



# Tumor-Associated Macrophages: Therapeutic Targets of Cancer

Yubin Li, Xuyao Zhang, Xian Zeng, Shaofei Wang, and Hongbin Wang

## Abstract

It has been widely known that macrophages play critical roles during infection and immune responses. While as tumor-infiltrating myeloid cells, macrophages accumulated in the tumor microenvironment (TME) could either suppress tumor proliferation through producing pro-inflammatory cytokines or promote tumor growth via activating tumor proliferation, metastasis, and angiogenesis, tumor-associated macrophages (TAMs), the most widely infiltrating macrophages in the TME contributing to immunosuppressive microenvironment establishment, could promote

tumor invasion and weaken conventional therapeutic effects. Thus, targeting TAMs is becoming a promising strategy for cancer therapy. Here we will discuss the role of TAMs and their crosstalk with tumor in TME, including immune suppression, tumor progression, tumor metastasis, and angiogenesis. In addition, the role of TAMs in cancer therapy, including chemotherapy, radiotherapy, and immunotherapy, will be presented. Next, TAMs as a promising therapeutic target for cancer treatment will be illustrated, including targeting recruitment and localization of TAMs, targeting TAMs inhibition, and targeting TAMs reprogramming. Finally, the potential of TAMs as drug delivery systems will also be summarized. The chapter highlighted TAMs as promising therapeutic targets of can-

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cer, which might be useful for drug development and evaluation of targeting TAMs for cancer therapy.

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**Keywords**

Tumor-associated macrophages · Cancer therapy · Drug delivery systems

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## 1 Introduction

Macrophages are white blood cells of the innate immune system that can reside in all tissues of the body and contribute to the development, tissue homeostasis, diseases, and tumor microenvironment (TME). They are given different names based upon their locations, for example, microglia cell in brain, Langerhans cells in skin, Osteoclasts in bone, alveolar macrophages in lung, and Kupffer cells in liver (Epelman et al. 2014; Ginhoux and Guilliams 2016). Tissue-resident macrophages are derived from at least three distinct sources: the yolk sac, the fetal liver, and the bone marrow (Guerriero 2018). Macrophages-mediated innate immunity plays a crucial role in maintaining tissue homeostasis by engulfing and digesting invading pathogens, bacteria, damaged tissue, and malignant cells (Haniffa et al. 2015; Yona and Gordon 2015; Guerriero 2019). They also contribute to specific adaptive immune responses of T lymphocytes activation by antigen presentation. Besides, macrophages can modulate immune system through the secretion of various cytokines, chemokines, and the activation of complement system (Guerriero 2019). Macrophages can be characterized *in vitro* into two types: classically activated M1 macrophages and alternatively activated M2 macrophages. While M1 type macrophages are induced by  $T_H1$  cytokines such as  $INF-\gamma$ , M2 type macrophages are induced by the  $T_H2$  cytokines such as IL-4/IL-13. Macrophages represent up to 50% of leukocytes in the TME. Clinical data revealed that the poor prognosis is associated with the abundance of tumor-associated macrophages (TAMs) in 80% of human cancer. It was originally thought the macrophages were

recruited to malignant sites to kill the cancer cells. However, accumulating evidence demonstrated that tumor cells recruit macrophages to promote tumor malignancy and progression, like macrophages are recruited to a wound site to assist in healing. In this book chapter, we will discuss the role of TAMs and their interplay and crosstalk with T effector cells and tumor cells in TME, including immune suppression, tumor progression, tumor metastasis, and angiogenesis. In addition, the role of TAMs in cancer therapy, including chemotherapy, radiotherapy, and immunotherapy, will be presented. Moreover, TAMs as a therapeutic target for cancer treatment will be illustrated, including the recruitment of TAMs, the signaling pathways involved in TAMs activation, and the production of cytokines and chemokines that are involved in tumor angiogenesis. Then current strategies targeting TAMs for cancer therapy including targeting TAMs inhibition, recruitment, localization, and reprogram will be discussed. Lastly, the potential of TAMs as drug delivery systems will also be summarized.

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## 2 Macrophages and Tumor-Associated Macrophages (TAMs)

