

Roles of Tumor-Associated Macrophages in Tumor Environment and Strategies for Targeting Therapy

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Tumor-associated macrophages (TAMs) constitute a significant component of the tumor microenvironment. This work reviewed the latest progress in comprehending the function of TAMs and their strategies for cancer therapy. TAMs are highly

updated strategies for targeting TAMs and the TAM-targeting agents currently being

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Abstract

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Introduction

heterogeneous and plastic and exhibit different functional phenotypes in response to different signal stimuli. The emergence of single-cell technologies allows us to revisit their diversity in cancer. When their pro-inflammatory function is activated, antitumor tumor-associated TAMs support and activate adaptive immune cells to eliminate cancer cells through T macrophages cell-mediated killing. In the context of cancer, anti-inflammatory TAMs play a variety of ► inflammation pro-tumor functions, such as releasing cytokines to promote the recruitment of bone marrow cells, promoting tumor angiogenesis, and inhibiting cytotoxic T cell function. immunotherapy The plasticity of TAMs makes them a potential tumor therapeutic target, so finally, we

evaluated in clinical trials.

tumor microenvironment

their complex interplay between the TME and tumor cells, as

well as the subsequent progression of the tumor. TAMs perform the "double-edged sword" function in the genesis and progression of tumor cells, with heterogeneous characteristics from antitumor and anti-inflammatory properties to pro-tumor and pro-inflammatory properties.² In tumor tissues, TAMs respond to different stimuli in the TME to acquire different functional phenotypes, indicating that they have plasticity.³ In the early stage of tumor initiation, the immune system controls cancer development during the immunosurveillance stage, when macrophages mediate phagocytosis and elimination of cancer cells and present cancer neoantigens to T cells. Subsequently, with the progressive activation of pro-inflammatory pathways, the properties of TAMs gradually change to help tumors bypass antigen recognition and antitumor immune response mechanisms,

garnered growing interest in recent times on account of

In recent decades, significant advancements have been

achieved in the realm of cancer research. We now under-

stand that cancer is a complex ecosystem. The initiation and progression of cancer are influenced by both the intrinsic

properties of cancer cells and their interactions with the

many constituents of the tumor microenvironment (TME) in

which they are situated. TME contains a wide diversity of immune cells, cancer-associated fibroblasts, extracellular

matrix (ECM), and other secreted molecules.¹ Tumor-associ-

ated macrophages (TAMs), which are among the most

numerous immune cell populations within TME, have

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thereby promoting tumor cell proliferation and survival.⁴ Based on clinical outcomes, there is a strong correlation between the degree of TAM infiltration and poor prognosis of several types of malignancies, and the significant role of TAMs in tumor progression and metastasis,^{5–8} as well as their discussion concerning tumor immunotherapy,^{2,6,9,10} suggesting that TAMs may be a viable target for immunotherapeutic interventions.

Herein, we review the origin and functional phenotypes of TAMs, discuss and update the recent progress of the research on the influence of TAMs on tumor progression, and summarize current targets and strategies for cancer therapy with TAMs.

Origin and Diversity of Macrophages

A great deal of research on macrophage cellular biology has been conducted since immunologist Metchnikoff proposed the concept of phagocytes in the 19th century, and substantial progress has been made in this area. We can now understand the origin and classification of macrophages¹¹ and here, in this review, we just briefly present it as an introductory background. Macrophages derive from two main sources in adult tissues. One originated from circulating monocytes generated by hematopoietic stem cells in the bone marrow (BM). After entering the bloodstream, monocytes migrate to various tissues, where they undergo differentiation into macrophages that are particular to the respective tissues. Examples of such tissue-specific macrophages are osteoclasts in bone, histiocytes in connective tissue, and Kupffer cells in the liver.¹² Macrophages originating from adult BM can be primarily categorized into "classical" inflammatory monocytes (Ly6C^{Hi} CX3CR1^{Low} in mouse, CD14²⁺ CD16⁻ in human) and "nonclassical" patrolling monocytes (Ly6C^{Low} CX3CR1^{Hi}CCR2^{Low} in mouse, CD14⁺CD16²⁺ in human). The recruitment of "Classical" inflammatory monocytes to the site of infection, tissue injury, and tumor is known to play a crucial role in the regulation of the inflammatory response. The "Nonclassical" patrol monocytes have a protective function, recognizing and detecting pathogens in the blood circulation and maintaining vascular integrity, rarely extravasating into tissues to differentiate into macrophages.¹³ Additionally, they contribute to the removal of tumor debris in the hosts with tumors, as well as the recruitment and activation of natural killer (NK) cells.¹⁴

Macrophages are also derived from the fetal liver (FL) or yolk sac (YS) during embryonic development. During prenatal development, embryonic progenitors are responsible for initiating the formation of fetal tissue macrophages, which subsequently become tissue-resident macrophages (TRMs). These TRMs remain throughout an individual's lifespan, existing independently of circulating monocytes.^{15,16} Lineage-tracing studies have provided evidence indicating that microglia predominantly originate from YS macrophages,¹⁷ while Langerhans cells are mixed from YS and FL monocytes.¹⁸ Alveolar macrophages¹⁹ and Kupffer cells²⁰ are mostly derived from FL monocytes, and BM monocytes can also undergo differentiation into Kupffer cells, with a minor contribution.²¹ In other tissues, such as the intestine, dermis, heart, and pancreas,^{22–25} there is a coexistence of macrophages produced from BM monocytes and TRMs. Over time, the TRMs are gradually replaced by BM-derived macrophages. In addition to their common functions of pathogen defense, inflammatory response causing and fading, immune surveillance, and cell debris elimination, macrophage populations in these different organs have tissue-specific functions. For example, brain-resident macrophages, and microglia, participate in synaptic remodeling during development.^{26,27} Kupffer cells in the liver participate in the elimination of microorganisms and cell debris from the blood and lipid metabolism.²⁸ Osteoclasts in bone fuse to form multinucleated cells that participate in bone resorption and support hematopoiesis.²⁹

Plasticity and Phenotype of Macrophages

Macrophages are highly plastic, and their phenotypes can be modulated by several stimuli present in TME, such as immunosuppressive cytokines generated by regulatory T cells, chemokines, tumor cell products, and also by the cytokine pool of type-1 T helper (Th1) and type-2 T helper (Th2) cells. According to their activation status, function, and secretion of cytokines, macrophages are often defined as classically activated M1 macrophages (pro-inflammatory) and alternatively activated M2 macrophages (anti-inflammatory).

M1 macrophages can be induced to activate by pro-inflammatory cytokines (interferon- γ [IFN- γ]) from NK and Th1 cells, bacterial products (lipopolysaccharide, LPS) from microbial pathogens, and granulocyte-monocyte colony-stimulating factor (GM-CSF), which play a significant part in tumor resistance by promoting an inflammatory response and killing intracellular infection pathogens. M1 macrophages typically exhibit characteristics associated with antigen-presenting cells, including heightened levels of major histocompatibility complex class II (MHC II) expression and costimulatory molecules (CD68/CD80/CD86), as well as significantly enhanced phagocytosis and tumor-killing activity.³⁰ Besides, by secreting cytokines and chemokines including interlukin-12 (IL-12), C-X-C motif ligand (CXCL9), and CXCL10, M1 macrophages promote the polarization and recruitment of Th1 and Th17 lymphocytes.³ Additionally, they release IL-23 and tumor necrosis factor to stimulate the pertinent function of adaptive immune cells.³ Furthermore, to enhance their cytotoxic capabilities, M1 macrophages secrete reactive oxygen intermediates and nitric oxide.31

M2 macrophages are primarily induced by cytokines, including IL-4 and IL-13, from Th2 cells as well as transforming growth factor- β (TGF- β). These cytokines play a crucial role in immune modulation, tissue remodeling and angiogenesis, and the facilitation of tumor progression.^{32,33} M2 macrophages regulate the TME by the secretion of chemokines, namely CC-chemokine ligand 2 (CCL2) and CCL17, which serve to attract Th2 cells and T regulatory cells.³⁰ In contrast to the functional role of M1 macrophages, M2 macrophages possess the ability to release cytokines with anti-inflammatory properties, including IL-4, IL-10, and TGF- β , as well as pro-angiogenic molecules such as matrix metalloproteinases and vascular endothelial growth factor (VEGF).³² Besides, M2 macrophages also showed a notable upregulation of the mannose receptor CD206, alongside a downregulation of pro-inflammatory cytokines. The M2 macrophage population can be further categorized into distinct phenotypes, namely M2a, M2b, M2c, and M2d.³⁴

In the majority of cancer types, the signals emanating from cancer cells or normal cells inside the TME prompt TAMs to undergo a distinctive shift in their macrophage phenotype, transitioning from a pro-inflammatory state to an anti-inflammatory state.⁹ During the initial phases of tumorigenesis, TAMs exhibit an M1-like phenotype before transitioning to the M2 phenotype. The anti-inflammatory M1 phenotype of classical polarization and the pro-inflammatory M2 phenotype of alternative polarization represent two relative extremes. This simple binary classification fails to adequately capture the complexity of the polarization state of macrophages, as numerous subpopulations exhibit mixed heterogeneity.

