated in PDL-1-deficient mice, while anti-PD-L1 blockade effectively controls the growth of PD-L1-deficient tumors in wild-type hosts, demonstrating that PD-L1 on host cells (specifically antigen-presenting cells) is a key target of anti-PD-1/PD-L1 blockade therapy.[142] Although myeloid PDL-1 seems to play an important role in the regulation of T cells, further clinical investigations are necessary, given several studies showing a reduced clinical response of anti-PD-1 blockade in patients with PD-L1-negative tumors.[143,144]

In the TME, a subset of macrophages and DCs also express PD-1.[145,146] While NFAT activation followed by T cell receptor stimulation upregulates PD-1 expression in T cells,[147,148] NF-κB activation by TLRs upregulates PD-1 expression in macrophages.[146] The functional significance of the activation-induced upregulation of PD-1 was first demonstrated by a mouse model of *Listeria monocytogenes* infection. Using mice lacking PD-1 and adaptive immunity (i.e., *Rag1*$^{-/-}$*Pdcd1*$^{-/-}$ mice), Yao et al. demonstrated that PD-1 negatively regulates proinflammatory cytokine production and bacterial control by DCs.[145] In the TME, an increased frequency of PD-1$^{+}$ TAMs was associated with advanced colorectal cancer stages. Intriguingly, therapeutic blockade of PD-1/PD-L1 improved the control of tumors in immunodeficient *NOD-scid IL2rynull* (NSG) mice by increasing the phagocytosis activity of TAMs against tumor cells,[149] suggesting that anti-PD-1/anti-PDL-1 blockade might also augment macrophage-mediated antitumor immunity. Recently, several lines of evidence suggested that cis interactions between PD-L1 and PD-1 or PD-L1 and B7-1 (CD80) on dendritic cells had a strong impact on T cell activation by competitively inhibiting the trans interaction between PD-L1 on APCs and PD-1 on T cells. While Zhao et al. showed that the PD-1/PD-L1 cis interaction on APCs inhibits the PD-1/PD-L1 trans interaction,[150] two different groups showed that the PD-L1 and B7-1 cis interaction was involved in the trans inhibition.[151,152] Given that only a small fraction of tumor-infiltrating DCs coexpress PD-L1 and PD-1 (20% in tumor-infiltrating DCs),[150] the B7-1/PD-L1 cis interaction might play a dominant role over the PD-1/PD-L1 cis interaction in APCs. Indeed, Sugiura et al. showed that the genetic abrogation of the B7-1/PD-L1 cis interaction hampered antitumor immunity as well as autoimmunity[151] In summary, myeloid PD-1 and PD-L1 have diverse roles in antitumor immunity, including (1) myeloid PDL-1-induced T cell suppression; (2) the myeloid cis interaction of PD-L1/B7-1 and/or PD-L1/PD-1; and (3) myeloid-intrinsic PD-1 functions (i.e., the negative regulation of phagocytosis).

### Tim-3

T cell immunoglobulin mucin-3 (TIM-3) was originally identified as an inhibitory molecule on Th1 cells and CD8 T cells that negatively regulates autoimmunity.[153] Its ligand, galectin-9, induces cell death and tolerance in activated T cells,[154,155] supporting that Tim-3 is an inhibitory immune checkpoint on T cells. As Tim-3 is frequently coexpressed with PD-1 on T cells in cancer patients, Tim-3 has been recognized as a new target of ICB.[156] Subsets of myeloid cells also express Tim-3, and myeloid Tim-3 has multifaceted roles in innate immune responses. First, Tim-4, another member of the TIM protein family, is known to provide an "eat me signal" to macrophages through the recognition of phosphatidylserine (PS) exposed on apoptotic cells.[157] Likewise, Tim-3 can also recognize PS on apoptotic cells and contributes to cross-presentation by CD8$^{+}$ DCs.[158] Indeed, coblockade of Tim-3 and Tim-4 by mAbs dampens the clearance of apoptotic cells and leads to the production of anti-double-stranded DNA,[158] indicating that Tim-3, together with Tim-4, contributes to immune homeostasis by the clearance of dying cells. By contrast, Tim-3 on tumor-associated DCs appears to have a negative impact on antitumor immunity. Chiba et al. showed that Tim-3 is expressed