Macrophages, the major phagocytic cells in immune system, are derived and matured from monocytes that leave from the circulation system and settle in spleen, lymph nodes, alveoli, and tonsils. Macrophages are also existing in the brain as microglia, in the skin as Langerhans cells, in bone as osteoclasts, and in the liver as Kupffer cells. These tissue-resident macrophages originate from at least three embryonic sources: erythro-myeloid progenitors in the yolk sac and in the fetal liver and macrophage/dendritic cell progenitor cells in the bone marrow that give rise to monocytes (Davies et al. 2013). Tissue-resident macrophages are primarily derived from both the yolk sac and the fetal liver. They reach their tissues during embryonic development, progressing to adulthood, and sustain through proliferation independent of hematopoietic stem cells. Borrow

marrow-derived macrophages have a short life span and are only generated in the tissue under inflammatory conditions (Guerriero 2018). In cases of infection, macrophages, through their receptors on the surface, are able to undergo phagocytosis, the process of ingesting of foreign antigens by lysosomes, such as small particles, whole cells, and bacteria. In addition, macrophages in the peripheral lymphoid tissues serve as the major scavengers of abnormal cells and cellular debris. Importantly, macrophages present a vital role in processing antigen to T lymphocytes to activate specific adaptive immune responses. Activated macrophages can release many inflammatory mediators, such as cytokines, chemokines, enzymes, reactive oxidative species, coagulation factors, and growth factors (Ginhoux and Jung 2014; Olingy et al. 2019; Varol et al. 2015). All macrophages express colony-stimulating factor-1 receptor (CSF-1R), which binds to CSF-1 or alternatively to the interleukin IL-34 and regulates macrophage differentiation, proliferation, and survival. The presence and functional states of macrophages are mainly regulated through by CSF-1, granulocyte macrophage CSF (CSF2/GM-CSF), and chemokines.

The activation status of macrophages can be classified into two subpopulations: M1 and M2 macrophages. M1 (also known as classically activated) macrophages, induced by TH<sub>1</sub>-type cytokines such as INF- $\gamma$  and GM-CSF and through Toll-like receptor 4 (TLR4) engagement of lipopolysaccharides (LPS) from gram negative bacteria, give rise to pro-inflammatory, antiviral, antibacterial, antitumoral phenotypes with powerful killing effects on invading pathogens and at the same time destructive effects on normal tissues (Fleetwood et al. 2007; Arnold et al. 2014). Activated macrophages are potent effector cells that can kill tumor cells and microorganisms, trigger massive proinflammatory cytokines production, and activate cytotoxic T lymphocytes. M2 (also known as alternatively activated) macrophages are stimulated by the TH<sub>2</sub> cytokines IL-4/IL-13, IL-10, CSF-1 and play important roles in anti-inflammatory humoral responses and pro-repair, pro-tumoral, and antiparasitic phenotypes (Davies et al. 2013; Murray et al.

2014). Subgroups of M2 macrophages were divided into M2a (IL-4), M2b (INF $\gamma$  + complexed immunoglobulin (Ig)), and M2c (dexamethasone) (Szulzewsky et al. 2015). The nomenclature of M1 or M2 macrophages describes two extremes states of macrophage activation and functions that have direct in vitro relevance during the infection of bacteria or parasites. In vivo, macrophages are not clearly divided into M1 and M2 classification. Indeed, a study demonstrated that over 60 percent of the upregulated genes in TAMs from brain tumors had no overlap with that from M1 or M2 (M2a, M2b, or M2c) ex vivo macrophages phenotypes, suggesting macrophages activated in TME may not be reflected by ex vivo stimulation of monocytes (Hambardzumyan et al. 2016). Recent studies revealed that there are populations of CD169+ and TCR+ macrophages in vivo, which cannot simply be described as M1 or M2 term and seem to play roles in maintaining homeostasis, immune regulation, and tolerance (Chavez-Galan et al. 2015; Crocker and Gordon 1986; Martinez-Pomares et al. 1996; Martinez-Pomares and Gordon 2012). TCR $\beta$  gene was discovered rearrangement in the early stage of bone marrow macrophages differentiation. TCR+ macrophages express chemokine (C-C motif) ligand 2 (CCL2) with strong phagocytic functions, which are different from traditional macrophages (Kaminski et al. 2013). It was suggested to move away from the ambiguity of the M1-M2 characterization of TAMs and to define TAMs based on functional, transcriptional, or epigenetic status, which will demonstrate more clear characterization of TAMs (Guerriero 2018).

TAMs are usually referred to as macrophages recruited from circulating monocytes to tumors and influenced by the presence of cancer to promote tumor malignancy, survival, proliferation, angiogenesis, metastatic dissemination, and chemoresistance (Solinas et al. 2009; Qian and Pollard 2010; Kitamura et al. 2017). Rather than a homogenous population, TAMs actually can originate from different sources and exhibit either pro-tumoral or sometimes antitumoral roles. Each population has a unique transcriptional landscape based on the type, the stage, and the

immune composition of the tumors they infiltrate. M2 macrophages are defined as TAMs in a narrow sense because active TAMs have various properties similar to M2 macrophages (Murray et al. 2014; Chavez-Galan et al. 2015). The TME consists of various cell types, such as tumor cells, granulocytes, macrophages (~50%), mast cells, fibroblast and epithelial cells. Secreted cytokines and chemokines, such as CCL2, CCL11, CCL16, and CCL21, are major determinants for the infiltration of macrophages. Increasing evidence demonstrated that tumor cells recruit macrophages to support tumor growth. Clinical data indicated that poor prognosis in over 80% of human tumor is associated with increased TAMs, which not only lack the phagocytic functions of tumor cells, also promote tumor cells dissemination. High density of TAMs in tumor has been associated with increased vascular density, chemotherapy resistance, and worse outcome in various cancer types, such as colorectal, breast, ovarian, nonsmall cell lung cancer, melanoma, Hodgkin's lymphoma, and multiple myeloma (Guerriero 2018).