In recent years, to address the limitations of the aforementioned approach, researchers have attempted to redefine TAM subpopulations and functions by using some newly emerging technologies, such as single-cell RNA sequencing (scRNA-seq) and mass cytometry by time-of-flight, with some progress. At present, investigations have been performed to evaluate the heterogeneity of TAMs and explore subgroup indicators in several tumor types, including non-small cell lung cancer (NSCLC) lung cancer, renal cell carcinoma, breast cancer, glioblastoma (GBM), etc.^{35–39} The study on lung cancer involved a comparative analysis of the genetic profiles of monocytes and macrophages in mice and humans, utilizing scRNA-seq technology. It was observed that the transcriptional programs used to distinguish TAMs from monocytes are conserved between mice and humans and that human and mouse macrophage subpopulations express many of the same genes despite their species-specific and complex phenotypic variability.³⁶ The findings of a comparative investigation on GBM in human and mouse subjects yield a similar conclusion,³⁹ which suggests that we can link macrophage heterogeneity across species through genetic signatures. In a human monocyte and macrophage scRNAseq study, one extracted 178,651 mononuclear phagocytes (MNPs) from 13 healthy and pathological tissues and selected 41 scRNA-seq datasets from them to construct the human MNP-VERSE.⁴⁰ In addition, monocytes and macrophages were isolated to establish MoMac-VERSE and reveal specific cell subsets that are extensively present in numerous tissues. The MNP-VERSE identifies six major MNP subsets, including cDC1, cDC2, mregDC, classical monocytes, nonclassical monocytes, and macrophages. MoMac-VERSE further identified five major subpopulations of macrophages, namely HES1, TREM2, IL4I1, C1Q, and proliferating macrophages. Among them, TREM2 and IL4I1 macrophages may be primarily monocyte-derived and exhibit immunosuppressive properties, whereas HES1 macrophages show an embryonic profile, express LYVE1, and seem to be reprogrammed into fetal macrophages during cancer development.⁴⁰

In a single-cell omics review of macrophage diversity published in 2022, the authors found that seven TAM subpopulations are retained in nearly all cancer types by reviewing recent cancer studies on scRNA-seq.⁴¹ The authors suggested renaming these TAM subpopulations based on their anticipated functions, recruitment mechanisms, and distinctive gene expressions. Additionally, the authors provided descriptions of the subpopulations' gene expression signatures and potential functions in tumor progression. The seven distinct TAM subgroups are as follows: interferon-primed TAMs, immune regulatory TAMs, inflammatory cytokineenriched TAMs, lipid-associated TAMs, pro-angiogenic TAMs, RTM-like TAMs, and proliferating TAMs. The utilization of single-cell multi-omics technology presents novel approaches for categorizing TAM subpopulations, hence enhancing our comprehension of the heterogeneity of TAMs in both mice and humans. Furthermore, the individualized investigation of the characteristics and functions of TAMs across various types of malignancies presents the potential to facilitate precise immunotherapy in the future.

The Role of TAMs in Tumor Progression

TAMs, being the most pervasive infiltrating leukocytes in the TME, are of significant importance in elucidating the relationship between inflammation and cancer. A growing body of research indicates that the degree of TAM infiltration is closely associated with unfavorable prognosis and drug resistance in patients with multiple cancers. TAMs play a variety of functions in tumor progression, not only directly affecting different stages of tumor development, including tumor proliferation, metastasis, and immune escape, but also indirectly regulating the immunosuppressive environment by engaging with other cells within the TME (**-Fig. 1**). Gaining a comprehensive understanding the function that TAMs play in the advancement of tumors could facilitate the discovering of novel therapeutic targets.

Tumorigenesis and Metastasis

The two different origins of TAMs play different roles in tumor progression, with TRMs supporting tumor cell proliferation in vivo, and BM-derived TAMs promoting tumor accumulation and spread.⁴² TRMs establish colonization inside tissue-specific niches during embryonic development. Consequently, they are already present within metastatic target organs before cancer grows and may facilitate metastasis by mediating local tissue changes. In a murine model of metastatic breast cancer, researchers observed that alveolar macrophages accumulate in the premetastatic lung via proliferation mediated by the complement C5a receptor. This accumulation leads to a reduction in the quantity and maturation of lung dendritic cells (DCs) and inhibition of Th1 cell responses, thereby enhancing lung metastasis.⁴³ In addition, the accumulation of TAMs derived from TRMs was found to be unaffected by CCR2 deficiency in another mouse model of lung cancer. Tumor cells could grow efficiently in vivo in the absence of BM-derived TAMs, suggesting that TRMs alone are capable of facilitating tumor cell



Fig. 1 The role of TAMs in tumor progression. TAMs contribute to the advancement of tumors by direct interaction with tumor cells or indirect interactions with other cells in the TME, thereby regulating the immunosuppressive environment. Initially, tumor cells recruit circulating monocytes and MDSCs from peripheral blood into tumor tissues and induce their differentiation into TAM. Moreover, TAMs are actively involved in the intricate mechanisms underlying tumor cell invasion and metastasis. TAMs contribute to the induction of EMT in tumor cells, the degradation of ECM, the facilitation of blood vessel dilation, and the perivascular cell recruitment. Furthermore, TAMs engage in interactions with several cell types, such as CTL, NK, and Treg, hence exerting regulatory control over the tumor immune microenvironment. CTL, cytotoxic T lymphocyte; EMT, epithelial-mesenchymal transition; MDSCs, myeloid-derived suppressor cells; NK, natural killer cells; TAMs, tumor-associated macrophages; TME, tumor micro-environment; Treg, regulatory T cells.

development, while BM-derived TAMs may contribute to tumor cell dissemination.⁴² Tumor cells can attract circulating monocytes from the peripheral circulation into tumor tissues by the secretion of various cytokines and chemokines, including CCL2, colony-stimulating factor 1 (CSF-1), and complement C5a. At the same time, TAMs in turn secrete cytokines that promote the proliferation and survival of tumor cells. These cytokines include epidermal growth factor (EGF), VEGF, platelet-derived growth factor, and TGF-β. In breast cancer, CCL2 secreted by TAMs can activate the PI3K/Akt/mTOR signaling pathway, form the endocrine resistance feedback loop in TME, and further promote tumor proliferation.⁴⁴ In ovarian cancer, the secretion of EGF by TAMs leads to the activation of EGFR on tumor cells, thereby upregulating VEGF/VEGFR signaling in neighboring tumor cells and promoting tumor cell proliferation and migration.