on tumor-associated DCs and that Tim-3 can bind high-mobility group protein 1 (HMGB1).[159] Dying cell-derived HMGB1 and nucleic acids can stimulate innate antitumor immunity through TLRs and RIG-I, but Tim-3 on tumor-infiltrating DCs can inhibit the adjuvant effect in a competitive manner by the sequestration of an HMGB1-nucleic acid complex.[159] Another group also showed that an anti-Tim-3 blocking mAb in combination with paclitaxel chemotherapy augmented antitumor immunity against breast cancer. However, the authors demonstrated that this combination efficacy was primarily mediated by the upregulation of CXCL9 in $CD103^+$ DCs and the subsequent recruitment of $CD8^+$ T cells. They also showed that blocking galectin-9, but not HMGB1, induced CXCL9 upregulation (as also seen post-anti-Tim-3 mAb), suggesting that the galectin-9-Tim-3 interaction, rather than the HMGB1-Tim-3 interaction, plays a predominant regulatory role in tumor-infiltrating DCs.[160] Despite the controversy around these mechanisms, both studies highlighted that Tim-3 on DCs might be a potential target to harness innate immunity against cancer, especially in combination with chemotherapeutic agents. The galectin-9-Tim-3 pathway may be implicated in immunosuppression in various types of tumors, given that galectin-9 is highly expressed.[161] Notably, the galectin-9-Tim-3 interaction might critically contribute to acute myeloid leukemia (AML) progression. Kikushige et al. identified Tim-3 as a surface marker expressed on $CD34^+CD38^-$ leukemia stem cells from AML patients but not on normal hematopoietic stem cells.[162] Intriguingly, AML cells release galectin-9, which provides an autocrine loop for self-renewal of AML.[163] It remains unknown whether blockade of the galectin-9-Tim-3 interaction could have an impact on the antileukemia immune response.

### Signal regulatory protein α

Paired receptors are characterized by (1) high homology with extracellular domains and (2) the presence of activating and inhibitory members due to their differential transmembrane and cytoplasmic domains.[164] Targeting the inhibitory counterpart of paired receptors has emerged as a possible approach to augment innate immune responses against cancer. Signal regulatory protein α (SIRPα) is a member of the SIRP paired receptor family, expressed on monocytes, macrophages and neutrophils, and contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) in its cytoplasmic domain. Upon recognition of CD47, SIRPα recruits Src homology 2 (SH2) domain-containing protein tyrosine phosphatase 1 and 2 (SHP1/2), leading to negative regulation of phagocytosis. The paired counterpart, SIRPβ, is known to augment phagocytosis activity through recruitment of the activating adaptor DAP12. However, only SIRPα can bind CD47,[165,166] and there is no competition between SIRPα and SIRPβ for CD47 ligand. Thus, therapeutic blockade of the interaction between SIRPα and CD47 either by anti-CD47 mAb or anti-SIRPα mAb augments macrophage-dependent antitumor immunity.[167–169] CD47 is widely expressed on normal cells, which prevents autologous phagocytosis by providing a "don't eat me signal" to macrophages. The overexpression of CD47 is reported in various types of tumor cells and is often correlated with poor prognosis.[167,168,170] Interestingly, the MYC oncogene is known to upregulate CD47 as well as PD-L1,[7] possibly providing resistance to immune surveillance. Additionally, by analyzing the superenhancers associated with CD47 in different cancer cell lines, Betancur et al. showed that TNF/NF-κB signaling drives CD47 overexpression,[5] indicating that the inflammatory TME might confer phagocytosis resistance to tumor cells. In a recent phase I clinical trial, anti-CD47 mAb (Hu5F9-G4) in combination with rituximab (anti-CD20 mAb) was tested in patients with relapsed and refractory B cell lymphoma.[171] As an on-target effect, anemia induced by phagocytosis of erythrocytes was the most common adverse event, although therapy-related anemia is transient and manageable. Notably, the combination therapy has shown promising clinical efficacy with a complete response rate of 33% and 45% in patients with diffuse large B cell lymphoma and follicular lymphoma, respectively.[171] CD47 blockade is also able to elicit antitumor T cell responses. Mechanistically, upon the phagocytosis of tumor cells by anti-CD47, DCs are activated via STING by the recognition of tumor DNA, leading to better cross-priming.[172] Thus, enhancing phagocytosis in combination with immunotherapy and/or chemotherapy will have broad therapeutic potential.

