

# Role of macrophages in tumor progression and therapy (Review)

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**Abstract.** The number and phenotype of macrophages are closely related to tumor growth and prognosis. Macrophages are recruited to (and polarized at) the tumor site thereby promoting tumor growth, stimulating tumor angiogenesis, facilitating tumor cell migration, and creating a favorable environment for subsequent colonization by (and survival of) tumor cells. These phenomena contribute to the formation of an immunosuppressive tumor microenvironment (TME) and therefore speed up tumor cell proliferation and metastasis and reduce the efficacy of antitumor factors and therapies.

The ability of macrophages to remodel the TME through interactions with other cells and corresponding changes in their number, activity, and phenotype during conventional therapies, as well as the association between these changes and drug resistance, make tumor-associated macrophages a new target for antitumor therapies. In this review, advantages and limitations of the existing antitumor strategies targeting macrophages in Traditional Chinese and Western medicine were analyzed, starting with the effect of macrophages on tumors and their interactions with other cells and then the role of macrophages in conventional treatments was explored. Possible directions of future developments in this field from an all-around multitarget standpoint were also examined.

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**Abbreviations:** TME, tumor microenvironment; TAMs, tumor-associated macrophages; TH, T helper; IFN- $\gamma$ , interferon  $\gamma$ ; TLR, Toll-like receptor; MHC II, major histocompatibility complex II; ROS, reactive oxygen species; IL, interleukin; CSF1, colony-stimulating factor 1; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; TGF- $\beta$ , transforming growth factor  $\beta$ ; EMT, epithelial-mesenchymal transition; MMPs, matrix metalloproteinases; GC-MSCS, gastric cancer mesenchymal stem cells; ECM, extracellular matrix; CTCs, circulating tumor cells; EGF, epidermal growth factor; PIGF, placental-derived growth factor; CAFs, cancer-associated fibroblasts; HCC, hepatocellular carcinoma; CRC, colorectal cancer; MPE, malignant pleural effusion; NETs, networks; STING, stimulator of interferon genes; TCM, Traditional Chinese medicine; SCFv, single-chain variable fragment; CSF1R, colony-stimulating factor 1 receptor; CpG ODN, CpG oligodeoxynucleotides, ICI, immune checkpoint inhibitor; RBC, red blood cells

**Key words:** tumor-associated macrophage, tumor microenvironment, immunity cells, antitumor strategy, targeted therapy

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## 1. Introduction

The tumor microenvironment (TME) is a complex and special environment consisting of interrelated components and has a bidirectional impact on tumor growth. Immune cells are the main component of the TME (1). An important subset of immune cells in this context are macrophages, which can change their phenotype and status during tumor progression. These changes have a dual effect on tumor growth. Therefore, targeting macrophages as part of an antitumor strategy is a promising approach.

Previously, it was maintained that macrophages develop from circulating monocytes derived from bone marrow hematopoietic stem cells. Subsequently, macrophages were found to

originate from the yolk sac or fetal liver in healthy individuals and to not be involved in bone marrow monocyte circulation during local self-renewal (2). Macrophages can be subdivided into three categories. The first one is monocyte-derived tumor-associated macrophages (TAMs), which are the main immune cells in the TME. Accounting for approximately half of tumor-infiltrating immune cells (3), the other two categories are tissue-resident macrophages and myeloid suppressor cells from the yolk sac and fetal liver (4). This review is primarily focused on TAMs.

Studies on macrophage polarization are being actively conducted, and the prevailing theory in this regard is that macrophages can be categorized into classically activated M1 macrophages and alternatively activated M2 macrophages on the basis of interactions of the cytokines secreted by CD4<sup>+</sup> T helper (TH) cell subpopulations (5). Macrophages stimulated by interferon  $\gamma$  (IFN- $\gamma$ ) and Toll-like receptor (TLR) ligands [e.g., lipopolysaccharide (LPS)] or granulocyte-macrophage colony-stimulating factor (GM-CSF) acquire a distinct proinflammatory M1 phenotype characterized by the secretion of proinflammatory cytokines and high levels of major histocompatibility complex II (MHC II). Production of nitric oxide and reactive oxygen species (ROS) inhibits tumor growth. Conversely, TH2 cytokines such as interleukin (IL)-4, IL-13, and IL-10 polarize macrophages toward the anti-inflammatory M2 phenotype characterized by the production of anti-inflammatory cytokines that promote tumor progression (6). M2 macrophages further differentiate into M2a-M2d macrophages according to different stimuli, where M2d cells are considered TAMs (7) (Fig. 1).

For the present review PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) was used to retrieve data. The keywords searched included 'macrophages', 'tumor', 'tumor associated macrophage', 'tumor microenvironment', 'immune cell interaction', 'cancer treatment' and 'TCM antitumor'. The time frame of the studies included was ~5 years, and was extended to 10-20 years for very few studies when necessary. The final inclusion criteria were relevance to the topic, date of publication and whether the experimental theory was suitable. A total of 151 studies were selected as references.

## 2. Role of macrophages in tumor growth

Macrophages show high plasticity and heterogeneity. It is generally considered that M1 macrophages play an antitumor part in the early stages of tumor progression, and gradually transform into M2 macrophages to stimulate tumor growth. Generally, TAMs are M2 macrophages (8).

The number and phenotype of macrophages vary at different stages of tumor progression. The number of macrophages markedly increases during the early stages of tumor growth (9). Nonetheless, the association between macrophages and clinical indicators is still uncertain, and whether macrophages can be used as prognostic indicators at early cancer stages remains to be determined. At early stages of lung cancer, macrophages are significantly recruited to the tumor site and manifest a mixed phenotype, but no significant association has been identified with major clinical indicators such as tumor size and stage (10). With cancer progression, tumor cells generate relevant signaling molecules to induce macrophages

to recruit themselves to the tumor site and to polarize toward the M2 phenotype for tumor growth promotion. For example, colony-stimulating factor 1 (CSF1) produced by tumor cells can facilitate TAM aggregation and polarization toward the M2 phenotype by inhibiting CD8<sup>+</sup> T-cell recruitment to accelerate the tumor's own growth (11). As a tumor enlarges, macrophages promote its growth by secreting a series of signaling molecules including vascular endothelial growth factor (VEGF), proinflammatory factor IL-6, anti-inflammatory mediator IL-10, ROS and the corresponding proteases, tumor necrosis factor (TNF), and transforming growth factor  $\beta$  (TGF- $\beta$ ) (12). Tumor cells stimulate their own malignant progression by interacting with macrophages, and as the tumor progresses, macrophages become an indicator of tumor malignancy and reflect cancer prognosis (8) (Fig. 2).

Metastasis is the leading cause of death in patients with cancer. As a cascade process, metastasis consists of four steps, i.e., tumor cell detachment from the primary site, invasion of blood vessels or lymphatic vessels, migration to distant tissues, and proliferation at the new site (13,14). Epithelial-mesenchymal transition (EMT) is the first step in epithelial cell carcinoma metastasis (15). Epithelial cells are usually involved in human metastases (16) and can acquire the characteristics of mesenchymal cells at this step (17). Tumor cells can promote EMT by influencing macrophages. For example, gastric cancer (GC) cells interact with macrophages by secreting TNF to generate CXCL1 and CXCL5, which trigger the CXCR2/STAT3 pathway to facilitate EMT and enable the migration of GC cells (15). GC mesenchymal stem cells (GC-MSCs) can accelerate metastasis by secreting IL-6 and IL-8 to launch the JAK2/STAT3 signaling pathway. This event contributes to macrophage M2 polarization and stimulates EMT (18). Macrophages may participate in every stage of tumor metastasis. Secreted matrix metalloproteinases (MMPs) can help tumor cells degrade the extracellular matrix (ECM). In addition, MMP2 and MMP9 can promote the production of new tumor blood vessels and facilitate metastasis in many respects (19). Tumor cells penetrate blood vessels and enter the bloodstream as circulating tumor cells (CTCs) (20). In one study, mouse T241 fibrosarcoma cells and Lewis lung carcinoma-derived macrophages activated by IL-6 and TNF were coimplanted in zebra fish, and this experiment confirmed that M2 macrophages promote metastasis by driving endosmosis, suggesting that M2 macrophages are necessary for the intravascular metastatic stage (21). As CTCs enter distant tissues, the complex interaction between tumor and target tissues results in the establishment of a microenvironment that is conducive to the survival of CTCs, which is a key step for colonization by tumor cells. TAMs are implicated in the formation of a premetastatic niche (22).