### 3 TAMs and Cancers

#### 3.1 Crosstalk of TAMs and Cancer Cells in TME

Various molecular mechanisms have been documented to play roles in mediating cancer cells that escape from the attacks of macrophages. Programmed cell death protein (PD-1) belongs to CD28 superfamily and plays a significant role in immunosuppression on T effector cells and TAMs. PD-L1 on the surface of tumor cells binding to PD-1 on effector T-cells and macrophages helps tumor cells escape from the attacks of T effector cells as well as TAMs by inhibiting cytokine expression, activation, and proliferation of effector T-cells, and macrophage phagocytosis (Boussiotis et al. 2014; Yu et al. 2015; Gordon et al. 2017; Katsuya et al. 2016). The cluster of differentiation 47 (CD47) molecule, the “do-not-eat-me” signal, is recently characterized as a self-molecule that protects host cells from destruction

by macrophages (Jaiswal et al. 2009; Zhao et al. 2016). CD47 expressed on the membrane of tumor cells binding to signal regulatory protein alpha 1 (SIRP1 $\alpha$ ) on macrophages will inhibit phagocytosis by blocking accumulation of myosin IIA at the phagocytic synapse (Okazawa et al. 2005; Barclay and Van den Berg 2014). Antibody-mediated inhibition of human CD47 enhances macrophage-mediated phagocytosis (Tseng et al. 2013). Targeting CD47/SIRP1 $\alpha$  signaling pathway is currently being tested in clinical trials (Pathria et al. 2019; Chao et al. 2012). In breast and ovarian cancer, CD24 was found as a dominant innate immune checkpoint, which could enhance tumor escape by providing “do-not-eat-me” signal through the interaction with inhibitory receptor sialic-acid-binding Ig-like lectin 10 (Siglec-10) expressed on TAMs. Suppressing the crosstalk between CD24 and Siglec-10 with respective antibodies or ablating the genes of CD24 or Siglec-10 can obviously promote phagocytic function of TAMs to tumors with CD24 expression (Barkal et al. 2019). Recent study revealed another recognition mechanism that can deter phagocytotic effects of macrophages on tumor cells, which is the interaction between leukocyte immunoglobulin like receptor subfamily B member 1 (LILRB1) on macrophages and major histocompatibility complex (MHC) class I component  $\beta$ 2-microglobulin on tumor cells. Either blocking  $\beta$ 2-microglobulin or anti-LILRB1 antibody can significantly enhance macrophage phagocytosis and inhibit tumor growth (Barkal et al. 2018). In TME, exosomes were recently discovered as important carriers for proteins, nucleic acid, which can affect survival, growth, and metastasis of cancer cells (Milane et al. 2015; Syn et al. 2016). A recent study found that TAMs characterized as M2-polarized phenotype can secrete exosomes and promote the metastasis of gastric cancer. Apolipoprotein E (ApoE) was identified richly in those exosomes, which can activate phosphatidylinositol 3-kinases (PI3k)-Akt signaling pathway to drive epithelial mesenchymal transition (EMT) and cytoskeleton rearrangement of cancer cells (Zheng et al. 2018). Another study found that TAMs-derived exosomes promote the resistance of PDAC to gem-

citabine by transferring miR-365 into cancer cells (Binenbaum et al. 2018). Exploring the roles of macrophage-derived exosomes and their components in cancer development and metastasis will open a new door for the discovery of cancer treatment. Recent study revealed that complement-mediated inflammation could promote tumorigenic progression (Medler et al. 2018). In a mouse squamous cell carcinomas (SCC) model, TAMs-triggered plasminogen produces C5a that is independent of C3 activation. Activation of C5aR by C5a in macrophages and mast cells leads to immunosuppressive macrophages that can inhibit CD8+ T-cell activation (Medler et al. 2018). Combined therapy of C5aR antagonist PMX-53 and paclitaxel significantly inhibits tumor growth as compared to PMX-53 or paclitaxel alone. Moreover, inhibition of C5aR signaling with PMA-53 significantly enhances antitumor efficacy of checkpoint inhibitor anti-PD-L1 antibody, indicating anticomplement therapy might be beneficial for cancer immune therapy (Affara et al. 2014; Zha et al. 2017).