### Inhibitory leukocyte immunoglobulin-like receptors

Members of the leukocyte immunoglobulin-like receptor (LILR) family belong to the superfamily of paired receptors and can be divided into inhibitory LILRBs (LILRB1-5) with cytoplasmic ITIM-like domains and activating LILRAs (LILRA1-A2 and A4-A6) with a positively charged arginine residue in the transmembrane domain, which is coupled with the immunoreceptor tyrosine-based activation motif (ITAM)-containing Fc receptor γ-chain (FcRγ).[164,173] It remains unknown how these activating and inhibitory paired receptors cooperatively regulate immune activation and regulation, as the ligands of LILR family receptors have not been fully identified. Inhibitory LILRB1 and LILRB2 are known to recognize classic MHC class 1 (HLA-A, HLA-B and HLA-C) and nonclassic MHC class 1 (HLA-E, HLA-F and HLA-G) in cis and in trans[174] and are the best characterized receptors among LILR family receptors. The murine ortholog of human LILRB2, inhibitory paired Ig-like receptor B (PIR-B), also recognizes MHC class 1.[175] The cis interaction between PIR-B and MHC-1 on DCs negatively regulates the priming of CD8 T cells by competing with the interaction between MHC-1 and CD8α. Adoptive transfer of antigen-pulsed $Pirb^{-/-}$ DCs showed better antitumor control by evoking effective antigen-specific CTL responses compared to WT DCs.[176] Intriguingly, PIR-B also critically regulated the maturation of myeloid cells.[177,178] Ma et al. showed that $Pirb^{-/-}$ MDSCs preferentially differentiated into M1-like macrophages in the TME and spleens and that the $Pirb^{-/-}$ deficient TME showed better tumor control.[177] Blocking mAbs against human LILRB2 were also developed, and these demonstrated reprogramming of TAMs from non-small-cell lung cancer patients into an inflammatory M1-like phenotype.[179] Additionally, given that both SIRPα and LILRBs negatively regulate cellular activity via ITIM/SHP-1/2, LILRBs might also transmit a "don't eat me signal" in monocytes and macrophages. Indeed, Barkal et al. recently showed that the expression levels of MHC class 1 molecules on tumor cells were positively correlated with resistance to phagocytosis and that blockade of LILRB1, but not LILRB2, on macrophages unleashed tumor MHC class-1-induced negative regulation of phagocytosis.[180] Importantly, the expression levels of LILRB1 were higher than LILRB2 in human primary macrophages isolated from spleens and tumor ascites, suggesting that LILRB1 is a key target to augment phagocytosis. Thus, targeting inhibitory LILRB receptors might be a potential approach to augment macrophage-mediated antitumor immune responses. Nonetheless, studies are necessary to understand the complex cis and trans binding of ligands as well as the possible roles of activating LILRA receptors.