Angiogenesis is a crucial factor in metastasis. There is evidence that the degree of tumor angiogenesis is positively correlated with the number of M2 macrophages in the tumor. Macrophages are major promoters of angiogenesis in the TME and act by secreting vascular growth factors and MMPs (23,24). Common proangiogenic factors include VEGF, epidermal growth factor (EGF), placental-derived growth factor (PlGF), platelet-derived growth factor, TGF- $\alpha$  and TGF- $\beta$ , and angiogenins 1 and 2. Among them, the top proangiogenic factors are PlGF and members of the VEGF family, i.e., VEGF-A,

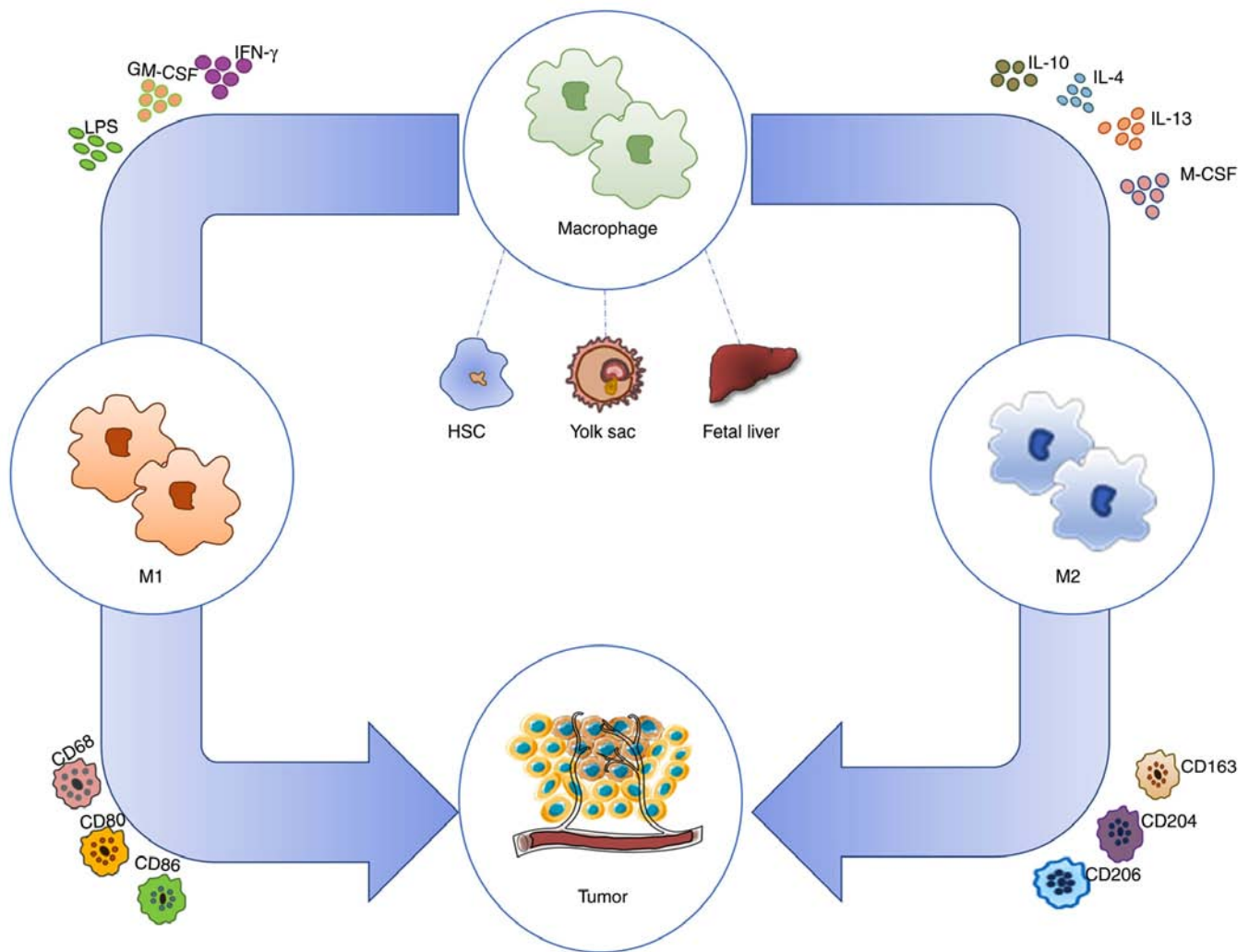


Figure 1. Origin and polarization of macrophages. In the TME, monocytes recruited by tumor cells interact with different cytokines to form TAMs with different functions. TH1 cells such as IFN- $\gamma$  and LPS polarize TAMs towards type M1. M2 is activated by cytokines secreted by TH2, such as IL-10 and IL-13. M1 and M2 are the two extremes of macrophages. TME, tumor microenvironment; TAMs, tumor-associated macrophages; TH, T helper; IFN- $\gamma$ , interferon  $\gamma$ ; IL, interleukin; LPS, lipopolysaccharide.

VEGF-B, VEGF-C, and VEGF-D (14). In triple negative breast cancer, macrophages can secrete VEGF to produce PCAT6, which upregulates VEGFR2 to accelerate angiogenesis and facilitate metastasis (25). MMPs can degrade all components of the ECM, among which MMP2, MMP9 and MMP14 are closely associated to tumor angiogenesis (26). Changes in the number and activity of macrophages can affect protease production, reduce angiogenesis, and slow tumor growth. By infecting tumor-bearing mice with *Plasmodium*, Wang *et al* revealed that *Plasmodium* hemozoin can reduce the number of infiltrating TAMs and decrease the expression of MMP9 and MMP2, thus suppressing tumor angiogenesis and slowing down metastasis and tumor growth (27).

### 3. Interaction between macrophages and the tumor immune microenvironment

The TME serves as a place where tumor cells and stromal cells can interact, including fibroblasts, endothelial cells, and both innate- and adaptive-immunity cells (28). The migration of leukocytes into the TME gives rise to the tumor immune

microenvironment (29). Macrophages constitute half of these leukocytes and play a major role in the TME (30). Therefore, focusing on the interactions between macrophages and other cells in the TME offers unique opportunities for cancer treatment (Fig. 3).

*Interactions between macrophages and cancer-associated fibroblasts (CAFs).* CAFs are the most abundant nontumor cells in the TME and perform a prominent function in tumor growth and metastasis. CAFs can simultaneously affect macrophage recruitment and polarization toward the M2 phenotype (31). CAFs are recruited and attach to macrophages under the influence of endostatin in hepatocellular carcinoma (HCC) and secrete GAS6 to promote macrophage M2-type polarization; injection of human antibody IgG78 into tumor-bearing mice specifically attenuated the impact of endostatin on the interaction of CAFs with macrophages, thereby slowing tumor growth (32). When cultured *in vitro*, CAFs from different tumors have been found to produce a large number of cytokines to drive the differentiation of monocytes into M2 macrophages; among these cytokines, the presence of the most

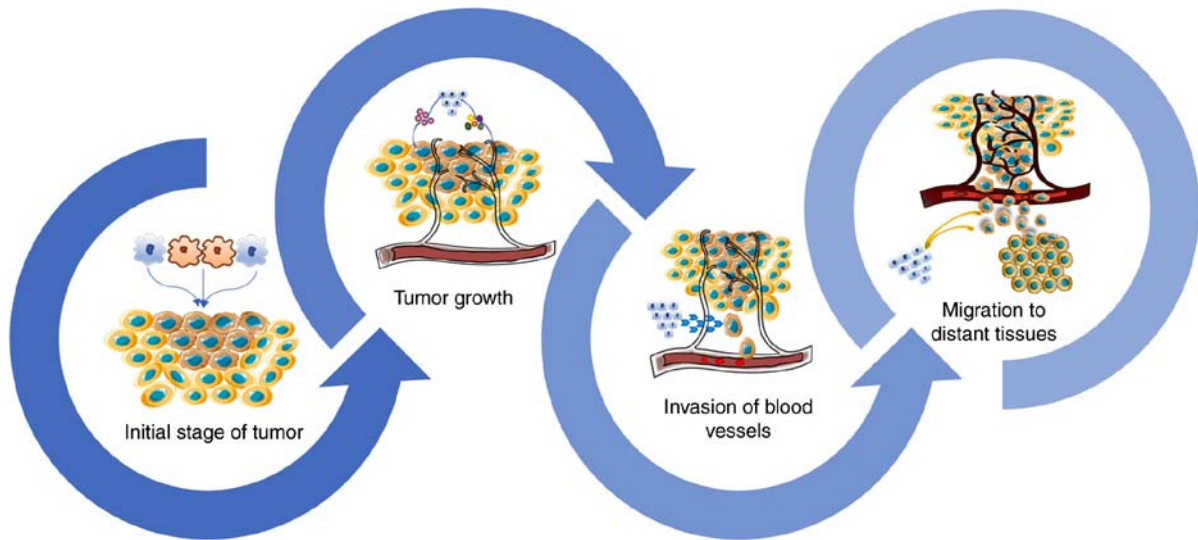


Figure 2. Role of macrophages in different stages of tumor progression. Macrophages begin to recruit to the tumor at the initial stage of the tumor, and then promote the growth of the tumor by interacting with the tumor cells during the tumor progression.