### 3.2 TAMs and Tumor Progression

It was originally considered that macrophages were recruited to tumor sites to kill the cancer cells. However, accumulating evidence has revealed that TAMs can cause tumor progression and development through secretions of various chemokines and cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10) (Zhou et al. 2020). IL-6, secreted from tumor-associated endothelial cells and TAMs, has been involved in carcinogenesis and progression of tumors by regulating local inflammation, cell cycle, tumor angiogenesis, and stem cell self-renewal. IL-6 mediates the regulation of signal transducer and activator of transcription 3 (STAT3) phosphorylation, which leads to EMT, the overexpression of snail and Bcl-2, and anti-apoptotic effect in cancers (Gao et al. 2018; Yadav et al. 2011). IL-8, highly secreted by TAMs, is involved in angiogenesis, tumor metastasis, and suppression of immunity (Williams et al. 2016). Monitoring IL-8 level can predict the

outcome of immunotherapy of checkpoint blockade (Sanmamed et al. 2017). Exogenous IL-8 causes the activation of JAK2/STAT3/snail signaling pathway by enhancing the expression of p-JAK2 and p-STAT3, further promoting EMT and carcinogenesis (Deng et al. 2013). IL-8 and chemokines, strongly expressed in inflammatory breast cancer (IBC), promote macrophages recruitment and transforming to M2 macrophages, which can secrete high level of IL-8 and chemokines, leading to a feed-forward cytokine/chemokine loop that drives the EMT of IBC (Valeta-Magara et al. 2019). In addition, TAMs also secrete IL-10, transforming growth factor- $\beta$  (TGF- $\beta$ ), and inflammatory mediators, such as prostaglandin E2 (PGE2) and matrix metalloprotease-7 (MMP-7), which can inhibit antigen-presenting process for T-cell activation. During chronic inflammation, toll-like receptor 4 (TLR4) stimulates M2 to secrete cytokine IL-10 and activation of TLR4 by LPS significantly enhanced the EMT of pancreatic cancer cells (Deng et al. 2013). IL-10 also increases the expression of inhibitor of CIP2A via the PI3K signaling pathway and promotes tumor aggressiveness in lung adenocarcinoma (Sung et al. 2013). IL-10 plasma level showed significant correlation with tumor progression, indicating IL-10 plays a critical role in tumor progression (Sato et al. 2011).

### 3.3 TAMs and Tumor Metastasis

The underlying mechanisms and processes of cancer metastasis are very complex. The cancer cells start from local invasion, intravasation, and ultimate extravasation at peripheral sites. The disseminated cancer cells need to escape the attack from immune system, survive in the blood or lymphatic circulation, settle down at a distant site, and proliferate in the new hostile environment (Ruffell and Coussens 2015; Cassetta and Pollard 2018). Macrophages have been implicated in all stages of the metastatic processes. High infiltration of macrophages, overexpression of CSF-1, and their chemoattractant CCL2 in human solid tumors are linked to poor clinical prognosis (Cassetta and Pollard 2020). Pollard

and his colleagues have demonstrated that in a mouse mammary gland, tumor model genetic depletion of CSF-1 (similar to the depletion of macrophages) can slow the tumor progression and significantly inhibit metastasis, suggesting TAMs play crucial roles in cancer metastasis (Joyce and Pollard 2009). The phenomenon was recapitulated in other preclinical mouse model studies of the development of other cancer types, such as lung cancer, pancreatic cancer, and glioblastoma (Zabuawala et al. 2010; Welm et al. 2007; Rolny et al. 2011; Gocheva et al. 2010; Qian et al. 2011). Metastatic cancer cells recruit bone marrow-derived classical monocytes through CCL2-CCR2 signal mechanism (Qian et al. 2011). These extravasated monocytes differentiate into metastasis-associated macrophages (MAMs) through involvement of chemokine CCL3-CCR1 autocrine signaling (Mummalaneni et al. 2014; Kitamura et al. 2015). MAMs were characterized to express the markers CD11b, VEGF receptor 1, CXCR3, and CCR2, which are different from resident macrophages and MAM precursor cells. MAMs mediate cancer cells metastasis through promoting extravasation and tumor growth, as well as inhibiting cytotoxic T-cells function. Macrophages open up a gate for the cancer cells to escape when arriving at the circulating vessels. In addition, macrophages produce other molecules that help cancer cell invasion (Sangaletti et al. 2008). Cathepsin proteases can remodel the matrix and release sequestered growth factors and TGF $\beta$  generated from MAMs that drive epithelial to EMT of cancer cells (Laoui et al. 2011; Quail and Joyce 2013; Bonde et al. 2012). Macrophages in the lung interact with metastatic cancer cells and support their survival through vascular cell adhesion protein 1 (VCAM1)-dependent and Akt-dependent mechanism. Moreover, MAMs crosstalk to metastatic cancer cells to retain MAMs in the metastatic foci, which can further support cancer growth (Kitamura et al. 2015). TAMs inhibit the function of cytotoxic T-cells, which might be one of the mechanisms that help cancer cells escape from attack of T-cells and promote cancer metastasis (Kitamura et al. 2017). TAMs can directly inhibit T-cell cytotoxicity