Tumor metastasis is a phenomenon in which tumor cells depart from the primary tumor site and establish colonies in other organs via circulatory or lymphatic systems. This process is widely recognized as a leading contributor to the death of cancer patients.⁴⁵ TAMs induce epithelial– mesenchymal transition (EMT) of tumor cells by the secretion of a variety of cytokines and inflammatory mediators, including IL-6, IL-8, and TGF- β . This secretion process serves to augment the invasive capabilities of tumor cells throughout the metastatic phase. Studies in pancreatic ductal adenocarcinoma (PDAC) and NSCLC have demonstrated that intratumoral macrophage density, EMT markers, and intraepithelial TGF- β levels are positively correlated with tumor grade. TAMs effectively induce EMT through TGF-B and activation of β -catenin pathways in intratumoral cancer cells, thereby promoting tumor metastasis.^{46,47} In addition, the ECM plays a crucial role as a tissue barrier against tumor metastasis.⁴⁸ TAMs facilitate the degradation of the ECM and the connections between cells and ECM by secreting matrix metalloproteinases (MMP9, MMP-12), serine proteases, and cathepsin, thereby promoting the spread and metastasis of tumor cells.^{49–51} In colorectal cancer (CRC), TAMs play a role in promoting EMT in tumor cells by regulating the JAK2/ STAT3/miR-506-3p/FoxQ1 axis to enhance tumor cell migration, invasion, and metastasis of tumor cells. Additionally, this regulatory axis leads to the production of CCL2 through a positive feedback loop, which contributes to macrophage recruitment and affects tumor progression.⁵²

The process of angiogenesis, which is essential for tumor cell metastasis, is coordinated by tumor cells and tumor stromal cells. TAMs have a significant role in several stages of angiogenesis, encompassing processes such as basal membrane disintegration, activation and migration of endothelial cells, proliferation of endothelial cells, and the development of new blood vessels.⁵³ TAMs support vascular dilatation and perivascular cell recruitment by producing pro-angiogenic factors, including VEGF, as well as angiogenic CXC chemokines such as CXCL8 and CXCL12.^{54,55} Studies have shown that the TAM subpopulation expressing angiopoietin-1 receptor (TIE2) has pro-angiogenic activity, and promotes

tumor angiogenesis and tumor metastasis, confirming the key role of TAM in tumor angiogenesis.⁵⁶ By targeting the TIE2 signaling pathway with drugs, angiogenesis can be reduced to inhibit tumor growth and metastasis.^{57,58} In the evaluation of angiogenic characteristics of TAMs using scRNA-seq, TAMs expressing the SPP1 gene were found across various tumor types (breast cancer, lung cancer, ovarian cancer, pancreatic adenocarcinoma, CRC), preferentially expressed genes related to angiogenesis. Compensatory pro-angiogenic features are also present in tumors with no SPP1 gene expression TAMs, such as VCAN in melanoma, INHBA in gastric cancer, and FN1 in kidney cancer, and high expression of these genes is associated with worse clinical outcomes and poor prognosis.⁵⁹

TAM-Mediated Immune Suppression

In addition to interacting with tumor cells, TAM can also interact with a diverse array of other cell types within TME, including T lymphocytes, B lymphocytes, and NK cells. The interactions between these cells not only impact the functionality and phenotype of TAMs in the TME but also further contribute to tumor growth by promoting immune escape.⁶⁰

T Cell

TAMs promote immunosuppression through different mechanisms. For example, TAMs impede the activation of CTL and NK cells through the secretion of immunosuppressive cytokines, IL-10, and TGF-β, while also enhancing the expression of reg, thereby promoting the immunosuppressive microenvironment.⁶¹ The presence of T cell immune checkpoint ligands (programmed cell death ligand 1, PD-L1) in TAMs may serve as a significant mechanism for TAM-mediated immunosuppression. In hepatocellular carcinoma (HCC), GBM, and pancreatic cancer (PC), PD-L1 expressed by TAMs binds to the T cell suppressor receptor programmed cell death protein 1 (PD-1), inducing apoptosis of infiltrating T cells.^{62–64} Another novel immunoregulatory ligand, V-domain Ig suppressor of T cell activation (VISTA), also exerts similar immunosuppressive functions by negatively regulating CD4⁺ T cell responses.⁶⁵ In addition, TAMs play an indirect antitumor immune role by secreting the chemokine CCL22, which facilitates the migration of Treg cells to the TME.⁶⁶ Treg subsequently mediates tumor immune escape by inhibiting CTL and NK activity through multiple mechanisms.⁶⁷ Recent studies have found that Treg can also promote the transformation of TAM to M2-like phenotype by inhibiting INF-γ production in CD8⁺ T cells.⁶⁸ Furthermore, studies based on a single-cell spatial transcriptomics approach and flow cytometry have demonstrated interactions occurring between TAMs and exhausted CD8 T cells (T^+_{ex}) in TME.⁶⁹ TAMs, which express multiple T cell suppressor receptor ligands, trigger weak T cell receptor stimulation and initiate the exhaustion program in CD8 T cells by capturing CD8 T cells in antigen-specific long-lasting synaptic interactions. At the same time, the T⁺_{ex} produces chemokines and growth factors (CSF1, MIF), which recruit more monocytes to the tumor site and prompt them to differentiate into tumorigenic TAMs.^{37,69,70}

Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) represent a diverse group of myeloid cells, including progenitors of macrophages, granulocytes, and DCs, which have immunosuppressive activity and promote tumor immune escape. Based on phenotypic and morphological characteristics, MDSCs mainly consist of two subgroups: polymorphonuclear-MDSC (PMN-MDSC) and monocytic-MDSC (M-MDSC).⁷¹ M-MDSCs are recruited to the peripheral lymphoid organs and tumor sites in response to CCL2, CCL5, CSF1, and other cytokines, and further differentiate into TAMs under the action of other factors. For example, hypoxia-inducible factor 1α (HIF- 1α) in TME can induce the differentiation of MDSCs to immunosuppressive TAMs.⁷² Studies have also shown that hypoxia might induce the increase in CD45 tyrosine phosphatase activity inside tumor MDSCs, which inhibits the activity of the STAT3 transcription factor, thus promoting the differentiation of MDSCs into TAMs.⁷³ In addition to differentiating into TAMs, MDSCs can also impede the immune response of T cell antigen-specific and nonspecific mechanisms, thus promoting immune escape from tumors.^{74,75} Examples include the restraint of T cell activation, incapacitation of activated T cells, suppression of NK cell cytotoxicity, and facilitation of macrophage polarization toward phenotypes that promote tumor growth.⁷⁶

Therapeutics Targeting TAMs

In consideration of the involvement of TAMs in various immunosuppressive processes in TME, TAM-targeting strategies have received increasing attention. In preclinical models and clinical trials, therapeutic approaches targeting TAMs have shown some promise with varying degrees of success. In general, therapeutic strategies that focus on macrophages to suppress their function of promoting tumor progression, or activating their antitumor activity, can be divided into three main directions: (1) inhibition and depletion of TAM recruitment; (2) reprogramming TAMs; (3) chimeric antigen receptor (CAR)-macrophages. Therefore, in this section, we review potential targets related to TAMs and strategies for anticancer therapy (**- Fig. 2, - Table S1-S13** [available in the online version]).

Inhibition and Depletion of TAM Recruitment

CSF-1R Blockade

CSF-1R is expressed on myeloid lineage cells, including monocytes, macrophages, and DCs, which regulates cell migration, differentiation, and survival through binding with CSF-1 or IL-34.^{77–81} CSF-1R signal controls the genetic signatures of TAM.⁸² The highly activated CSF-1/CSF-1R axis promotes EMT in inflammatory breast cancer in a special way, in which E-cadherin remains stable while vimentin expression is elevated.⁷⁹ Evidence shows that activated CSF-1R recruits and polarizes monocytes into M2-like macrophages, which accelerates tumor progression.^{83,84} Besides, activated CSF-1R also impedes the efficacy of multiple therapies, such as anti-PD-1 agents and chemotherapy.^{77,83,85} Interestingly, both CSF-1 and IL-34 are expressed by tumor-



Fig. 2 Therapeutic strategies targeting TAMs in cancer therapy. This diagram shows several therapeutic strategies for TAMs targeted in current preclinical models and clinical trials, which mainly consist of three parts: (1) inhibition and depletion of TAM recruitment; (2) reprogramming TAMs; (3) CAR-macrophages. Targeted therapy of TAMs can directly reduce tumor burden and indirectly regulate tumor microenvironment, depleting M2 macrophages and transforming them into M1 macrophages. CAR, chimeric antigen receptors; TAMs, tumor-associated macrophages.

specific T cells in these experiments. Besides myeloid cells, cancer cells could also express CSF-1R, which confers resistance to EGFR kinase inhibitors.⁸⁶ Clinically, CSF-1R expression is positively correlated with the stage or metastasis of prostate carcinoma.^{87,88} Among patients with either metastatic or node-negative breast cancer, a high CSF-1R level predicts a poor prognosis.^{89,90} In the study conducted on a cohort of patients with lung cancer, co-expression of IL-34 and CSF-1 is associated with poor prognosis and advanced stage.⁸⁰