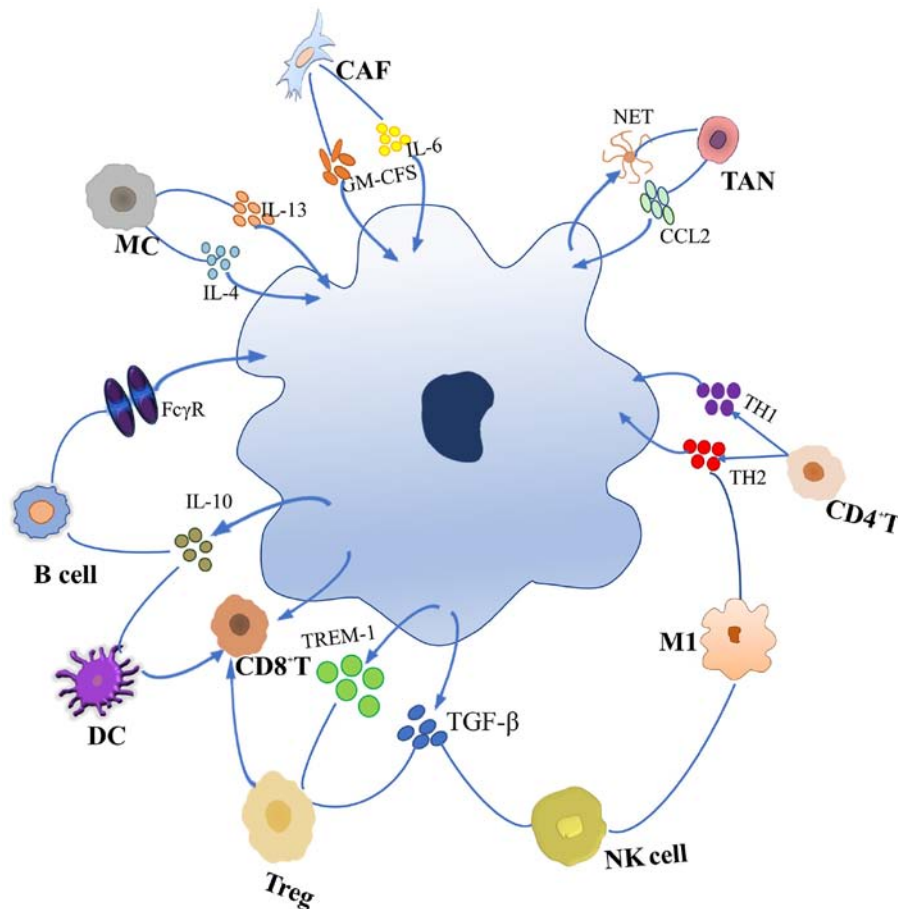


Figure 3. Interactions between TAMs and other cells in the TME. The TME mainly includes stromal cells, fibroblasts and immune cells. The interaction between TAMs and other components of the TME is a unique entry point to study the antitumor effect of macrophages. TAMs, tumor-associated macrophages; TME, tumor microenvironment.

representative factors IL-6 and GM-CSF has been confirmed in a variety of tumors, and a combination of these two factors can promote M2 polarization of TAMs and affect cancer prognosis (33). The ECM, a set of connective substances produced

by CAFs, primarily consists of special fibrin, and is related to all steps of metastasis. As the most important stromal cells, CAFs determine the hardness and structure of the ECM. The interaction between CAFs and TAMs can contribute to ECM



remodeling and tumor metastasis (34). There have been few studies on the effect of TAMs on CAFs. Nonetheless, a positive correlation between the numbers of TAMs and CAFs has been found in prostate cancer, and macrophages can induce the transformation of stromal fibroblasts into CAFs (31). Macrophages stimulate fibroblast activation via paracrine production of TGF- $\beta$ 1 in nontumor tissues. MMP9 production promotes fibroblast migration (35,36). The interaction between these two factors can synergistically accelerate tumor progression. In neuroblastomas, TAMs are mainly distributed near CAFs, and the two cell types can promote one another and jointly influence tumor growth (37). CAFs and TAMs jointly affect the clinical stage and prognosis of patients with cancer. In oral squamous cell carcinoma, CAF grade was found not only to be an independent prognostic factor of tumor progression but also to accelerate tumor progression by influencing the number and phenotype of TAMs and by creating an immunosuppressive TME (38). Tumor cells can also promote their own growth by influencing both CAFs and TAMs. Exosomes derived from colorectal cancer (CRC) cells can reduce the secretion of substances by fibroblasts and convert them into CAFs, stimulating the polarization of macrophages toward the M2 phenotype, thereby promoting CRC cell growth (39).

The two cell types mutually facilitate tumor growth, and their interplay results in the emergence of immunosuppressive activities, suggesting that a better understanding of their action is necessary for exploring effective antitumor strategies that are based on the targeting of macrophages.

*Interaction between macrophages and immune cells.* The immune cells in the TME include innate- and adaptive-immunity cells. Innate-immunity cells include macrophages, mast cells (MCs), neutrophils, dendritic cells (DCs), myeloid inhibitory cells, and natural killer (NK) cells. Adaptive-immunity cells include T and B cells (40).

*Interaction between macrophages and T cells.* After TAMs, T cells are the most important immune cells in the TME. They can recognize tumor-associated antigens and play a key role in tumor destruction (41). T cells differentiate into different subtypes with different immune functions according to the surface proteins and cytokines produced (42).

Regulatory T cells (Tregs) are a subset of CD4<sup>+</sup> T cells that promote tumor immune escape by suppressing antitumor immunity and contributing to tumor progression. In a cohort analysis of prostate cancer patients, a positive correlation was found between the numbers of Tregs and M2 macrophages, which was associated with shorter survival (43). Tregs can alter the TAM phenotype by acting on CD8<sup>+</sup> T cells. Liu *et al* revealed that tumor-derived Tregs in a mouse model could regulate metabolic adaptation of TAMs by inhibiting IFN- $\gamma$  production by CD8<sup>+</sup> T cells and promoting TAM conversion into the M2 type (44). In a hypoxic TME, TAMs express triggering receptor expressed on myeloid cell 1 in a manner dependent on hypoxia-inducible factor 1 $\alpha$  and upregulate Treg-related chemokine CCL20 to facilitate Treg recruitment to the tumor (45). In that study, there was two-way promotion between the two cell types. TAMs expressing a macrophage receptor enhanced Treg activity in non-small cell lung cancer (46). Tregs can control polarization and

the number of peritoneal macrophages at a specific site of immune-cell aggregation in the abdominal cavity. Similarly, in the peritoneum of patients with epithelial ovarian cancer, miR-29a-3p and miR-21-5p in exosomes secreted by M2 macrophages increased Treg production by downregulating the STAT3 protein (47). The interaction between the two cell types is not simple linear promotion. In malignant pleural effusion (MPE) of patients with lung cancer, TAMs produced chemokine CCL22 under the influence of TGF- $\beta$  thus driving Treg recruitment to MPE, and the IL-8 produced by Tregs in MPE could in turn promote TGF- $\beta$  expression in TAMs. In addition, IL-8 was revealed to increase the production of CCL22 and enhanced the immunosuppression in MPE (48). Depletion of Tregs can slow tumor growth in tumor-bearing mice but can also cause an increase in the number of CSF1 receptor (CSF1R)<sup>+</sup> TAMs and defeat the purpose of this form of therapy. The inhibition of Tregs and TAMs can also greatly enhance the antitumor action (11).

Tregs can interact with TAMs in a variety of ways, and the crosstalk between Tregs and TAMs suggests that the interaction between macrophages and other components of the TME should be considered carefully. To maximize the therapeutic effect, joint targeted measures should be taken instead of targeting only macrophages.

CD8<sup>+</sup> T cells are the first choice for targeted T-cell immunotherapy. They are the main antitumor lymphocytes that can directly recognize and kill tumor cells and play a crucial part in the tumor immune cycle. The concentration of CD8<sup>+</sup> T cells infiltrating tumor tissues is closely related to the efficacy of antitumor immunity, and depletion of CD8<sup>+</sup> T cells can suppress antitumor immunity (42). Studies on the crosstalk between TAMs and CD8<sup>+</sup> T cells have mostly been focused on the effect of TAMs on CD8<sup>+</sup> T cells through their interactions with Tregs, and there are few studies on the direct interplay between TAMs and CD8<sup>+</sup> T cells. In a mouse model of lung cancer, it was demonstrated that TAMs could inhibit CD8<sup>+</sup> T-cell activation through direct cell-cell contact. CD8<sup>+</sup> T cells kill TAMs through their unique antigen-specific cytotoxicity, although TAMs can become resistant to the cytotoxic effect of CD8<sup>+</sup> T cells by down-regulating the expression of cell survival genes (49). There is a negative correlation between the numbers of CD8<sup>+</sup> T cells and TAMs in GC primary foci and abdominal metastatic foci; in a comparison of the GC primary foci with the metastatic foci, it was found that the numbers of CD8<sup>+</sup> T cells were significantly lower in the abdominal metastatic foci than in the primary foci, and the prognosis was poor. Concurrently, M2 infiltration was significantly higher in the abdominal metastatic foci than in the primary foci (50). Additionally, TAMs express DC-specific C-type lectin (DC-SIGN) in bladder cancer. Most DC-SIGN<sup>+</sup> TAMs are M2 macrophages that highly express immunosuppressive cytokines. Blocking DC-SIGN using neutralizing antibodies can promote the antitumor activity of CD8<sup>+</sup> T cells. CD8<sup>+</sup> T-cell proliferation is enhanced by PD1 therapy (51).

Thus, it can be concluded from the existing literature that while TAMs reduce the activity of CD8<sup>+</sup> T cells, CD8<sup>+</sup> T cells can kill TAMs. Finding the equilibrium point of their interaction can reduce tumor immune escape and improve the efficacy of immunotherapy.