through the depletion of L-arginine via release of arginase 1, which is essential for the re-expression of the T-cell receptor (TCR) after antigen engagement on T-cells. In addition, TAMs generate cytokines such as IL-10 and TGF $\beta$  that lead to immunosuppressive microenvironment by inhibiting CD4+ and CD8+ T-cells and inducing Treg cells expansion in the TME. TAM-mediated release of chemokines, like CCL2, CCL3, CCL4, CCL5, and CCL20, contributes to the recruitment of Treg cells in the TME (Noy and Pollard 2014). Moreover, TAM-induced immune suppression is caused by the expression of inhibitory receptors, such as nonclassical major histocompatibility complex class I (MHC-I) molecules such as HLA-E and HLA-G, which can inhibit activation of NK cells and T-cells through the interaction with CD94 and leukocyte immunoglobulin-like receptor subfamily B member 1 (LIR1), respectively (Morandi and Pistoia 2014). The expression of T-cell immune checkpoint ligands by TAMs, such as PDL1, PDL2, B7-1, and B7-2, can directly inhibit T-cell functions (Santarpia and Karachaliou 2015; Buchbinder and Desai 2016). It is of great interest to target TAMs for anticancer metastasis therapy since both preclinical and clinical data have established the proof of principle.

### 3.4 TAMs and Angiogenesis

Angiogenesis is a key event in tumor growth and progression. Cancerous tissues are characterized by hypoxia and unequal vascularization, which actually can modify macrophage distribution and function. TAMs accumulate preferentially in the inadequately vascularization areas of tumors, which have low oxygen (Mantovani et al. 2002). Under hypoxic condition, macrophage migration is suppressed and TAMs are immobilized in non-vascular, necrotic, hypoxic regions of tumor, from which macrophages cooperate with cancer cells to promote angiogenesis (Grimshaw and Balkwill 2001; Leek et al. 1996, 1999; Lewis et al. 2000). In breast cancer, TAMs overexpress hypoxia-inducible factor-2 $\alpha$  (HIF-2 $\alpha$ ) and HIF-1, which are transcription factors to induce the

secretion of pro-angiogenic factors, such as endothelial growth factor VEGF, bFGF, and chemokines CXCL8 and CXCL12 to promote angiogenesis (Talks et al. 2000; Crowther et al. 2001; Lin and Pollard 2007; Hughes et al. 2015). Other factors such as TGF $\beta$ , WNT7B, TNF, and thymidine phosphorylase also contribute to angiogenic process by recruitment and activation of endothelial cells or other cells like fibroblast or pericytes that support the generation of vascular networks in microenvironment (Cassetta and Pollard 2018; Yeo et al. 2014; Hirano et al. 2001). A subpopulation of TAMs with the expression of the angiopoietin 1 receptor (TIE2) was identified to play essential roles in tumor angiogenesis. Depletion of these TAMs suppresses tumor growth and metastasis (De Palma et al. 2005; Mazziere et al. 2011) (Fig. 1).

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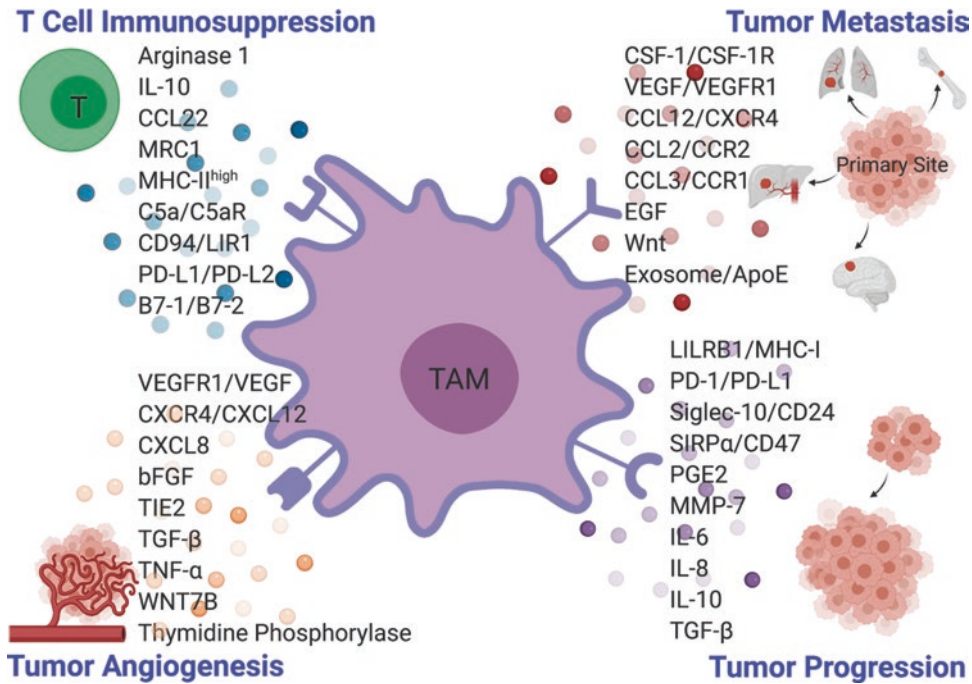
## 4 TAMs in Cancer Therapy