### Sialic-acid-binding Ig-like lectin 10

Siglecs are a family of sialic acid-binding immunoglobulin-like receptors. Among Siglecs, Siglec-1 (also known as CD169) is well known as a marker expressed on a unique subset of macrophages in lymphoid organs that contributes to hematopoiesis and cross-presentation.[181,182] Sialic-acid-binding Ig-like lectin 10 (Siglec-10) contains two ITIM signaling motifs in its cytoplasmic domain, and the interaction with its ligand CD24 ameliorates inflammatory responses possibly through the recruitment of SHP1/2.[183–185] Despite the fact that overexpression of CD24 is correlated with poor prognosis in several cancers,[186,187] the functional impact of the CD24-Siglec10 axis on antitumor immune responses was not

clarified. Recently, Barkal et al. showed that TAMs in patients with breast or ovarian cancer express Siglec-10. They also showed that genetic ablation or therapeutic blockade of the CD24-Siglec10 interaction dramatically improved macrophage phagocytosis activity against tumor cells.[188] Thus, together with the SIRP1α-CD47 interaction, the CD24-Siglec10 axis has emerged as a new regulator "don't eat me signal". For clinical application, on-target adverse events will need to be carefully monitored, given that CD24 is also expressed on normal cells such as B cells, neutrophils, and nonhematopoietic cells.[189]

Scavenger receptors

Macrophages play a central role in the clearance of dying cells and cellular debris, and thus, they express a wide variety of scavenger receptors. In 1979, Goldstein et al. first identified scavenger receptors on macrophages that recognize and uptake acetylated low-density lipoprotein (LDL).[190] It is now appreciated that scavenger receptors comprise a group of structurally diverse membrane proteins with eight classes (class A–H) and that they have a strong impact on inflammatory responses, tissue repair and remodeling and innate immune responses through the recognition of a broad range of ligands, including DAMPs/PAMPs.[191] Notably, M2-polalized tissue repairing macrophages are often identified by their surface expression of scavenger receptors, such as CD206 (mannose receptor), scavenger receptor-A (SR-A), and CD163 (receptor for the hemoglobin-haptoglobin complex),[192,193] which might be explained by the fact that scavenger functions are particularly important during tissue repair and remodeling phases. Indeed, several scavenger receptors are often upregulated on TAMs and have been studied as possible therapeutic targets.[194]

SR-A (also known as CD204) recognizes a wide range of ligands, including-modified LDLs, heat shock proteins, proteoglycans, and various PAMPs. It was reported that high expression levels of SR-A on TAMs correlated with tumor invasiveness and angiogenesis in patients with lung and esophagus squamous carcinoma.[195,196] Initially, Wang et al. showed enhanced efficacy of tumor vaccines in mice deficient in SR-A.[197] Recently, Neyen et al. showed that coculture of TAMs and tumor cells can enhance tumor invasiveness in an SR-A-dependent manner, and they screened for putative ligands of SR-A by mass spectrometry. Although the authors have not identified a key ligand for SR-A on TAMs, they showed that a small-molecule inhibitor for SR-A called 4F markedly inhibited tumor growth and the metastasis of ovarian and pancreatic cancers.[198] However, the molecular mechanisms of SR-A-mediated reprogramming of TAMs have yet to be clarified.

Similar to SR-A, macrophage receptor with collagenous structure (MARCO) is a member of the class A scavenger family, expressed on DCs and tissue-resident macrophages. MARCO can recognize various ligands, including oxidized LDL, crystalline silica, nucleic acids, and bacterial lipopolysaccharides.[199] Since these ligands are also recognized by TLRs, MARCO can modulate inflammatory responses by TLRs. Intriguingly, MARCO and SR-A can facilitate the activation of cytosolic PRRs (TLR3 and NLRs) by their ability to internalize ligands, while the rapid internalization/sequestration of ligands attenuates inflammatory responses by cell surface PRRs (i.e., TLR4).[200] In DCs, MARCO negatively regulates migration activity, and thus, tumor lysate-pulsed *MARCO*$^{-/-}$ DCs show superior antitumor efficacy to WT DCs when adoptively transferred.[201] Recently, Georgoudaki et al. showed that MARCO was expressed on TAMs with M2-like phenotypes and that anti-MARCO mAb inhibited tumor growth and metastasis by directing TAMs into M1-like phenotypes.[202] Intriguingly, the therapeutic efficacy of this anti-MARCO mAb was dependent on the inhibitory Fc receptor (FcγRIIb), although the exact mechanism remains unknown.