The anticancer impact of another category of T cells, CD4<sup>+</sup> T lymphocytes, has not been well studied. There are four subsets of CD4<sup>+</sup> T cells associated to antitumor immunity, i.e., TH1, TH2, TH17, and Tregs. Tregs were previously aforementioned separately because of their close association with TAMs and CD8<sup>+</sup> T cells. CD4<sup>+</sup> T cells not only have the auxiliary regulatory function of TH cells but can also directly kill tumor cells lacking MHC II expression by secreting perforin and granzyme B *in vivo* (52). CD4<sup>+</sup> TH1 cells are associated with TAM typing, and *in vitro* coculturing of these TH1 cells with peritoneal exudate cells (PECs; which can represent TAMs) results in the repolarization of M2-like PECs in order to acquire an M1-like phenotype and function. Adoptive transfer of CD4<sup>+</sup> T cells *in vivo* has been employed for constructing an ovalbumin-expressing melanoma mouse model. CD4<sup>+</sup> T cells have been reported to diminish tumor invasion by speeding up the repolarization of M2 to M1 macrophages through homologous TH1 activities (53). Macrophages can stimulate the differentiation of juvenile lymphocytes into TH1 cells, which have a tumoricidal effect similar to that of M1 macrophages and can kill nearby tumor cells. In the MOPC315 model, TH1 cells stimulate a shift of macrophages toward the M1 phenotype by producing IFN- $\gamma$ . Eventually, the interaction between the two cell types results in the elimination of tumor cells after 10–12 days (54). TH2 cells mostly take part in infection and allergic reactions (55). In tumors, TH2 cytokines promote the M2-type polarization of macrophages, and M2 macrophages can contribute to a TH2-driven response (56).

With further research on the antitumor action of CD4<sup>+</sup> T cells, the interaction between CD4<sup>+</sup> T cells and macrophages can be used to alter the polarization state of macrophages to improve their ability to kill tumor cells.

*Interaction between macrophages and DCs.* Mature DCs link the innate immune system to the adaptive immune system through their unique cross-rendering functions; as the main antigen-presenting cells, they can internalize extracellular antigens and provide them to CD4<sup>+</sup> T cells, present tumor antigens to CD8<sup>+</sup> T cells, and promote the activation of cytotoxic T lymphocytes. The association between DCs and the activation of T cells forms the basis of tumor immunotherapy (57,58). DCs have different subtypes, including plasmacytoid DCs, conventional DCs (cDC1 and cDC2 cells), and monocyte-derived DCs. Among these, cDC1 cells are the main antigen-presenting cells and are especially important for the activation of CD8<sup>+</sup> T cells (59,60). Single-cell sequencing has revealed that TAMs and cDCs form the core network of cellular action in tumors (61). To date, DC studies have largely dealt with DC vaccines, but DC vaccines have poor immunogenicity. On the other hand, various TAM inhibitors combined with a DC vaccine can improve the efficacy of the latter. In a mesothelioma mouse model, a TAM inhibitor combined with a DC vaccine was found to increase the infiltration by CD8<sup>+</sup> T cells, reduce PD-L1 expression, and enhance TAM depletion. Improving the TME composition increases the efficacy of antitumor immunity (62). IL-10 also affects the immunogenicity of DCs, and TAMs are the main source of IL-10 for breast cancer cells. IL-10 attenuates the activation of CD8<sup>+</sup> T cells by reducing the production of IL-12 by DCs, thereby affecting the efficacy of chemotherapy. In a colon cancer

model, a combination of a TAM inhibitor, IL-10 antagonist, and DC vaccine significantly increased CD8<sup>+</sup> T-cell infiltration and optimized tumor shrinkage (63,64).

A combination of a DC vaccine and TAM inhibitor has been shown to significantly improve the immunogenicity of DC vaccines. The combination of the two modalities offers another feasible approach to the targeting of TAMs for improving the efficacy of antitumor immunity in the future.

*Interaction between macrophages and NK cells.* NK cells are a part of the innate immune system and can directly target and kill tumor cells without sensitization. By secreting cytokines, NK cells can promote mutual crosstalk of immune cells in the TME, and conversely, the cytokines in the TME can reduce the killing ability of NK cells (65). NKs can interact with macrophages in different polarization states. In coculture of TAMs (or PECs), bone marrow-derived M2 macrophages, and NK cells, TAMs produced a large amount of TGF- $\beta$  and reduced the expression of CD27 in NK cells through cellular contact, thus altering the phenotype of NK cells; the CD27<sup>low</sup> NK cells have a higher activation threshold and poor cytotoxicity (66). Coculture of M1-type or LPS-treated M0 and M2 macrophages with resting NK cells can increase IFN- $\gamma$  and CCR7 production by means of IL-18. CCR7 can promote NK cells to a lymph node metastasis and upregulate their cytotoxicity, and the activated NK cells kill the remaining TAMs (67). TAM status can be affected by enhancement of NK cell activity during treatment, and TAMs, as an essential component of the TME, correlate with cancer prognosis. In a melanoma mouse model, anti-MARCO antibodies improved prognosis by activating NK cells and increasing their activity in lymph node metastases thereby driving the M1-type polarization of macrophages (68). Sorafenib is a tyrosinase inhibitor. In coculture of NK cells with TAMs, sorafenib could stimulate the production of proinflammatory cytokines, promote NK cell migration to TAMs, increase NK cell degranulation and IFN- $\gamma$  secretion by TAMs, and amplify the killing ability of NK cells (69).

Because of the complexity of the TME, NK cells cannot become fully active and cannot exert a cytotoxic action there, and macrophages with different polarization states have different effects on NK cell activity. Incorporating NK cells into macrophage-reprogramming therapy can strengthen its suppressive influence on tumors.

*Interaction between macrophages and tumor-associated neutrophils (TANs).* Neutrophils make up the largest class of circulating myeloid leukocytes, can quickly respond to invasive pathogens, and are the first line of immune defense (70). In the past, neutrophils have not been considered a significant factor in tumor growth due to their short cell cycle. Subsequent studies have revealed that the cell cycle of neutrophils in the TME is significantly prolonged, and tumor cell-derived cytokines and/or chemokines contribute to the TME accumulation of neutrophils *in vivo* (71,72). Similar to M1 and M2 macrophages, neutrophils can be categorized into N1 and N2 populations based on their different functions. TGF- $\beta$  expression can render them prone to differentiation into tumor-promoting N2 cells, whereas IFN- $\beta$  can contribute to the conversion of TANs into the antitumor N1 type (73,74). Most data indicate that TANs can promote tumor growth,

but invasive TANs are positively correlated with prognosis in only a small number of tumor types (75). Neutrophils affect tumor growth mainly by secreting proteases, generating ROS, altering angiogenesis, and speeding up metastasis. TANs and TAMs have similarities and play partially overlapping roles in tumor progression, which means that they can jointly enhance tumor growth. Both are almost always found in cluster intra-hepatic cholangiocarcinoma tissues, increase downstream target expression, and stimulate tumor growth and metastasis by generating Oncostatin M and IL-11 to trigger the STAT3 pathway (76). TANs and TAMs are both important sources of MMPs in primary tumors, and MMP2 and MMP9 are closely related to tumor angiogenesis (19). Nevertheless, a quantitative association between TAMs and TANs in tumors has not been clearly identified, and a negative correlation has been found in TNBC: A decrease in the number of TAMs accompanied by an increase in the number of TANs (71). In an HCC mouse model, the tumor volume and the metastatic rate in mice injected with TANs and tumor cells was demonstrated to be significantly increased as compared with the mice injected with the tumor cells alone. TANs can secrete CCL2 and CCL17 to recruit macrophages and Tregs in order to induce tumor invasion and angiogenesis and increase tumor microvascular density, thereby accelerating tumor growth. Experiments performed *in vitro* indicate that TANs can recruit macrophages in the same way (77). Neutrophil-derived CSF1 can promote the polarization of macrophages toward an immunosuppressive (Ly6C<sup>low</sup>/M2) phenotype in a nontumor model resulting in transplant tolerance in mice (72). In addition, activated neutrophils can release a special form of reticular ultrastructure, which is mostly composed of DNA and granular proteins that can assemble into special structures called neutrophil extracellular bactericidal networks (NETs). These can be formed after neutrophil necrosis or apoptosis to combat microorganisms. Macrophages of different phenotypes can dissolve NETs, and the proinflammatory type (M1) has the strongest effect on the dissolution of NETs (78).

In view of their overlapping sources and functions, as well as the uncertainty about their mutual influence in the TME, the correlation between TAMs and TANs in the TME cannot be exploited at present. Nonetheless, the interaction between the two cell types occurs in terms of almost every parameter of tumor growth, and research on the methods for strengthening the interaction between the two can make their crosstalk useful for improving the efficacy of cancer treatment.

*Interaction between macrophages and MCs.* MCs were first considered to be the primary effector cells of allergic reactions, and are mainly distributed in tissues and at the junction point of the host. In a tumor, MCs are mostly located at the edges of the tumor or near blood vessels; according to protease expression in MC granules, MCs are subdivided into two categories, namely, MCTs expressing only trypsinlike proteases and MCTCs expressing trypsinlike, chymotrypsin, and other proteases (79,80). MC population is highly heterogeneous and performs a dual function in tumor growth, but MCs largely contribute to tumor growth by influencing angiogenesis. In tumors, the interaction between MCs and macrophages is primarily manifested in the recruitment of macrophages (81) and induces the polarization of macrophages toward the M2

phenotype through the production of cytokines IL-4 and IL-13 (82). On the one hand, the association between the number and activity of MCs and TAMs in different tumors is unclear. It is reported that a large number of MCs in lymphoma can suppress TAM activity and reduce the promotion of the TAMs involved in tumor growth (83). On the other hand, immunohistochemical analysis of CRC tissues from patients has shown that there is a positive correlation between the numbers of MCs and TAMs (84), while there is no significant association between the two numbers in oral squamous cell carcinoma (85). Considering that both cell types are highly heterogeneous, they have dissimilar influences on tumor growth in different tumors due to the disparity in the number and state of infiltrating cells in the TME as well as in cancer stage and in the location of macrophages and MCs in the tumor. Nonetheless, because both are closely related to tumor angiogenesis, there is a distinct association between the number of both types of cells and new angiogenesis in various tumors. For example, both MC and TAM counts were independent of angiogenesis in non-small cell lung carcinoma (86). In HCC, however, both counts were positively correlated with new angiogenesis, with TAMs highly correlating with new angiogenesis (87).