TAMs are the dominant cell populations recruited into the TME and are shaped by cancer cells to present M2 properties. The considerable functional plasticity enables TAMs to rapidly adapt to microenvironmental perturbations from various cancer treatment modalities (ranging from radiotherapy, chemotherapy, targeted therapy to immunotherapy) in TME, and clinical consequences of such interactions between TAMs and cancer therapies vary across different cancer types and different therapeutic categories (Vitale et al. 2019; De Palma and Lewis 2013; Larionova et al. 2019; Neophytou et al. 2020). In some cases, therapeutic agents may achieve their pharmacological effects via the function of TAMs. For example, accumulating evidence indicated that metformin suppresses tumorigenesis, cancer cell proliferation, and tumor metastasis by modulating functional states of TAMs. Metformin induced the expression of M1-related cytokines IL-12 and TNF- $\alpha$  and reduced M2-related cytokines IL-8, IL-10, and TGF- $\beta$ , to revert the polarization of TAMs to M2 phenotype by cancer cells and thus may recover tumor immune surveillance (Ding et al. 2015; Chiang et al. 2017; Ma et al. 2020; de Oliveira et al. 2019). However, in other

cases, TAMs may hamper efficacy of drug treatment by forming an immunosuppressive TME.

### 4.1 TAMs in Radiotherapy

Radiotherapy is the most commonly used modality for clinical treatment of solid tumors. In addition to its direct cell-killing effects, radiotherapy profoundly modified TME, and especially, TAMs are usually reshaped by radiations to exhibit bidirectional consequences (pro-inflammatory or pro-tumorigenic phenotypes) depending on the radiation doses and cancer types (Mantovani and Allavena 2015). On the one hand, immunogenic cell death induced by radiation could activate innate and adaptive immune systems to trigger antitumor immune responses where TAMs are closely involved in (Golden et al. 2014). For example, local low-dose gamma irradiation programs TAMs to iNOS<sup>+</sup> M1 state, which subsequently promotes the recruitment of tumor-specific T-cells to TME and then evokes T-cell immune response (Klug et al. 2013; Prakash et al. 2016). On the other hand, accumulating evidence has demonstrated that TAMs mediated radio-resistance in multiple cancers (Wu et al. 2017). Radiotherapy may polarize TAMs to their M2 state to allow immune escape and compromised antitumor effects. For instance, an in vivo study revealed that radiation at 3 Gy in prostate cancer upregulated CSF1 expression on cancer cells, which in turn recruited TAMs (express CSF-1R) to TME and then promoted tumor regrowth (Xu et al. 2013). In glioblastoma, irradiation increased the number of M2 TAMs by upregulating CSF-1R expression, which finally lowers the tumor sensitivity to radiotherapy (Stafford et al. 2016). Bone marrow-derived macrophages (BMDM $\phi$ ) promoted tumor growth after ionizing radiation at 20 Gy. The underlying mechanism involved in the upregulation of VEGF expression resulted from autocrine or paracrine of TNF $\alpha$  in BMDM $\phi$  cells, and removing TNF $\alpha$  could re-sensitize tumors to ionizing radiation (Meng et al. 2010). Recent data demonstrated that fibroblast growth factor 2 (FGF2) is critical for the transition of TAMs toward M2 phenotype



**Fig. 1** The role of tumor-associated macrophages (TAMs) in promoting tumor progression, tumor angiogenesis, tumor metastasis, and T-cell immunosuppression. Multiple mechanisms of molecular interactions (CSF-1/CSF-1R, VEGF/VEGFR1, CCL12/CXCR4, CCL2/CCR2, CCL3/CCR1, PD-1/PD-L1, LILRB1/MHC-I, Siglec-10/CD24, SIRP $\alpha$ /CD47, C5a/C5aR, and CD94/