As a member of the receptor tyrosine kinase family, CSF-1R could be inhibited by small-molecule chemicals. In an immune-compromised neuroblastoma mice model, BLZ945 helps chemotherapies suppress the tumor, which does not rely on T cells but on the depletion of TAM.⁹¹ A combination of Pexidartinib (PLX3397) and DC vaccination cooperatively inhibits mesothelioma in a mouse model,⁹² indicating the need for boosting the immune system in some tumor types. Therefore, it is not surprising that neither Pexidartinib nor PD-1 blockade is sufficient to prolong overall survival (OS) in a subcutaneous CT26 colon cancer model.93 However, in some preclinical models, CSF-1R inhibitors polarize rather than deplete macrophages. In the presence of GM-CSF, BLZ945 polarizes M2 to M1 in mice bearing proneural glioma.94 Similarly, macrophages in HCC and proneural glioma lose their M2 phenotype after the administration of Pexidartinib.^{83,95} It is necessary to mention that induced apoptosis and re-polarization are not mechanically incompatible, as a bispecific inhibitor named 3D-185 targeting CSF-1R and FGFR could kill part of the macrophages while polarizing the rest.⁹⁶

Antibodies represent another effective strategy to inhibit CSF-1R signaling. An anti-mouse CSF-1R antibody depletes most of the TAM and shows good tumor-suppressive efficacy when combined with a PD-1 inhibitor.⁹⁷ Anti-CSF-1R antibody combined with cisplatin induces class I IFN in breast lobular cancer in another study.⁹⁸ It could also cooperate with GM-CSF-secreting tumor vaccine and anti-PD-1 agent to suppress PDAC when treated before and after the therapy schedule.⁹⁹ This may be because GM-CSF recruits monocytes, which are polarized to M1 macrophages by anti-CSF-1R antibody. As previously reported, GM-CSF protects macrophages from being killed by CSF-1R inhibitors and mediates the re-polarization.^{83,94}

Although CSF-1R antibodies seem to be potential immune modulators, they perform poorly clinically. In a phase I clinical trial evaluating AMG-820, patented by Amgen, 32% of patients with mixed types of solid tumors had stable disease.¹⁰⁰ The investigators attribute the poor response to the high proportion of CRCs and the single treatment with AMG-820. However, even when combined with nivolumab after radiotherapy, half of the patients treated with cabiralizumab (BMS) still showed progressive disease.¹⁰¹ Similar results have been seen with other anti-CSF-1R antibodies. Roche developed emactuzumab (RG7155), which blocks CSF-1R signaling through its binding to the extracellular domains 4 and 5 of CSF-1R and prevents the receptor from dimerizing the interface.¹⁰² Preclinically, emactuzumab efficiently induces apoptosis of macrophages both in vitro and in vivo. Clinically, like PLX3397, emactuzumab exhibited potential in treating diffuse-type giant cell tumor (dT-GCT) patients.¹⁰³ Disappointingly, however, when combined with paclitaxel, emactuzumab showed no further benefit in patients with advanced solid tumors,¹⁰⁴ leading Roche to discontinue the trial in 2017. At the 2019 American Society of Clinical Oncology, Eli Lilly announced the results of a phase I study, which aimed to evaluate the safety and preliminary efficacy of LY3022855 in patients with metastatic breast cancer or castration-resistant prostate cancer (CRPC). LY3022855 shows limited activity with no complete or partial responses observed in cohorts. Cabiralizumab, patented by Five Prime, failed to meet its primary endpoints in a phase II trial (NCT03336216) in advanced PC. Despite the disappointing clinical news from anti-CSF-1R antibodies, small molecules pexidartinib combined with paclitaxel demonstrated favorable tolerability and exhibited preliminary encouraging efficacy in patients with advanced solid tumors.¹⁰⁵

The unfavorable clinical data of anti-CSF-1R antibodies may come from multiple aspects. It is found that IL-4 protects CD206⁺ macrophages from being depleted by CSF-1R blockade.¹⁰⁶ Glioma-bearing mice have an elevated proportion of CD8 T cells expressing IL-4, which induces macrophages toward the wound-healing phenotype.¹⁰⁷ Thus, it may cause concern to test IL-4 levels when selecting patients who may benefit. Infiltration of PMN-MDSCs may also be responsible for the resistance of CSF-1R blockade.^{78,102} CSF-1 inhibits the expression of CXCL1/8, chemokines that impede the recruitment of PMN-MDSCs into TME via CAF. However, blocking CSF-1R could reverse inhibition and create a more immunosuppressive environment. PLX3397 combined with paclitaxel benefits patients with advanced solid tumors far better than emactuzumab.¹⁰⁵ It may be a result of the simultaneous c-kit blockade activity of PLX3397, as c-kit mediates the recruitment and expansion of MDSCs.¹⁰⁸ Besides, depletion of TAM may induce T-reg infiltration as a feedback loop.¹⁰⁹ CSF-1R inhibitor could also reduce IL-15 secretion, which is required for NK cell activation and promotes cancer metastasis.¹¹⁰ What's more, CSF-1R blockade may hamper the antigen-presenting process as the differentiation and expansion of DCs are highly dependent on CSF-1R.^{111,112} The therapeutic agents chosen to be combined with the CSF-1R inhibitor also influence the outcome. CSF-1R blockade enhances the efficacy of cisplatin or oxaliplatin, which is not observed with docetaxel. It may explain why emactuzumab fails to benefit patients when combined with paclitaxel, as docetaxel is the derivative of paclitaxel, and they share similar mechanisms to arrest cell circle.98

In conclusion, whether CSF-1R inhibitors are powerful or not to treat solid tumors remains debatable. Further preclinical evidence is still required to define the value of depleting macrophages in the tumor.

Predominant Chemokines

Chemokines, representing a large family, are proteins responsible for immune cell migration. Among dozens of chemokine receptor pairs, the predominant pair for macrophage mobilization is CCR2/CCL2 (C–C chemokine receptor type2/C–C chemokine ligand 2). CCR2/CCL2 interaction is widely accepted for its role in mediating monocyte migration and TAM polarization during cancer initiation and metastasis.^{113–115} Expressed on monocytes or malignant cells, CCR2 engages with CCL2 secreted by tumor cells, stromal cells, or macrophages to mediate migration and metastasis.^{114–116} CCR2-positive macrophages in metastatic foci or primary sites are associated with an immunosuppressive environ-

ment.^{117,118} Similar to CSF-1R, CCR2/CCL2 level predicts poor prognosis in several tumor types, including oral squamous cell carcinoma, clear cell renal carcinoma, metastatic CRC, esophageal squamous cell carcinoma, etc.^{119–122} For several specific examples, ductal carcinoma in situ expressing CCR2, which tends to co-localize with CCL2-secreting fibroblasts, counts against the OS of patients.¹²³ Tumor-associated neutrophils expressing CCL2 recruit CCR2-positive macrophages to lung cancer sites and promote M2 polarization and cancer metastasis.¹²⁴ It seems reasonable to block the CCR2/CCL2 axis to treat cancer. The preclinical studies seem to identify the value of CCR2/CCL2 as a target. Combined with anti-PD-1 agents, CCR2 antagonists suppress TAM infiltration while activating CD8 T cells in cutaneous T cell lymphoma.¹²⁵ In a chemo-resistant ovarian tumor model, Carlumab (anti-CCR2 antibody) cooperates with paclitaxel or several other chemo drugs to suppress tumor growth.¹²⁶ Likewise, CCL2-neutralizing antibody improves the sensitivity of immunologically resistant tumors, such as PDAC, to radio-therapy in a mice model.¹²⁷

Clinically, in a cohort of patients with metastatic PDAC, PF-04136309 (CCR2 inhibitor) fails to provide further benefits beyond nab-paclitaxel or gemcitabine. No obvious monocyte accumulation in BM, a sign of efficacy, is observed in those patients.¹²⁸ However, in another phase I trial, PF-04136309 significantly improves the ratio of partial response seen in patients with locally advanced PDAC when combined with FOLFIRINOX (5-fluorouracil, folinic acid, irinotecan, and oxaliplatin).¹²⁹ In patients displaying response, monocytes decrease in the peripheral blood while accumulating in BM, which is a good hint for prognosis.¹³⁰ On the contrary, the clinical results of CCR2 or CCL2 blocking antibodies are discouraging. Although MLN1202, a CCR2-blocking antibody, partially suppresses tumor-bone metastasis in patients with solid tumors (NCT01015560), it fails to control cancer progress.¹³¹ Neither does it show persuasive efficacy in patients with CRPC.¹³² Though carlumab seems to be effective preclinically, it does not perform well in clinical trials on solid tumors.¹³³ Although well tolerated in patients with solid tumors, the combination of carlumab with chemotherapy proved to be inefficient in achieving long-lasting and sustained inhibition of serum CCL2 concentration, resulting in a gradual increase in free CCL2 levels during treatment.