Due to the high heterogeneity of the two cell types, targeted measures can be taken during cancer treatment according to the correlation between the two in a given tumor, and the inhibition of tumor neovascularization can be better utilized to reduce metastasis.

*Interaction between macrophages and B cells.* B cells play a crucial role in adaptive immunity because they can present antigens to T cells. They can also produce immunoglobulins and have an immunomodulatory influence on antitumor immunity (88). B cells can exert different actions on tumors depending on their phenotypes and interactions with other components of the TME. IL-10 is key for the ability of B cells to influence the phenotype of macrophages and can accelerate the polarization of macrophages toward the M2 phenotype without affecting the number of macrophages (89). Depletion of B cells was revealed to promote the M1-type repolarization of macrophages in a mouse model of squamous cell carcinoma (88). Macrophages upregulate CD40/CD40L via the TLR4-MyD88 pathway through cell-to-cell contact with B cells to support the activation of tumor exosomes and enhance the antigen-presenting function of B cells (90). Because B cells are the source of some hematological cancers, the depletion of B cells by antibodies binding to the B-cell-specific surface molecules, CD19 and CD20, has become an important treatment method.

By expressing FcγR, macrophages interact with immunoglobulins produced by B cells thereby affecting the depletion of B cells by the anti-CD19 and anti-CD20 therapy. Therefore, altering the number and activity of macrophages during treatment can affect the therapeutic effects of monoclonal antibodies (91). Upregulation of type I IFN gene expression by stimulator of interferon genes (STING) in lymphoma increases the production of type I IFN and enhances macrophage phagocytosis *in vitro* and *in vivo*. As a consequence, the FcγR A:I ratio of macrophages is increased and the depletion of B cells by anti-CD20 therapy is enhanced (92).

B cells, as independent regulators of the macrophage phenotype, can be used in relatively independent therapeutic approaches for the treatment of relevant tumors, and the interaction between B cells and macrophages is expected not only to improve the efficacy of monoclonal antibodies but also to become a major method for reprogramming macrophages.

#### 4. Antitumor strategies targeting macrophages

Based on the impact of macrophages on tumor progression, current treatment strategies targeting macrophages in tumors are mainly focused on altering macrophage recruitment, promoting M1-type polarization of macrophages, depletion of macrophages, delivery of antitumor drugs through macrophages, and on using these cells in combination with other therapies (93).

*Treatment of tumors by targeting macrophages by means of Traditional Chinese medicine (TCM).* In terms of the TME, TCM can slow down tumor growth by affecting the activity of immune cells; hence, TCM can change the composition of the TME (94). The effect of TCM on macrophages largely manifests itself by altering the polarization state of macrophages and inhibiting the recruitment of macrophages to tumors (95).

*TCM affects macrophage polarization.* M2 macrophages in tumor tissues are associated with a poor prognosis. Inhibition of the polarization of macrophages toward the M2 phenotype or activation of M1 macrophages is a major method for targeting macrophages during cancer treatment (96). Monotherapies and complex formulations of TCM affect tumor growth by regulating the M1/M2 ratio in the TME. For example, *Tripterygium wilfordii* can slow tumor growth by affecting apoptosis, angiogenesis, and drug resistance, among other effects. Triptolide, the active ingredient of *T. wilfordii*, can inhibit macrophage M2 polarization through dose-dependent cytotoxicity *in vivo* and *in vitro*. It also reduces the expression of IL-10 and TGF- $\beta$ 1 *in vivo* and in this way decreases the production of TH2 cytokines. Thus, the M2 polarization of TAMs is blocked thereby weakening the recruitment of TAMs to the tumor matrix and diminishing tumor growth (97). In addition, rhubarb contains emodin, a natural anthraquinone derivative. Emodin reduces lung infiltration by M2 macrophages by inhibiting STAT6 and C/EBP $\beta$  pathways and attenuates lung metastasis of breast cancer in mice (98). Ginsenosides can regulate the communication between macrophages and lung cancer cells when the two cell types are cocultured, they can reduce the protein expression of VEGF, MMP2, and MMP9 and they can repolarize M2 macrophages into the M1 phenotype to slow tumor growth and metastasis (99). The clinical dose of sorafenib can effectively inhibit tumor growth but has strong adverse effects, whereas a subclinical dose exerts milder adverse effects but has poor antitumor properties. Combining an injection of a compound kushen injection with a low dose of sorafenib in the treatment of HCC results in an increase of the M1/M2 ratio. The TME is remodeled upon the triggering of the TNF receptor TNFR1 and the downstream launch of NF- $\kappa$ B and MAPK P38 pathways, and these events cause the production of soluble molecules that indirectly increase the number and activity of CD8<sup>+</sup> T cells. These

phenomena lead to an improvement of the antitumor action of low-dose sorafenib and a reduction in its adverse effects (100). Yupingfeng powder, composed of *Astragalus membranaceus*, *Atractylodes atractylodes*, and Fangfeng, increases STAT1 phosphorylation in a dose-dependent manner, contributes to the M1 polarization of TAMs and to the remodeling of the TME, enhances the antigen-presenting function of M1 macrophages, and promotes the degranulation of CD4<sup>+</sup> T cells. As a consequence, the growth of tumor cells is slowed down, and the survival of tumor-bearing mice is prolonged (101).

*TCM affects macrophage recruitment.* TAM recruitment to tumors can enhance the stemness of cancer stem cells and accelerate metastasis (102). Monocyte precursors are the main source of TAMs, and inhibition of monocyte recruitment into tumor tissue and of the subsequent influence of macrophage infiltration is one approach to TAM-targeting therapy (103). By influencing the levels of chemokines, growth factors, and CSFs produced by cancer cells and stromal cells in the TME, the recruitment of monocyte macrophages by tumors can be suppressed (104). Studies (105–107) on the effect of TCM on macrophage recruitment have mostly addressed the influence of macrophage recruitment through the CCL2-CCR2 axis. CCL2 is also called monocyte chemoattractant protein-1, and its expression is associated with metastasis and macrophage recruitment. The corresponding receptor CCR2 is located on the surface of TAMs, and TCM can effectively inhibit metastasis by acting on the CCL2-CCR2 axis to reduce macrophage recruitment (31). Dahuang Zhechong pill was revealed to significantly reduce the expression of CCL2 in the liver of CRC tumor-bearing mice, decrease macrophage recruitment, alleviate liver fibrosis, destroy the premetastatic niche, and diminish metastasis (105,106). Dihydroisotanshinone I, an active ingredient of *Salvia miltiorrhiza*, can reduce the expression of CCL2 in THP-1 cells or RAW 264.7 cells cocultured with lung cancer cells, it can inhibit the recruitment of macrophages by tumor cells, and it can hinder the migration of lung cancer cells (107).

The number of TAMs in the TME, their polarization state, and its progression are closely related to cancer prognosis, and research aimed at macrophage-based tumor targeting has recently begun. The impact of TCM on macrophages for reshaping the TME is an effective antitumor strategy targeting macrophages. However, there are few studies on the specific mechanisms by which TCM acts on macrophages to cause the observed antitumor effects. Therefore, because changing the status of macrophages through TCM to reshape the TME can be regarded as an important means of targeting macrophages, further research in this area would be warranted.

*Conventional medicine targets macrophages to treat tumors.* With respect to the influence of macrophages on tumors, the approach of conventional medicine has consisted of targeting macrophages to treat tumors from two perspectives: Either inhibiting or supporting the presence of macrophages in tumors.

*TAM depletion.* Based on the tumor-promoting properties of M2 macrophages, selective depletion of TAMs and retention of other macrophage subtypes in the TME are expected to



alter the immunosuppressive properties of the TME and its resistance to treatment. Liposomes can engulf mouse M2 macrophages, and the number of lymphatic vessels and blood vessels in mice treated with chlorophosphonate-containing liposomes was reduced along with the number of macrophages (108). Chlorophosphonate liposomes can also engulf macrophages and suppress angiogenesis in tumor-bearing mice but affect not only M2 macrophages but also tissue macrophages and other immune cells (109,110). Considering that chlorophosphonate cannot selectively and completely target macrophages, the development of a strategy for selective depletion of macrophages is a relevant research direction in the field of macrophage-targeted therapies. In mice with PD-1-resistant melanoma, selective killing of CD163<sup>+</sup> macrophages by doxorubicin-bound antibody-conjugated lipid nanoparticles abrogated tumor growth without affecting the total number of macrophages (111). Bivalent T-cell engagers specifically recognize M2 macrophage markers and redirect endogenous T cells to M2 macrophages. In malignant ascites, FR $\beta$  bivalent T-cell engager therapy significantly increases the number of T cells that specifically deplete M2 ascites macrophages and reshape the TME (112). Although the combination with nanomaterials can better kill TAMs selectively, there is uncertainty about the long-term consequences of the TME alterations after macrophage depletion in tumors; for example, studies show that when macrophages are depleted, monoclonal antibodies cannot deplete B cells (113); further research is needed to determine whether the strategy involving macrophage depletion can be used in the clinic.