Clever-1 (common lymphatic endothelial and vascular endothelial receptor-1, also known as stabilin-1 or FEEL1) is a class H scavenger receptor that recognizes SPARC (secreted protein acidic and rich in cysteine) and modified LDL proteins.[203] Clever-1 is expressed on endothelial cells, a subset of monocytes and tumor-associated macrophages.[204,205] Palani et al. first showed that Clever-1$^{hi}$ monocytes can potently suppress Th1 responses, compared to the Clever-1$^{lo}$ subset, and that Clever-1 is markedly downregulated under M1 polarization by LPS and TNF, raising the possibility that Clever-1 might be a marker for immunosuppressive monocytes/macrophages.[205] Indeed, using mice with myeloid-specific ablation of Clever-1, they also demonstrated that the absence of clever-1 in macrophages abrogated their immunosuppressive activities, leading to better control of tumors.[206] Importantly, this phenotypic switch was associated with increased glycolytic activity and elevated mTOR signaling in macrophages, indicating that the inhibition of Clever-1 metabolically alters TAMs. Although it is appreciated that metabolic switches in the TME confer immunosuppressive phenotypes to TAMs,[207,208] therapeutic strategies to specifically redirect TAMs have not been established. Targeting scavenger receptors might inhibit the uptake of specific substrates and/or metabolic intermediates that are required for immunosuppressive activity by TAMs.

## CONCLUDING REMARKS

Recently, it became possible to reinforce T cell-mediated immune responses against cancers by ICB therapy or adoptive T cell transfer therapy. By contrast, it is still challenging to alleviate immunosuppression by targeting MDSCs and/or TAMs. This problem might be explained by various factors, including the high turnover of myeloid cells, functional compensation among different myeloid subsets, and myeloid cell heterogeneity and plasticity. While the high abundance of TAMs or neutrophils in the TME are generally associated with poor prognosis in many types of cancer,[61,209] a high infiltration of these cells predicts favorable prognosis in certain types of cancer, such as colorectal cancer.[210,211] Undoubtedly, an in-depth understanding of immunosuppressive networks in the TME will provide a clue to overcome myeloid-mediated immunosuppression and to harness innate antitumor immunity. It should be noted that MDSCs/TAMs are known to support cancer stem cells,[212–214] suggesting that myeloid-directed therapeutic approaches have broad implications in a wide range of cancer therapies in addition to immunotherapy. We do not yet know the outcomes of many ongoing clinical trials where immunosuppression by MDSCs/TAMs is being targeted; however, reprogramming these cells by targeting myeloid checkpoints seems to be a potential new approach to harness innate antitumor immunity. While the roles of inhibitory receptors and scavenger receptors on myeloid cells have been widely studied in the context of host defense against infection, their roles in the innate immune response against tumors remain largely uncharacterized. These receptors on myeloid cells might be druggable targets for immunotherapy. Finally, while the combination of T cell-based immunotherapy and myeloid-directed therapy will be a rational approach, treatment strategies will need to be carefully optimized to minimize the risk of adverse events. The hyperactivation of monocytes and macrophages might trigger cytokine release syndrome and/or hemophagocytic lymphohistiocytosis,[215,216] whereas prolonged dysfunction or depletion of myeloid cells might render patients susceptible to severe infection and/or organ injury. It is well appreciated that the depletion of intratumor regulatory T (Treg) cells is an ideal therapeutic approach to augment antitumor immunity, but systemic depletion of Treg cells causes serious immune-related adverse events.[217] Likewise, the efficacy-to-toxicity ratio might be improved by specific targeting of intratumor immunosuppressive myeloid cells using nanoparticle-mediated delivery of drugs[218] or photodynamic immunotherapy.[219] Alternatively, adoptive transfer of macrophages with chimeric antigen receptors for phagocytosis might effectively eliminate tumor cells.[220] We are at an interesting

point in the translation of cancer immunotherapies where an improved knowledge of myeloid cell checkpoints will be critical.

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## ADDITIONAL INFORMATION

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