Explanations for the unfavorable results of anti-CCR2/CCL2 antibodies are rare. However, several studies provide clues. Researchers using an osmotic pump to continuously deliver a CCL2-neutralizing antibody to mice bearing breast cancer find out that the antibody makes the situation worse, in which the CCL2 level in the serum gets even higher during the delivery.¹³⁴ Tumor-associated neutrophils may also be responsible, as more neutrophils infiltrate into the TME when CCR2 is given.¹³⁵ Besides, in a clinical study with PF-04136309, T cells in the TME expressed more PD-1, indicating an exhausted state of T cells.¹²⁸ What's more, CCR2-positive monocytes could help to resolve fibrosis in PDAC and make a contribution to chemotherapy.¹³⁶ Unfortunately, none of these studies fully illustrate the mechanisms behind the phenomenon. Like CSF-1R, the clear role of CCR2/CCL2 in tumors should be further illustrated.

As another crucial molecule expressed in monocytes, CCR5 has gained attention in recent years as an interesting target for cancer therapy. It retains macrophages after monocytes are recruited and differentiate in the metastasis site.¹¹³ The CCR5/ CCL5 axis induces VEGF expression and endothelial cell differentiation for angiogenesis.^{137,138} During the progression of Hodgkin's lymphoma, tumor cells escalate CCL5 levels by recruiting mesenchymal stromal cells, which further promotes TAM infiltration.¹³⁹ In patients with GBM, CCR5/CCL5 signaling promotes the immunosuppressive phenotype of TAM and is inversely correlated with prognosis.¹⁴⁰ Clinical trials show the preliminary efficacy of maraviroc (a CCR5 inhibitor) in patients with metastatic CRC. Leronlimab, a humanized anti-CCR5 antibody, is being tested on patients bearing triple negative breast cancer (TNBC).¹⁴¹ These restrike the hope of treating solid tumors with chemokine/chemokine receptor blockade.

Antiangiogenesis Therapeutics

VEGF-secreting TAMs accumulate in hypoxic breast cancer, suggesting the link between TAM and angiogenesis.¹⁴² The Ang1/2-Tie2 axis is another important vessel modulator. Ang1 and Ang2 share a similar structure but have different roles in angiogenesis. Ang1 activates Tie2 and stabilizes the vessels, while Ang2 antagonizes Ang1.^{143,144} Angiopoiein-1 receptor (Tie2)-positive TAM, which tends to show M2-polarized phenotype,^{55,145} is reported to promote angiogenesis.^{146,147} Tie2 macrophages could be recruited to tumor sites for angiogenesis and mediate resistance to VEGF inhibitors.¹⁴⁸ Correspondingly, overexpression of angiopoietin-2 (Ang-2) recruits more Tie2 macrophages and enhances immature blood vessel formation.¹⁴⁹ These research studies connect TAMs with angiogenesis.

Although it seems rational to block the Ang2–Tie2 axis for therapeutic purposes, single Ang2 antibody-MEDI3617 treatment fails to enhance OS in GI261 or U87 orthotopic models.¹⁵⁰ While *in vivo* experiment on MMTV-PyMT mice shows the efficacy of anti-Ang2 antibody-3.19.3 to suppress tumor growth, the hypoxic area in the tumor gets enlarged compared with the control group,⁵⁸ risking the chance of recruiting MDSCs,¹⁴⁸ even though no drug resistance is reported in the study.

VEGF could be downstream of the Ang2 signal.¹⁵¹ Bevacizumab treatment reduces IL-10-expressing circulating macrophage,¹⁵² which could be a result of Ang2-VEGF blockade. However, Ang2 impedes the normalization of blood vessels in the U87 orthotopic model with DC101 (anti-VEGFR2 antibody) treatment,¹⁵³ suggesting there may be a feedback loop between Ang2 and VEGF signals. Thus, blocking VEGF and Ang2 simultaneously seems more reliable.^{150,154,155} As pointed out, cediranib (a VEGFR tyrosine kinase inhibitor) could further suppress the growth of GI261 and U87 xenografts combined with MEDI3617 (anti-Ang2 antibody).¹⁵⁰ However, the combination does not suppress TAM infiltration nor enhance the M1/M2 ratio compared with single-agent treatment. To our surprise, blocking CSF-1 partially neutralizes OS benefits provided by combined therapy, suggesting that the benefits depend on macrophages. Another group treats GI261 (poorly vascularized) and MGG8 (highly vascularized) tumor models with anti-Ang2-VEGF bispecific antibodies (BsAbs) showing similar tumor-inhibitory activity but a different macrophage state.¹⁵⁴ Targeting VEGFA with bevacizumab unexpectedly increases the M2/M1 ratio,¹⁵⁶ and simultaneous blockade of both two targets significantly polarizes macrophages toward an M1-like phenotype with a higher M1/M2 ratio.¹⁵⁴

Clinically, a crossmab format BsAb blocking both Ang2 and VEGF-A, named vanucizumab, shows relative safety and initial patient responses in a phase I study.¹⁵⁵ However, in a phase II study conducted with patients of naïve metastatic CRC,¹⁵⁷ the BsAb failed to further prolong progression-free survival compared with bevacizumab. Another phase I trial showed superior efficacy of LY3127804, an anti-Ang2 mAb, combined with ramucirumab compared with LY3127804 treatment alone.¹⁵⁸ However, the overall response rate is not high, with only 4 in 42 patients showing partial response to LY3127804 combined with ramucirumab. Similar results are provided by other trials. Only 15% of patients treated with MEDI3617 plus bevacizumab show partial response.¹⁵⁹ In two separate clinical trials testing CVX-060 (NCT01441414/ NCT00879684), only a very small cohort (2 in 18 patients treated with CVX-060 plus axitinib) showed a partial response. The clinical trials do not provide exciting clues about the potential of targeting Ang2. More solid preclinical data are needed to find biomarkers that predict good responses to anti-Ang2 agents.

Though blocking the interaction between Ang2 and Tie2 receives intensive attention, the vascular stabilizing ability of the Ang1-Tie2 pathway reminds us of bevacizumab. Thus, activation of Tie2 may be a complementary way to block Ang2. Some therapeutics have been tested preclinically. ABTAA was designed based on this assumption. ABTAA blocks Ang2-Tie2 interaction and simultaneously activates Tie2.^{160,161} The purpose of the design is to normalize but not suppress vessels in the tumor. On the GI261 orthotopic model, ABTAA normalizes vessels with enhancing pericyte coverage and suppress TAM infiltration. In the Lewis Lung Cancer model, ABTAA further improves OS in combination with cisplatin.¹⁶¹ It provides another strategy to regulate Tie2 signaling in cancer treatment.

The studies discussed above highlight the possibility of utilizing antiangiogenesis therapeutics to modify macrophage polarization. However, we need further preclinical investigations and clinical trials to identify the thoughts.

Scavenger Receptor CD163

CD163 is a constituent of the scavenger receptor superfamily, characterized by its composition of nine scavenger receptor cysteine-rich domains. Physiologically, it is responsible for infection surveillance and promotes pro-inflammatory cyto-kine secretion.¹⁶² Pathologically, it interacts with and internalizes the Hb–Hp complex during hemolysis.¹⁶³ In tumors, CD163 is often recognized as an M2 hallmark, the level of which is inversely correlated with prognosis. For example, CD163 in macrophages predicts advanced cutaneous melanoma and poor prognosis.¹⁶⁴ CD163 can also be found on the membranes of tumor cells. A recent investigation has indicated

that CD163 takes part in the tumor growth of GBM.¹⁶⁵ Silencing of CD163 impairs the proliferation of tumor cells both in vitro and in vivo. Besides, cancer cells could be CD163-positive when fused with macrophages, showing stronger potential for metastasis.¹⁶⁶ There have been attempts to exploit CD163 to deliver anti-inflammatory drugs in ADC formats, such as antibody-dexamethasone.¹⁶⁷ Data show that the rate of drug internalization is really fast, which is a preferred characteristic of ADC. Direct cytotoxic drugs toward CD163-positive macrophages are also being explored with doxorubicin-loaded liposome coated with anti-CD163 antibody.¹⁶⁸ Recently. OncoResponse Company announced the preliminary activity of OR2805, an anti-CD163 antibody, on humanized NSG-SGM3 mice bearing lung cancer xenografts. A clinical trial is underway to test the initial efficacy of OR2805 when provided as a standalone treatment and in conjunction with a PD-1 inhibitor among patients with advanced solid tumors (NCT05094804).