**Inhibition of macrophage recruitment.** There are three major pathways that inhibit macrophage recruitment, and one of them is the CCL2 signaling cascade. Inhibition of CCL2 and its receptors can effectively suppress macrophage recruitment and reduce metastasis. As the receptor for CCL2, CCR2 is also associated with macrophage recruitment and polarization. By combining a CCR2-targeted single-chain variable fragment (SCFv) antibody with polyvalent nanoparticles, investigators have demonstrated that high-priced 58C-SCFV can suppress macrophage recruitment to the maximum extent possible and can support M2 macrophage repolarization into the M1 phenotype (114). Nevertheless, suppression of the CCL2 pathway may accelerate metastasis. In breast cancer, inhibition of CCL2 can isolate monocytes in bone marrow and effectively reduce metastasis, but cessation of CCL2 inhibition can release monocytes into bone marrow. These monocytes migrate to a tumor site, and with increased IL-6 levels resulting in a VEGF-A release and elevated angiogenesis, these cells lead to rapid metastasis (115). Strategies involving targeted CCL2- or CCR2-based suppression of macrophage recruitment should take into account the rapid recurrence of metastasis after discontinuation of anti-CCL2 therapy; combining this approach with nanomultivalent targeting to improve the effect of macrophage recruitment through the CCL2-CCR2 axis should also be considered. Tumor cells secrete macrophage CSF (M-CSF) to recruit macrophages to the tumor; therefore, the CSF1R axis is another effective route for altering macrophage recruitment. In sarcomas, pexidartinib (PLX3397), a CSF1-CSF1R signaling inhibitor, reduces macrophage recruitment, remodels the TME, and slows tumor

growth and metastasis (116). In a mouse model of melanoma, this CSF1R antagonist (PLX3397) was found to reduce the number of TAMs, and a combination with CD8<sup>+</sup> T-mediated immunotherapy significantly delayed the action of tumor growth-enhancing intratumor T cells (117). Similarly, CSF1R antagonists have greater efficacy when applied in combination with other therapies. The CSF1-CSF1R axis has significant effects on macrophage recruitment and tumor growth suppression, and the combination of CSF1R inhibitors with other therapies can delay tumor growth to a greater extent. VEGF is also associated with macrophage recruitment and polarization. VEGFR-1 is a tyrosine kinase receptor that induces the recruitment of TAMs and promotes tumor growth after upregulation of VEGF-A, VEGF-B, or PlGF (118). VEGF-A has been shown to affect monocyte macrophage recruitment via VEGF-R1 in melanoma models (119). VEGF-1 inhibitors can not only reduce macrophage infiltration in tumors but also enhance tumor suppression when used in combination with an immune checkpoint inhibitor (ICI) (71). Combining the method influencing macrophage recruitment and remodeling of the TME through VEGF with other therapies may become a major breakthrough for future combination therapies based on the targeting of macrophages.

**Macrophage reprogramming.** Repolarizing M2 macrophages into M1 macrophages is a more promising approach than the depletion of TAMs. Toll-like receptors (TLRs), as sensors in innate immunity, represent an essential pathway for macrophage reprogramming and influence inflammatory pathways through corresponding connexins. TLR7, as an important component of this pathway, can diminish IL-10 production by delivering LET-7-equivalent TLR7 agonists and by reprogramming macrophages to reshape the TME (120). Compared with TLR agonists, STING agonists can not only regulate Fc $\gamma$ R expression *in vitro* but also reverse the inhibitory Fc $\gamma$ R spectrum *in vivo*, thereby improving the triggering of Fc $\gamma$ R on TAMs and effectively stimulating macrophage reprogramming (92). Alteration of metabolic patterns of macrophages is an essential method of their reprogramming. M1 and M2 macrophages have different metabolic phenotypes, and M2 macrophages obtain energy mainly through fatty acid oxidation and oxidative phosphorylation. Inhibition of M2 macrophage-related metabolic pathways can implement macrophage reprogramming. For instance, via upregulation of RIPK3 in tumor tissues in HCC, ROS production can be increased and PPAR lysis can be promoted, thereby effectively suppressing fatty acid metabolism and reducing the polarization of macrophages toward the M2 phenotype and slowing tumor progression (121). Dimethyl malonate treatment of tumor-bearing mice can block succinic acid production in the oxidative phosphorylation pathway, reduce macrophage conversion to the M2 phenotype, and delay tumor growth (122). In addition, proliferation and polarization of macrophages can be changed by treatments affecting macrophage RNA. In mice with Dicer1 deletions, upregulation of M1 macrophage-related cytokines by cytotoxic-T-lymphocyte-derived IFN- $\gamma$  and increasing the proportion of M1 macrophages have been shown to improve tumor suppression (123). A combination of macrophage-targeting drugs with various vectors can better influence the reprogramming

of macrophages. For instance, combining a STAT3 inhibitor called corosolic acid with long-circulating liposomes to form corosolic acid-long-circulating liposomes acting on human macrophages can reduce STAT3 expression in CD163<sup>+</sup> macrophages to a greater extent, diminish IL-10 production, and promote the switching of macrophages to the M1 phenotype (124). A combination with nanoparticles to reprogram macrophages can improve the degree of reprogramming, and a combination of a photosensitizer and nanoparticles can alter the polarization state of macrophages by increasing ROS production and increasing T-cell infiltration to enhance tumor inhibition (125). Iron oxide nanoparticles can induce macrophage repolarization and decelerate tumor growth; in particular, negatively charged iron oxide nanoparticles can maximize the conversion of M2 macrophages to the M1 type (126). A CpG oligodeoxynucleotide (CpG ODN), which is a TLR9 agonist, can contribute to the repolarization of the macrophages, however it is prone to inflammatory processes *in vivo* due to its failure to penetrate cell membranes. In one study CpG ODN was encapsulated by the strong acidic nanocellular carrier ferritin and an M2 macrophage-targeting peptide. Intravenous administration of CpG ODN slowed down tumor growth in tumor-bearing mice and switched macrophages from the M2 to M1 type. Of note, it emerged that this method can reverse the phenotype of human macrophages, and there is hope that it can be translated to clinical practice (127). The combination of macrophage-specific targeting ligands and various carrier materials pushes the reprogramming to new heights. The combination of nanomaterials can implement the reprogramming of macrophages to a greater extent and is expected to gain popularity in clinical practice; this is a key research direction for the reprogramming of macrophages in the future.

Existing antitumor strategies targeting macrophages are mainly based on the functional characteristics of macrophages. These strategies have good efficacy either alone or in combination with other therapies.

## 5. Routine therapies combined with targeted macrophage therapy

Routine therapies of tumors mainly include chemotherapy, radiotherapy and immunotherapy. Macrophages undergo known changes during conventional tumor treatments, and synergistic effects may be achieved by targeting macrophages to perform relevant adjustments (Fig. 4).

**Macrophages in chemotherapy.** As the most common cancer treatment, chemotherapy not only exerts cytotoxic actions on tumor cells but also causes a woundlike reaction in the injured tissue, drives a release of cytokines altering immune-cell infiltration of the TME, and contributes to the aggregation of macrophages in the tumor. In addition, chemotherapy also promotes the M2 polarization of macrophages, which is associated with chemotherapy resistance (128). Chemotherapy-treated pancreatic ductal adenocarcinoma cells can recruit macrophages and support their differentiation into the M2 type, and M2 macrophages decrease gemcitabine cytotoxicity by secreting deoxycytidine (129); the depletion of TAMs by means of chlorophosphonate in

pancreatic ductal adenocarcinoma significantly increases the tumor sensitivity to gemcitabine and increases apoptosis of tumor cells (130). The depletion of macrophages can improve tumor sensitivity to chemotherapy drugs, but direct depletion of macrophages has uncertain long-term effects. It is safer and more effective to combine chemotherapy with inhibiting macrophage recruitment or changing the polarization state of macrophages. In PDAC mice, gemcitabine combined with blocking TAM effector cytokines TGF- $\beta$ 1 and GM-CSF could reduce M2-polarized TAM and generate more CT8<sup>+</sup> T cells to remodel the TME, thereby improving the inhibition of gemcitabine on tumor growth (131). CSF1R protein is highly expressed in tumor tissues, which is closely related to chemotherapy resistance of various cancers (132). Although anti-CSF1R alone can inhibit macrophage recruitment and reduce the number of macrophages in tumor tissues, it cannot directly affect tumor growth and metastasis. Furthermore, a combination of an anti-CSF1R therapeutic agent and a platinum chemotherapy drug can produce synergistic effects prolonging the survival of mice with breast cancer, and on this basis, combined with neutrophil inhibitors, it can further improve the efficacy (133). The efficacy of chemotherapy is correlated with the polarization of macrophages, and paclitaxel can exert LPS-like actions in mouse models of breast cancer and melanoma by inducing M1-type repolarization of M2 macrophages in a TLR4-dependent manner, thereby reducing the immune tolerance toward cancer cells (134). A peptide with hairpin structure combined with simvastatin was revealed to drive M1-type repolarization of M2 macrophages by acting on the liver X receptors/ATP-binding box transporter A1, reduce TGF- $\beta$  secretion, reverse EMT, and diminish paclitaxel resistance in lung cancer cells (Table I) (135).

Due to the association between macrophages and chemotherapy efficacy, combination treatments involving a macrophage inhibitor can effectively alleviate chemotherapy resistance by blocking macrophage recruitment to tumors or by altering macrophage polarization. In addition, if the interaction between macrophages and other cells is considered, combined strategies targeting other cells can further improve the efficacy of chemotherapy.