LIR1) and secreted growth factors (VEGF, EGF, and bFGF), cytokines (CSF-1, IL-6, IL-8, IL-10, TNF $\alpha$ , TGF $\beta$ ), chemokines (CXCL8, CXCL12, and CCL22), and other mediators (TIE2, PGE2, MMP-7, exosome, thymidine phosphorylase) are involved in TAMs-mediated different stages of tumor progression and T-cell immunosuppression

after radiotherapy, and blocking FGF2 increased percentage of M1-like TAMs, extended tumor growth delay, and prolonged long-term survival in mouse model (Im et al. 2020). In addition, the FGF2 receptor (FGF2R) has been reported to mediate radio-resistance in glioblastoma (Gouaze-Andersson et al. 2016). Those evidence indicated that reverting the M2 polarization either by direct depletion of TAMs or by targeting abovementioned key players (CSF-1/CSF-1R, TNF $\alpha$ , FGF2/FGF2R) represents a promising strategy for combinatory radiotherapy to improve clinical efficacy.

## 4.2 TAMs in Chemotherapy

The interactions between TAMs and chemotherapy have been widely cataloged to be involved in variations of clinical efficacy of a wide range of

cancer therapeutics among patients (Larionova et al. 2019). Cancer cells can reshape cellular metabolism and thus shift the phenotype of TAMs to protect themselves from drug exposure. For example, TAMs in M2 state have been well documented to be involved in chemoresistance in the treatment of pancreatic ductal adenocarcinoma (PDA) and CCR2 and CSF-1R played key roles in driving chemoresistance (Mitchem et al. 2013). Metabolomics profiling of TAMs and PDA cells revealed the underlying mechanism that TAMs promote pyrimidine biosynthesis from glucose and release deoxycytidine molecules to interact with PDA cells and finally drive the gemcitabine resistance, and blocking deoxycytidine release by simply limiting glucose supply could sharply sensitize PDA cells to gemcitabine exposure, providing novel mechanism of gemcitabine resistance from an intratumoral metabolic crosstalk perspective



(Halbrook et al. 2019). Further retrospective analysis in this study indicated that lower TAMs in microenvironment is a favorable factor for better drug response and improved disease-specific survival outcome of PDA patients. As deoxycytidine release was also observed in a panel of cancer cell lines and patient-derived cancer cells, release of pyrimidine molecules by TAMs may drive chemoresistance in other cancers.

Moreover, TAMs were revealed *in vitro* and *in vivo* to mediate chemotherapy resistance by enhancing survival factor activities or activating antiapoptotic programs in cancer cells (Correia and Bissell 2012; Castells et al. 2012; DeNardo et al. 2011). For instance, TAMs suppressed cytotoxic effects of Taxol, a widely used antimetabolic agent, on multiple cancer cell lines and on *in vivo* model models (Olson et al. 2017). TAMs in M2 state are able to release nitric oxide (NO) to protect cancer cell from apoptosis induced by cisplatin, leading to resistance in several cancer cell lines (Perrotta et al. 2018). Coculture studies *in vitro* using macrophages derived from bone marrow (BM) with mammary carcinoma cell lines demonstrated that macrophages confer to chemotherapy resistance to paclitaxel, doxorubicin, and etoposide and to gemcitabine in PDA cells (Mitchem et al. 2013; Shree et al. 2011). The activation of signal transducer and activator of transcription 3 (STAT3) in TAMs has been shown to promote PDAC cancer cells proliferation and survival, resistance to carboplatin *in vivo*, and tumor cell growth when synergized with IL-6 and other macrophage-derived factors such as milk fat globule-epidermal growth factor VIII (Jinushi et al. 2011; Taniguchi and Karin 2014; Yu et al. 2014). IL-6 can be generated by co-culture of BM-derived macrophages with neoplastic cells as well as from TAMs *in vivo* (DeNardo et al. 2009; Movahedi et al. 2010; Song et al. 2009). IL-1 $\beta$  can also induce IL-6 production in monocytes and osteoblasts (Mori et al. 2011; Tosato and Jones 1990). *In vivo* evidence of IL-6 being chemoresistance was shown in a murine lymphoma model (Gilbert and Hemann 2010). Nevertheless, the source and relevance of IL-6 during chemotherapy for solid tumor remains partly described. In a subcutaneous

MCF-7 breast cancer xenografts animal model, anti-CSF-1 neutralizing antibody can increase of chemosensitivity (Paulus et al. 2006). Soluble chemoprotective factors generated from TAMs depend on protease activity of cathepsin B and S. *In vivo* inhibition of cathepsin protease activity enhances the response of mammary carcinomas to paclitaxel (Shree et al. 2011). Macrophages are main source of tumor necrosis factor (TNF), which may be one of crucial factors mediating chemoresistance either directly through NF- $\kappa$ B activation or indirectly through induced IL-6 expression (Mori et al. 2011; Li and Sethi 2010). TNF $\alpha$  has been reported to mediate chemoresistance to MAPK inhibitors in melanoma through NF- $\kappa$ B-dependent expression of microphthalmia transcription factor (Smith et al. 2014). Taken together, it has a strong rationale to target TAMs that contribute chemoresistance for anticancer therapy.