CD163 could be a potential target for selective M2 depletion with antibodies, ADCs, or immunotoxins due to its narrow expression on M2. Furthermore, ADCs or immunotoxins may directly kill cancer cells that express CD163 in a complementary way.

Reprogramming TAMs

CD40 Agonist

Belonging to the tumor necrosis factor receptor (TNFR) family, CD40 is expressed in various types of cells such as B cells, DCs, macrophages, and even some tumor cells.^{169–172} The CD40–CD40L signal is of significant importance in the process of activating antigen-presenting cells and antigen

cross-presentation (**-Fig. 3**). Activation of CD40 has emerged as a promising approach to enhance the adaptive immune response within the TME.

Undeniably, inside the TME, T cells always seem to be poorly equipped policemen who should be responsible for suppressing tumor growth but always fail. However, this could be reversed by CD40 agonist/ICI combination when macrophages are activated.^{173–176} As pointed out, antigen-presenting cells expressing low secondary stimulatory signals could induce T cell exhaustion.¹⁷⁴ Therefore, the combination of CD40 agonist and anti-PD-L1 antibody is expected to elevate the secondary signal for fully activated T cells.^{173,175} In the AT3 mammary carcinoma model, CD40 agonist lowers PD1 on CD8 T cells and reverses resistance to anti-PD1 agents.¹⁷³ This could be partially attributed to the higher IL-12 level in TME, which is a hallmark of M1 and is critical for macrophages to secrete IFN-Y.^{172,177} Interestingly, in another report, higher PD-L1 expression on macrophages mediated by IFN-y leads to anti-CD40 agonist resistance in the MC38 tumor model.¹⁷⁵ These research studies establish a foundational framework for the combination of CD40 agonist and ICI. The combination of CD40 agonistic antibody with VEGFA/Ang-2 BsAb also displays further benefits.¹⁷⁸ As expected, this combination shows synergistic outcomes both in normalizing vessels and in activating macrophages with higher CD80 and CD86 expression.

Except for promoting APC activity and reversing T cell exhaustion, CD40 agonists could directly control the state of macrophages and tune them for tumor suppression. In a study combining anti-CSF-1R antibody and CD40 agonist to treat MC38-bearing mice, both the tumor size and treatment schedule affect the curative effect.¹⁷⁹ Combined therapy



Fig. 3 CD40–CD40L interaction licenses dendritic cells for activating CD8 cells. CD4⁺T cells interact with DCs through CD40–CD40L to cause cross-activation. Activated DCs secrete cytokines to promote T cell differentiation, and then cause CTL response. At the same time, CD4⁺ helper T cells obtained MHC and costimulatory molecules composed of synapses after being activated by DCs, and become CD4⁺ T helper APC, which leads to the interaction between CD4⁺ T cells and CD8⁺ T cells, resulting in CTL proliferation and memory formation. APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; DCs, dendritic cells; MHC, major histocompatibility complex.

shows higher efficacy only when the tumor reaches a larger size with more TAM infiltration. Moreover, the efficacy of combined therapy is abrogated when the anti-CSF-1R antibody is administered in advance, indicating the dependence of macrophages. It echoes another report, in which macrophages produce more pro-inflammatory cytokines like IL-12, and TNF- α while less IL-10.¹⁸⁰

CD40 agonist antibody could even suppress tumor cells independent of T cells. A partial response was observed in a patient diagnosed with PDAC when gemcitabine was administered in conjunction with CP-870,893 (CD40 agonist antibody with IgG2a format).¹⁷² Strikingly, very little T cell infiltration is observed in the primary lesion. It turns out that the macrophages, but not T cells, are the main force for tumor suppression. It is not odd, because an earlier report found that peritoneal macrophages activated by CD40 agonists could kill B16 tumor cells *ex vivo* even when T cells and NK were depleted.¹⁷⁷ The dependence on macrophages is further illustrated by an experiment conducted on B16 and NXS2 xenograft mice models *in vivo*.¹⁸¹ Thus, macrophages could be sharp swords polished by CD40 agonists.

Similar to other TNFRs, CD40 activates downstream signals only when the molecules are trimerized.^{182–185} Thus, an effective agonist should trigger the trimerization. Antibodies that could engage with FcyRIIB gain better agonistic activity because of cross-linking.^{186–189} However, it is the cross-linking, but not FcyRIIB, that is necessary for CD40 agonistic therapeutics. For example, Fabs multimerized by PEG ligation still show promising suppressive efficacy against BCL1 lymphoma. Moreover, multi-fused CD40L could fully activate CD40-expressing cells without further cross-linking.^{183,190,191} In addition to cross-linking, the rigid hinge structure imparts considerable agonistic activity to human IgG2 antibodies even when the Fc domain is depleted.^{171,189,192} CD40 agonists with the murine IgG2 format have been argued to have poor tumor suppressive activity.¹⁸⁶ Surprisingly, however, mlgG2 FGK4.5 shows encouraging efficacy in PDA, B16-OVA, and MMTV-PyMT mouse tumor models,^{172,178} indicating that binding epitopes also influence the effect of CD40 agonistic antibodies.^{187,188}

Clinically, the CD40 agonistic antibody shows a preliminary positive effect. Although SGN-40 with the IgG1 format is not effective enough against B cell lymphoma,^{193,194} hIgG2 selicrelumab (CP-870,893) in combination with gemcitabine improves clinical outcomes in patients diagnosed with metastatic melanoma.¹⁷⁶ In an additional phase I trial involving patients diagnosed with resectable PDAC, selicrelumab elevates infiltration of mature DCs, M1 macrophages, and T cells into the tumor.¹⁹⁵ In addition, both CD4⁺ and CD8⁺ T cells express more PD-1, which is a sign of activation. Systematic adverse effects of CD40 agonistic antibody could be avoided by intratumoral injection.¹⁹⁶ Patients with treatment-naïve melanoma show good responses to intratumoral administration of sotigalimab (APX005M) plus pembrolizumab (NCT02706353). The treatment is well tolerated. There are no dose-limiting toxicities and no discontinuations or deaths due to occurrence of treatment-related events. The overall response rate in this trial reached 50%. Sotigalimab is also showing potential in another trial enrolling patients diagnosed with anti-PD(L)1 refractory melanoma (NCT03123783). Partial responses were seen in 5/33 patients and the disease control rate was 48%. The data collected from PDAC and melanoma, which are thought to be highly immune-suppressed, are really attractive. However, antibodies like SGN-40 seem to be less clinically effective. This may result from different antibody epitopes or the different tumor types they treat. Also, the IgG1 format of antibodies like SGN-40 may kill macrophages or DCs by the ADCC effect. It is necessary to optimize antibody structure before initiating clinical trials.

CD47/SIRPa Antagonists

CD47, a "do not eat me" signal, is widely expressed on normal or malignant cells to prevent themselves from being phagocytosed when engaged with signal-regulatory protein α (SIRP α) (**-Fig. 4**).¹⁹⁷⁻¹⁹⁹ Preclinical evidence indicates that the CD47/SIRP α axis has a role in several pathways that contribute to drug resistance.^{200,201} Clinically, the CD47/ SIRP α axis is negatively correlated with prognosis in multiple types of cancer.^{202,203} Numerous findings make CD47/SIRP α an exciting target for boosting the immune system in tumors.

Preclinical studies targeting CD47/SIRP α with either fused protein or antibody show encouraging results. Hu5F9-G4 (IgG4 format) suppresses a wide range of small cell lung cancer cell lines *in vitro* in the presence of macrophages.²⁰⁴ It also shows promising effects against a patientderived xenograft model. Besides phagocytosis, selected CD47 antibodies in IgG2 format which show minimal Fc function could also directly induce apoptosis of tumor



Fig. 4 Blockade of CD47–SIRPα interaction promotes phagocytosis and antigen presentation. CD47 antibody and prephagocytic molecules such as calreticulin work synergically to enhance the phagocytosis of APC on tumor cells and activate CD8⁺ T cell-mediated immune responses by presenting tumor-specific antigens, thereby causing tumor killing. APC, antigen-presenting cell.

cells.²⁰⁵ Moreover, camel nanobodies with no Fc structure blocking CD47 are also efficient in suppressing SKOV3 cells engrafted on NSG (defective macrophages) mice.²⁰⁶ However, in most of the cases, single CD47 antibody treatment is not effective enough to inhibit the tumor. One way to optimize the efficacy is to combine therapeutic antibody with CD47 antibody. One group of researchers is trying to solve drug resistance to antiangiogenesis therapeutics in NSCLC with VEGFR1-SIRPα-IgG1-fused protein.²⁰¹ The effect of this fused protein is macrophage-dependent. Similarly, CD47 antibody cooperates with trastuzumab by prompting macrophage infiltration and M1 polarization in HER2-positive mammary carcinoma.²⁰⁷ The phagocytic activity of macrophages is at the core of these experiments.