**Macrophages in radiotherapy.** Radiotherapy can directly act on DNA and generate free radicals to induce apoptosis and kill tumor cells while having indirect effects on macrophages. As for the association between radiotherapy and the number of macrophages, it has been reported that radiotherapy can promote the accumulation of macrophages by affecting macrophage chemokines (136). It has also been suggested that radiotherapy can alter the gene expression of resident leukocytes, leading to a significant increase in macrophage numbers during the tumor regeneration period (14 days) after radiotherapy (137). The general theory in this field is that low-dose radiotherapy can encourage the polarization of macrophages toward the proinflammatory (M1) phenotype, whereas high-dose radiotherapy can promote the anti-inflammatory (M2) phenotype of macrophages. Nevertheless, no clear conclusion has been reached regarding the association between the radiotherapy dose and macrophage status. Research indicates that brachytherapy at 10 Gy

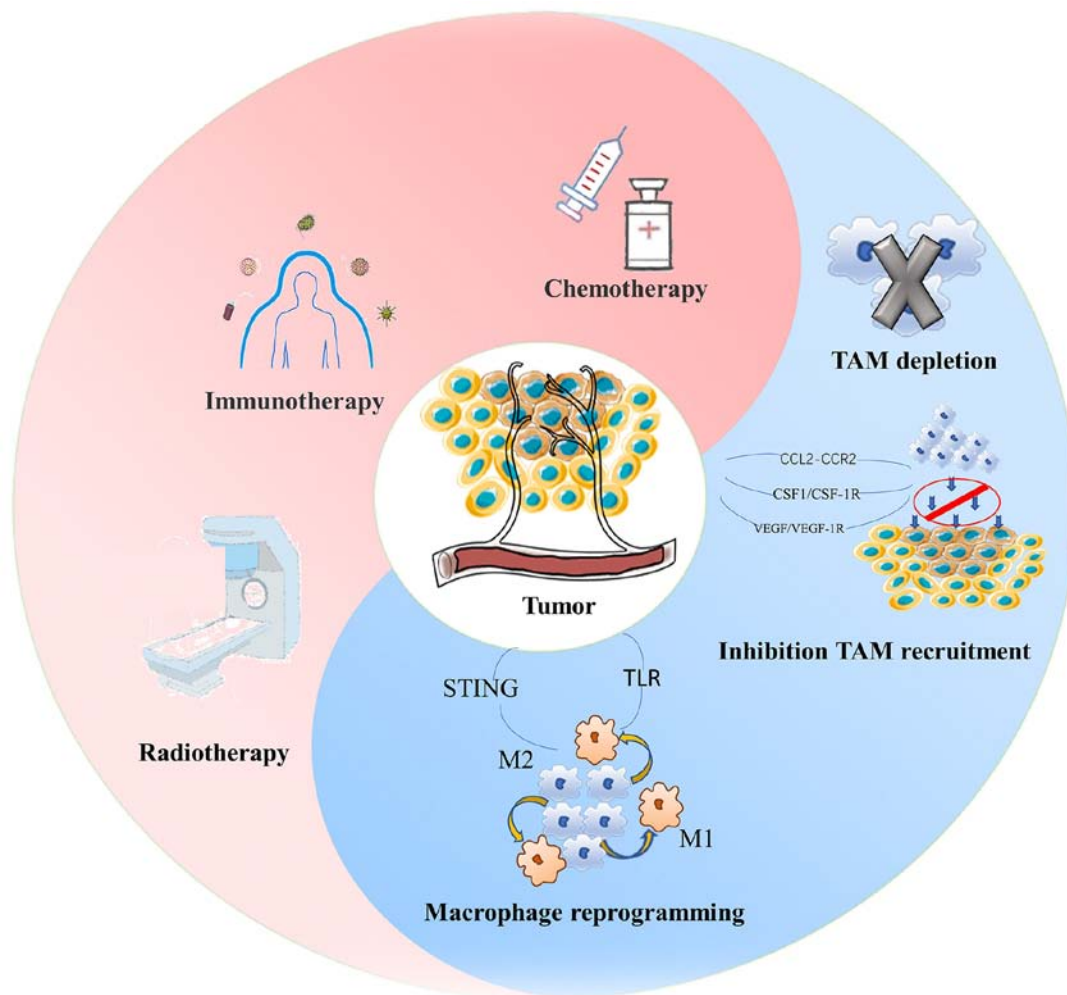


Figure 4. Strategies for targeting macrophages in combination with conventional cancer therapy. The strategies for targeting macrophages mainly include TAM depletion, Inhibition of macrophage recruitment and macrophage reprogramming. Combining these strategies with chemotherapy and radiotherapy immunotherapy is expected to improve the therapeutic effect of cancer. TAM, tumor-associated macrophage.

can most effectively reduce tumor growth in mice with melanoma, significantly increases the number of macrophages in tissues, and reduces the number of M2 macrophages (138). Other studies suggest that 10 Gy ionizing radiation can cause DNA damage in macrophages without affecting their activity and can reduce the anti-inflammatory phenotype but does not stimulate the conversion to the proinflammatory phenotype. *In vitro* manipulations of ionizing-radiation-exposed macrophages can produce a proinflammatory or anti-inflammatory phenotype (139,140). In advanced PDA, high-dose radiotherapy promoted macrophage accumulation and M2-type polarization through M-CSF, but different from conventional M2 macrophages, the M2 macrophages accumulated after radiotherapy expressed high levels of TNF- $\alpha$ . Notably, the association between radiotherapy and macrophage polarization is also time-dependent. Macrophage recruitment and M2 polarization can be observed in the early stage of radiotherapy, but such changes disappear after 8 weeks of radiotherapy (141). A combination of targeted macrophage inhibitors with radiotherapy can better suppress tumors; in mice receiving 10 Gy radiotherapy, the serum CSF1 protein content increased, and a combination with anti-CSF1 therapy reduced the number of TAMs and repolarization to the M1

type, prolonging the inhibition of tumor growth by radiotherapy (142). In breast cancer mice, radiotherapy combined with anti-CSF1 not only repolarized M2 macrophages to the M1-type, but also reduced the number of CD4<sup>+</sup> T cells and reshaped the immunosuppressive microenvironment (137). Radiotherapy can recruit TAM through M-CSF. The combination of  $\alpha$ -M-CSF mAb with radiotherapy can not only inhibit the recruitment of TAM and promote the repolarization of TAM to the M1 type, but also reduce the generation of tumor-promoting T cells and improve the inhibition of tumor growth (141). CD47 binds to the macrophage ligand SIRP $\alpha$  to inhibit macrophage phagocytosis. In breast cancer, HER2 activates NF- $\kappa$ B through the PI3K/Akt pathway to promote CD7 expression. The expression of CD47 is increased in breast cancer cells after radiotherapy, and the combination of radiotherapy and anti-CD47 anti-HER2 double receptor inhibition can maximize the phagocytosis of macrophages and improve the sensitivity of radiotherapy (143). Radiotherapy can cause local skin damage. Blocking macrophage recruitment after radiotherapy can alleviate local skin irritation and reduce local inflammation (26). Pulmonary fibrosis is a delayed side effect of thoracic radiotherapy. Pulmonary interstitial macrophages have an M2 phenotype after radiotherapy, which

Table I. Targeting macrophages in combination with chemotherapy.

Targets	Drugs	Chemotherapy	Tumors	Status	Last update posted	ClinicalTrials.gov identifier
CCL2/CCR2	CNTO 888	Gemcitabine Paclitaxel and carboplatin Docetaxel	Solid tumors	Completed	May 30, 2012	NCT01204996
	PF-04136309	Nab-paclitaxel Gemcitabine	Pancreatic ductal adenocarcinoma	Terminated	February 4, 2019	NCT02732938
CSF1/CSF1R	RO5509554	Paclitaxel	Solid Tumors	Completed	March 16, 2018	NCT 01494688
	GM-CSF	CHOP-R	Lymphoma	Terminated	January 18, 2018	NCT00455897
VEGF/ VEGFR-1	Ziv-aflibercept	Docetaxel	Fallopian tube cancer Malignant tumor of the peritoneum Recurrent ovarian epithelial cancer	Completed	February 26, 2019	NCT00436501
	Bevacizumab	Capecitabine	Breast cancer	Completed	June 18, 2014	NCT00109239
	Sevacizumab	FOLFIRI	Metastatic colorectal cancer	Unknown	April 20, 2016	NCT02453464
	VTX-2337	Paclitaxel	Ovarian epithelial cancer Tubal cancer Peritoneal cavity cancer	Completed	December 25, 2014	NCT01294293
STAT3	VTX-2337	Cyclophosphamide	Solid tumors	Terminated	September 5, 2018	NCT02650635
	Napabucasin	Paclitaxel	Non-small cell lung cancer	Terminated	June 15, 2021	NCT02826161
	Napabucasin	FOLFIRI	Colorectal cancer	Recruiting	June 18, 2019	NCT03522649

This table is according to <https://clinicaltrials.gov/>.

can induce fibroblast activation and produce the ECM. The combination of anti-CSF1R can specifically reduce interstitial macrophages and slow down the formation of pulmonary fibrosis (Table II) (144).