However, anti-CD47 antibodies could cause anemia or thrombocytopenia due to the ubiquitous expression of CD47 molecules across various cellular populations.^{198,208,209} Accumulating evidence suggests that illustrating the Fc function of CD47 antagonists may mediate the side effects.^{210–212} However, depletion of the Fc domain may impair the efficacy. Other ways should be considered to avoid the safety issue. One of the ways is to administer therapeutic antibodies without Fc function together with SIRPα-fused protein.^{204,210} Other ways include blocking the CD47/SIRPα axis with SIRPα antagonist instead of targeting CD47,²¹¹ considering the relatively restricted expression of SIRPα.^{213,214} Another solution is to screen for antibodies or peptides that target only malignant but not normal cells.^{205,206,208}

Currently, a considerable number of therapeutic interventions that block the CD47/SIRPα axis are being actively studied in clinical trials. Based on preliminary results, CD47-blocking peptides show attractive efficacy against hematologic malignancies. In a study enrolling patients with non-Hodgkin's lymphoma refractory to rituximab, 36 and 14% of patients administrated with magrolimab (Hu5F9-G4) in combination with rituximab (NCT02953509) show complete and partial response, respectively.²¹⁵ In another trial, individuals diagnosed with relapsed/refractory hematologic malignancies exhibited favorable tolerance to treatment and initial indications of antitumor efficacy were observed with TTI-621.²¹⁶ Patients with advanced solid tumors may also benefit from anti-CD47 therapy. The administration of magrolimab in conjunction with cetuximab (NCT02953782) prolongs median OS in patients with KRAS-mutant advanced CRC compared with historical controls.²¹⁷ In addition, treatment-related increases in macrophage immune cell infiltrates in patients with stable disease and baseline T cell infiltration were associated with longer OS. Attractive results are also reported from a phase I trial of AO-176. Among 27 patients with diverse advanced solid tumors, one patient had a confirmed partial response, and seven experienced stable disease. The drug was well tolerated.²¹⁸ An additional trial evaluating AO-176 in combination with paclitaxel (NCT03834948) in patients with solid tumors is ongoing. In addition, drugs targeting CD47 can also be combined with drugs targeting PD-1 or PD-L1 to improve antitumor efficacy. In phase I research including patients with advanced solid tumors, the BsAb IBI322, which

targets both CD47 and PD-L1, demonstrated a well-regulated safety profile and exhibited encouraging antitumor effects.²¹⁹ Of the 20 patients treated with active doses, 4 achieved partial response and 7 achieved stable disease.

TREM Inhibitors

The group of cell surface receptors known as triggering receptors expressed on myeloid cells (TREM) consists of members such as TREM1 and TREM2. These receptors belong to the immunoglobulin superfamily. The expression of TREM-1 was observed to be significantly upregulated on the surface of TAMs in HCC, colon, and lung cancer. Additionally, it was found that TREM-1 played a role in inhibiting the apoptosis of macrophages.^{220–222} In human NSCLC, the expression of TREM-1 in TAMs is associated with cancer recurrence and reduced survival rates in patients with NSCLC.²²² Additionally, studies conducted on mouse xenografted NSCLC models have demonstrated that inhibiting TREM-1 can effectively decrease tumor growth and extend the lifespan of mice.²²³ Likewise, the involvement of TREM-1 has been observed in the stimulation of Kupffer cells and tumor development in a mouse HCC model.²²⁴ In addition, in the hypoxic tumor environment of HCC, HIF-1 α induced increased expression of TREM-1 in TAMs, leading to immunosuppression.²²⁵ Furthermore, TREM-1 is highly expressed in myeloid cells in patients, which is associated with poor outcomes.²²⁶ GF9, signaling chain homooligomerization (SCHOOL) peptides, can induce potent antitumor activity achieving an ideal treatment/control (T/C) value of 19%, and prolonged mouse survival in three distinct human PC xenografted mouse models.²²⁷ PY159 is a humanized monoclonal antibody that acts as a TREM-1 agonist to promote myeloid cell reprogramming and promote antitumor immunity. In preclinical models, the administration of PY159 either as a standalone treatment or in conjunction with checkpoint inhibitors led to full remission of tumors in multiple mouse subcutaneous and orthotopic tumor models.²²⁸ The ongoing clinical trial (NCT04682431) is presently assessing the efficacy of a novel treatment approach in patients diagnosed with solid tumors who exhibit resistance and refractoriness to conventional standard-of-care therapies.

The primary localization of TREM-2 is shown on the cellular membrane of monocyte-macrophage lineages, encompassing macrophages, myeloid DCs, neutrophils, microglia, and osteoclasts.²²⁹ TREM2 reduces the release of pro-inflammatory cytokines and inhibits macrophage activation by binding to the adaptor DAP12.²³⁰ Recent studies on TREM2 have also shown that TREM2 has a significant role in the modulation of TAMs and MDSCs. For example, in a study of lung cancer, it was observed that individuals diagnosed with lung cancer as well as mice with tumors had a notable increase in the presence of TREM2⁺ monocytes in their peripheral blood, in comparison to the levels observed in healthy individuals serving as controls. Besides, there is a positive correlation observed between the levels of TREM2 on macrophages surrounding tumor cells in lung cancer patients and the tumor node metastasis stage.²³¹ In addition,

they further found that TREM2+ DCs secreted more IL-10 and less IL-12, and significantly inhibited T cell proliferation. In sarcoma, CRC, and breast tumor models, TREM-2 deficiencv can delay tumor growth and increase CD8 T cells within tumors. A study conducted using The Cancer Genome Atlas database revealed that TREM-2 expression exhibited an inverse correlation with both overall and relapse-free survival in CRC and TNBC cohorts.²³² Furthermore, the scRNA-seq analysis revealed that TREM2^{high} lipid-associated macrophages have immunosuppressive capacities and facilitate tumor growth in TNBC.²³³ Upregulation of TREM2 has also been linked to the advancement of tumors in glioma, HCC, and NSCLC.^{234–236} PY314 is also a humanized monoclonal antibody that depletes TREM-expressing TAMs by binding to TREM2 through antibody-dependent cell-mediated cytotoxicity or antibody-dependent cell-mediated phagocytosis. PY314 was evaluated in a phase Ia dose-escalation study in patients with advanced solid tumors (NCT04691375). The results indicated that it had favorable tolerability and an excellent safety profile when used alone or in combination with pembrolizumab.²³⁷

TLRs Agonists

Toll-like receptors (TLRs) are integral components of the innate immune system, serving as pattern-recognition receptors for the innate immune response. TLRs, mainly expressed by DC and macrophages, can respond to bacterial membrane components (such as LPS) and intracellular nucleic acids, trigger the release of pro-inflammatory cytokines, and enable macrophages to polarize toward the M1 phenotype and exert pro-inflammatory function.²³⁸ To take full advantage of the important function of TLR agonists in the immune system, there is ongoing development of TLR agonists as potential vaccine adjuvants and antitumor agents.²³⁹ Bacillus Calmette-Guerin (BCG), a type of mycobacteria, was used early in immunotherapy for bladder cancer. The administration of BCG, known to elicit a localized immune response against tumors when applied to the skin and tumor site, has been extensively utilized in cancer treatment due to its notable clinical activity.²⁴⁰ Understanding the role of BCG has facilitated the advancement of TLR agonists for intratumoral immunotherapy.

Rintatolimod (Ampligen), a TLR3 agonist, was evaluated in combination with Pembrolizumab and Cisplatin in a phase II clinical trial in patients with platinum-sensitive ovarian cancer (NCT03734692). Interim analysis results showed that the treatment regimen was well tolerated, with most patients experiencing mild to moderate adverse effects, and some patients exhibiting remission and experiencing a prolonged period without disease progression.²⁴¹ Poly-ICLC, another TLR3 agonist, is a synthetic compound consisting of double-stranded RNA. A pilot study of Poly-ICLC in patients with solid tumors showed favorable tolerability and produced local and systemic immune responses.²⁴² In addition, a multicenter phase II clinical investigation (NCT02423863) is currently examining the use of Poly-ICLC as a standalone treatment or in conjunction with anti-PD-1 or anti-PD-L1 therapies in patients with solid

tumors. BO-112 is a double-stranded synthetic RNA consisting of poly-IC and polyethyleneimine. In both preclinical animal models and an initial clinical trial involving human subjects, administration of anti-PD-1 mAb in combination with other treatments resulted in an augmented local IFN activity and CD8 T cell infiltration, achieving partial responses in 3 of 28 patients and stable disease in 10.²⁴³ The phase II clinical trial evaluated the combination of BO-112 with pembrolizumab in patients with advanced or metastatic melanoma (NCT04570332). Of 40 patients with evaluable responses, 10 achieved responses, 3 achieved complete response, 7 achieved partial response, and 17 achieved stable disease, showing a clear trend of clinical benefit.

Monophosphoryl lipid A (MPLA) is a TLR4 agonist used clinically as a vaccine adjunct. In an experimental model of breast cancer in mice, MPLA combined with IFN-γ has been shown to effectively remodel TAMs, resulting in the inhibition of both tumor development and metastasis. It can also promote the infiltration and activation of cytotoxic T cells by macrophage-secreted cytokines.²⁴⁴ Imiquimod (Aldara), a TLR7 agonist, has been approved by the Food and Drug Administration for the treatment of superficial basal cell carcinoma.²⁴⁵ BDC-1001 is a novel immune-stimulating antibody conjugate that is coupled by trastuzumab to a TLR7/8 agonist via a noncleavable linker. In a first-in-human phase I/II study, BDC-1001 is being investigated as a monotherapy and in combination with pembrolizumab in patients with advanced HER2-expressing solid tumors (NCT04278144).

CAR-Macrophages

Currently, cancer immunotherapy based on CAR has made notable advancements in clinical practice. In particular, CAR-T cell therapy has been demonstrated to achieve a rapid and accurate tumor-killing effect in hematological malignancies. However, due to the difficulty of entering solid tumors and the influence of immunosuppressive TME, T cells are difficult to play an ideal curative effect in solid tumors.²⁴⁶ Macrophages can be widely recruited into solid tumors with better infiltration into the TME, phagocytosis function, antigen presentation, and plasticity. Therefore, CAR-macrophages (CAR-M) have recently emerged as a viable therapeutic option for the management of solid tumors, exhibiting promising prospects for further development and application. CAR-M cells consist of extracellular signaling domains that can identify particular tumor antigens, transmembrane domains, and intracellular activation signaling domains.²⁴⁷ Genetically modified CAR-M cells possess the ability to selectively recognize and eliminate tumor cells. Additionally, these cells can modify the surrounding microenvironment by releasing pro-inflammatory cytokines. Furthermore, CAR-M cells can convey tumor antigens to T cells, thereby stimulating the immune response.

A study conducted by Klichinsky and colleagues involved the genetic modification of human macrophages using HER2-targeting CARs. The researchers then proceeded to assess the efficacy of these modified CAR-M cells in terms of their ability to eliminate tumors in xenografted mouse models.²⁴⁸ In the SKOV3 human ovarian cancer mouse model, a single infusion of human CAR-M cells can effectively diminish tumor burden and extend the OS of the mice. Moreover, in a humanized mouse model, CAR-Ms can express pro-inflammatory cytokines and chemokines, transform M2 macrophages into M1 macrophages, and recruit and present antigens to T cells, playing a tumor-killing role.²⁴⁸ Based on this CAR-M cell therapy, Klichinsky and Gill co-founded a company called Carisma Therapeutics and initiated a phase I clinical trial of CT-0508, a CAR-M therapy targeting HER2, in late 2019 for the treatment of patients with recurrent/refractory HER2-overexpressing tumors (NCT04660929). In addition to HER2, Carisma has also developed CAR-M cell therapies targeting prostate-specific membrane antigen (PSMA; CT-0729) and mesothelin (CT-1119), both of which are currently in preclinical stages. CT-1119, generated using the chimeric adenovirus vector Ad5f35, expresses a scFv-containing CAR-targeting human mesothelin for the treatment of mesothelin-positive solid tumors. Preclinical investigations have demonstrated that CAR-1119 exhibits targeted phagocytosis of several tumor cell lines expressing mesothelin, employing both CAR-dependent and antigen-dependent mechanisms. Moreover, it has been observed that CAR-1119 substantially decreases tumor load in vivo, as evidenced by mouse xenograft models of lung cancer.²⁴⁹ CT-0729 targets PSMA for the treatment of metastatic CRPC. In addition, MCY-M11 is a mesothelintargeting CAR developed by MaxCyte that uses mRNA transfection of peripheral blood mononuclear cells (precursors of macrophages) to express CAR-M cells. A phase I trial (NCT03608618) is underway for the treatment of advanced ovarian cancer and peritoneal mesothelioma. Moreover, the researchers developed a CAR-M with a CD147 signaling domain, which is mainly used to destroy the ECM and facilitate T cell entry into the tumor. CAR-147 macrophages showed antitumor effects through the upregulation of IL-12 and IFN- γ inside tumor tissue, and the infusion of CAR-147 macrophages resulted in substantial suppression of tumor growth in HER2-4T1 mouse models.²⁵⁰

Conclusion

As a key component of the TME, macrophages serve a critical function in maintaining homeostasis and regulating immunity. In recent years, numerous studies have demonstrated that macrophages are implicated in every aspect of tumorigenesis, progression, and metastasis. TAMs are distinctly heterogeneous, and the previous paradigm of naming macrophages as pro-inflammatory M1 phenotype and anti-inflammatory M2 phenotype based on the Th1/Th2 nomenclature failed to explain the complex features of TAMs in disease. M1 and M2 phenotypes are not necessarily mutually exclusive but may coexist. Therefore, we cannot consider them as completely different subsets of macrophages, but need to take into account the tissue environment in which they exist, the signaling they receive, and the genetic characteristics they exhibit to provide a more objective view of macrophages. Single-cell multi-omics technologies can analyze the plasticity of TAMs and the interaction between TAMs, tumor cells, and tumor-infiltrating T cells. The clustering of TAM subsets through distinctive molecular features will help us deeply understand the heterogeneity of macrophages, and thus more accurately target TAMs in clinical practice.

Prospect

The dual functional regulation of macrophages, with both pro-tumor and antitumor properties, renders them a promising candidate for tumor therapy. By regulating the signals received from cell surface receptors, the function of TAMs can be switched from pro-tumor to antitumor. Although many targeted therapies have been developed for TAMs, some agents have increased resistance or nonspecific injury due to the nonspecificity of the targets. For example, CSF-1R inhibitors could cause the recruitment of PMN-MDSC to TME. We need to consider how to bypass blocking CSF-1R in fibroblasts and precisely target CSF-1R in TAMs or try to use single-cell omics methods to analyze whether there is a potential molecular regulatory mechanism for drug-resistant TAMs. In another approach to CD47 antagonism, the presence of CD47 on platelets and red blood cells can result in the development of anemia and thrombocytopenia when CD47 antagonists are administered. It is also necessary to develop novel agents that can reduce nonspecific toxicity. Therefore, it is imperative for us to rationally select therapeutic targets that specifically target tumor-promoting TAMs and develop agents with better targeting. Furthermore, it can also be considered to combine with other immunotherapies for combination therapy or design BsAb drugs that target both TAMs and other immunosuppressive targets to take maximum advantage of TAM-targeting therapies. Currently, BsAb trials such as vanucizumab and IBI322 have shown initial results in solid tumors, paving the way for further development of new TAM-targeting agents using other BsAb platforms.^{251,252}

In conclusion, we still have a long way to go to achieve precision therapy with TAMs, and the combination of singlecell multi-omics analysis technologies promises to help us achieve this goal.

Supporting Information

Detailed information for representative clinical trials of TAM-targeting agents and strategies for anticancer therapy (**-Table S1**, available in the online version), and chemical structural and corresponding targets of small-molecule compounds mentioned in the text (BLZ945, pexidartinib, 3D-185, PF-04136309, maraviroc, BMS 813160, cediranib, rintatolimod) (**-Table S2**, available in the online version); as well as TAMs targeting-related studies including the significant progress, advantages, and limitations (**-Table S3**, available in the online version), are included in the Supporting Information (**-Table S1-S3**, available in the online version).

Conflict of Interest None declared.

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