TAMs attenuate DNA damage caused by radiation to tumor cells. The combination of radiotherapy and targeted macrophage strategy is an effective combination therapy based on the role of macrophages in radiotherapy, which can greatly improve the inhibition of tumor growth by radiotherapy and reduce the side effects caused by radiotherapy. Although there are still conflicting theories about the association between radiotherapy and macrophages, the main problem will be solved if investigators determine the optimal radiotherapy dose that repolarizes macrophages to the M1 phenotype or suppresses their tumor recruitment. Grasping the changes of macrophages at different time-points after radiotherapy and adopting the strategy of targeting macrophages combined with radiotherapy at the most appropriate time, can not only improve the growth inhibition of tumors but also alleviate the side effects brought on by radiotherapy.

*Macrophages in immunotherapy.* Although immunotherapy has unprecedented therapeutic effects, it is also accompanied by immune nonresponse and immune tolerance in most patients (145). Currently, the main immunotherapies include immune checkpoint inhibitors and adoptive cell therapy (146). An important reason limiting the efficacy of immunotherapy is the lack of antitumor T cells in the tumor cell region. TAMs can slow down the movement of CD8<sup>+</sup> T cells in the tumor matrix and inhibit the contact between CD8<sup>+</sup> T cells and the tumor. Based on the interaction between macrophages and T cells, the combination of anti-CSF1R-specific depletion macrophages and anti-PD-1 therapy can generate T-cell chemokines, promote the infiltration of CD8<sup>+</sup> T cells in tumor islets, and increase the stable contact between CD8<sup>+</sup> T cells and tumor cells, thereby reducing tumor load (147).

Macrophages express PD-1 and its ligand PD-L1, and the phagocytosis by PD-1<sup>+</sup> TAMs is poor in immunodeficient mice. A knockout of the PD-1 ligand called PD-L1 can reduce the tumor burden and restore macrophagic

Table II. Targeting macrophages in combination with radiotherapy.

Targets	Drugs	Radiotherapy	Tumors	Status	Last update posted	ClinicalTrials.gov identifier
CSF1/CSF1R	GM-CSF	Carbon-ion radiotherapy	Hepatocellular carcinoma	Withdrawn	September 9, 2019	NCT02946138
	rhGM-CSF	Radiotherapy	Lung cancer	Recruiting	July 11, 2017	NCT03113851
VEGF/ VEGFR-1	Bevacizumab	Radiation therapy	Glioblastoma	Active, not recruiting	October 23, 2020	NCT01730950
	Bevacizumab	Radiation (IMRT)	Glioma	Completed	May 23, 2017	NCT00595322
TLR	SD-101	Radiation therapy	B-cell lymphoma	Terminated	September 4, 2020	NCT02266147
	GLA-SE	Radiation therapy	Soft tissue sarcoma	Completed	November 6, 2019	NCT02180698
STAT3	CAS3/SS3	Radiation therapy	B-cell non-Hodgkin lymphoma	Recruiting	December 28, 2021	NCT04995536

This table is according to <https://clinicaltrials.gov/>.

Table III. Targeting macrophages in combination with immunotherapy.

Targets	Drugs	Immunotherapy	Tumors	Status	Last update posted	ClinicalTrials.gov identifier
CCL2/CCR2	BMS-813160	Nivolumab	Non-small cell lung cancer Hepatocellular carcinoma	Recruiting	October 15, 2021	NCT04123379
CSF1/CSF1R	Pexidartinib	Durvalumab	Pancreatic or colorectal cancers	Completed	September 5, 2021	NCT02777710
	Cabiralizumab	Nivolumab	Peripheral T-cell lymphoma	Active, not recruiting	September 10, 2021	NCT03927105
	PLX3397	Pembrolizumab	Advanced melanoma and other solid tumors	Terminated	March 5, 2020	NCT02452424
	LY3022855	Durvalumab/ Tremelimumab	Solid tumors	Completed	January 15, 2019	NCT02718911
VEGF/ VEGFR-1	ARRY-382 BLZ945	Pembrolizumab PDR001	Solid tumors Solid tumors	Completed Active, not recruiting	March 22, 2021 January 19, 2022	NCT02880371 NCT02829723
	Axitinib	Avelumab	Renal carcinoma	Recruiting	January 6, 2021	NCT04698213
TLR	Bevacizumab	Atezolizumab	Melanoma	Recruiting	September 9, 2021	NCT04356729
	TransCon TLR7/8 Agonist MGN1703	Pembrolizumab	Solid tumors	Recruiting	January 28, 2022	NCT04799054
STAT3	BBI608	Ipilimumab	Advanced solid malignancies Colorectal cancer	Active, not recruiting Completed	November 2, 2021 August 27, 2021	NCT02668770 NCT03647839

This table according to <https://clinicaltrials.gov/>.



phagocytosis without affecting the composition of the macrophage population (148). The number and activity of macrophages significantly change after treatment with an anti-PD-L1 antibody. Macrophages with high expression of CD86 and MHC II produce TNF and IL-12 and can acquire the proinflammatory (M1) phenotype. The anti-PD-L1 antibody can shift the polarization of macrophages toward the proinflammatory phenotype by canceling out the changes in macrophage metabolic pathways via the mTOR pathway to enhance tumor suppression (123). In solid tumors, chimeric antigen receptor (CAR) T-cell therapy is combined with macrophages to form CAR macrophages (CAR-MS) by means of gene transfer, which not only overcomes the inherent resistance of macrophages to gene manipulation, but also endows them with stable M1 phenotype and upregulates antigen presentation function to stimulate T cells. CAR-MS treatment can improve the phagocytosis of macrophages and promote tumor clearance *in vitro*, and can reduce tumor load and prolong survival time *in vivo* (149). In contrast to immune checkpoint inhibitors targeting T cells, macrophage checkpoint inhibitor 5F9 suppresses the 'don't eat me' signal of CD47, strengthens macrophagic phagocytosis of tumor cells, and enhances the antigen presentation function of macrophages. 5F9 is used in combination with rituximab in patients with rituximab resistance or recurrent B-cell lymphoma. With this combination, drug toxicity can be reduced and the rate of remission increased. However, CD47 blockade can accelerate the clearance of senescent red blood cells (RBC), thus the side effects are expected targeted anemia and a small amount of anemic-associated hemolysis (150). To date, the combination of targeting macrophages to improve immunotherapy is based on the effect of macrophages on immunotherapy, either by re-educating macrophages to change the phenotype of macrophages, or by combining CD47 with SIRP $\alpha$  to affect the phagocytosis of macrophages. Gene-edited magnetic nanoparticles (gCMs-MNs) with membrane coating can be constructed by combining genetic engineering with membrane coating, which can simultaneously affect macrophage phenotype and phagocytosis. The gCM bionic shell overexpressed SIRP $\alpha$  variant, enhanced the affinity of CD47, effectively blocked the CD47-SIRP $\alpha$  signaling pathway, and improved the phagocytosis function of macrophages. The MN core promotes the repolarization of TAM to M1 in TME, improves the antigen presentation function of macrophages, and promotes the accumulation of antitumor T cells. In addition, the gCM shell protects the MN core from immune clearance. The MN core feeds the gCM shell into the TME by feedback under magnetic navigation. Through nanomaterial binding genetic engineering, the combination of macrophage reprogramming and anti-CD47 will not only improve the inhibition of tumor growth, reduce metastasis and prolong survival, but also improve the targeted side effects caused by CD47 blockers, which has a great possibility of clinical application (151). The reversal of immunotherapy drug resistance through macrophages and the development of immunosuppressants targeting macrophages are promising areas of macrophage-based immunotherapy research (Table III).

Based on the role of macrophages in conventional tumor therapy, conventional tumor therapy is combined

with antitumor strategies targeting macrophages. This not only improves tumor inhibition, but also exerts tumor inhibition through the adaptive immune system by reshaping the TME. Concurrently, it can reduce the toxic and side effects of conventional treatment, and has a considerable possibility of clinical application. More importantly, compared with the development of new antitumor drugs, the combination of targeted macrophages and conventional therapy has a more profound clinical basis, with better safety and timeliness.

## 6. Conclusions

TAMs are a highly heterogeneous population of cells and have different functional phenotypes during tumor progression. Other cells that infiltrate the TME also undergo alterations. Therefore, the dynamic interaction between TAMs and other immune cells may be a major target for future treatments involving tumor-specific macrophages. The number and state of macrophages can be changed through the interaction of the TME itself to achieve the inhibition of tumor growth. Modern medicine has developed a series of targeting strategies for macrophages by changing the number and phenotype of macrophages, starting from the changes of macrophages in the process of tumor growth and their effects on tumor growth. Combining these strategies with existing anticancer therapies has significantly improved the inhibition of tumors. In-depth investigation into the interaction between macrophages and other components of the TME and into the different roles of macrophages in conventional therapies is a key step in the treatment of tumors by means of macrophages. Due to the impact of TCM on macrophages, designing a unique multitarget approach aimed at macrophages for cancer treatment is possible. These insights however, remain only in theory thus far. There are still very few clinical validations of targeted macrophage therapies, and most of the relevant research involves only animal experiments. Therefore, increasing the number of clinical studies on macrophages is essential for future research in this field.

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## Authors' contributions

YX wrote the manuscript, searched the literature and prepared the figures and tables. XW was involved in the design of the study, and revised the manuscript. CS provided article ideas, modified the tables and revised the manuscript. LL, JWa and

JWu performed literature research and collected relevant articles. Data authentication is not applicable. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

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### Competing interests

The authors declare that they have no competing interests.

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