### 4.3 TAMs in Cancer Immunotherapy

T-cell-based immunotherapies, such as adoptive T-cell therapy and immune checkpoint blockages (ICBs), are changing the traditional paradigm of cancer treatment in clinical practice (Galon and Bruni 2019). However, only a small fraction of cancer patients can benefit from those therapies. As the dominant immune cell population in TME, TAMs actively interact with other immune cells to interfere antitumor immunity derived from T-cell based immunotherapies, accounting for an important mechanism of immunotherapy failure in primary resistant patients (Li et al. 2019a). T-cell-based immunotherapies exert antitumor activity by boosting T-cell immunity in TME, while T-cells are closely influenced by TAMs. TAMs could not only inhibit T-cell function by suppressing naïve T-cell proliferation, but also inhibit cytotoxic T-cell responses via releasing various anti-inflammatory cytokines (e.g., IL-10, TGF- $\beta$ , PGE2) or leveraging inhibitory receptors (checkpoint molecules on TAMs such as B7-H4 and VISTA), which finally established an immunosuppressive TME and therefore led to compro-

mised clinical outcomes (De Palma and Lewis 2013; Ruffell et al. 2014).

#### 4.4 TAMs Aid Precision Cancer Treatment

As discussed above, TAMs profoundly influence antitumor activities of cancer therapies. This provides a series of insights to develop predictive or prognostic biomarkers for customized therapeutic regimes and to dig into detailed mechanisms for the discovery of novel TAM-related therapeutic targets, which will finally facilitate precision cancer medicine.

High-throughput omics profiling studies proposed the possibility that using the functional landscape of TAMs as predictive biomarker to guide precise patient stratification for improved therapeutic outcomes. In the TRIBE and FIRE3 clinical trial cohorts, gene mutation sequencing analysis demonstrated that mutation features of TAMs regulating genes could robustly predict progression-free survival of patients with metastatic colorectal cancer treated with bevacizumab plus FOLFIRI (Sunakawa et al. 2015). A recent analysis of multiomics profiles of bulk tumors from 348 patients reported that the infiltration level of M1 macrophages significantly correlated with drug response of ICBs in metastatic urothelial cancer (Zeng et al. 2020), indicating that genomics profile that encoded TAMs states may be an effective biomarker for patient selection in ICB therapy. In a phase IV clinical trial study of NSCLC, DNA methylation microarray profiling revealed that nonresponders to ICBs were characterized as those patients who exhibit high enrichment of TAMs, neutrophils, and cancer-associated fibroblasts in TME, highlighting profiles of TAMs as complementary biomarkers in addition to PD-L1 staining score and tumor mutation burden (Duruiseaux et al. 2018). Transcriptomic profiling of monocytes isolated from breast cancer patients and healthy controls identified TAM signatures, which are highly associated with aggressive subtypes and poor survival, and elucidated that SIGLEC1 and CCL8 were key regulators to govern the interactions

between TAMs and cancer cells in this scenario, suggesting novel biomarkers and potential therapeutic targets for breast cancer treatment (Cassetta et al. 2019).

Moreover, TAM transcriptomic profiling at single-cell level depicts more precise TAMs landscape and reveals more clues for personalized cancer therapy. Recent years, single cell sequencing technology is reforming our understanding of tumor heterogeneity. Transcriptomic landscape of tumor at single cell resolution is opening new revenue for personalized cancer treatment by providing individualized immune interaction profiles as novel evidence for tailored therapeutics design (Weissleder and Pittet 2020; Qian et al. 2020). Single-cell RNA-seq profiling of patients with lung cancer demonstrated that TAMs can be functionally categorized into up to 10 subtypes in lung cancer and especially, certain subtypes express both M1 and M2 markers (Weissleder and Pittet 2020), recapitulating that TAMs employ a spectrum of functional states rather than the conventional notion that TAMs simply consist of M1 and M2 subtypes to cope with the complexity of TME.

Interactions of TAMs and cancer therapeutics result in multifaceted effects on clinical outcomes of cancer therapies via various elucidated and unexplored molecular mechanisms. This provides promising opportunities to vanish immunosuppressive TME, sensitize resistant cancer cells to cancer treatment modalities, stratify patients to match optimal treatment strategies, and finally benefit cancer patients in clinical practice, by harnessing those interactions either via depleting tumor-promoting TAMs or re-directing them to their tumor-inhibiting phenotype.

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## 5 Target TAMs for Cancer Therapy

TAMs play essential roles during tumor proliferation, angiogenesis, invasion, metastasis, and immunosuppression and interfere with most standard cancer therapy (Pathria et al. 2019). Elevated number of circulating monocytes from blood and massive macrophage infiltration into tumor tis-

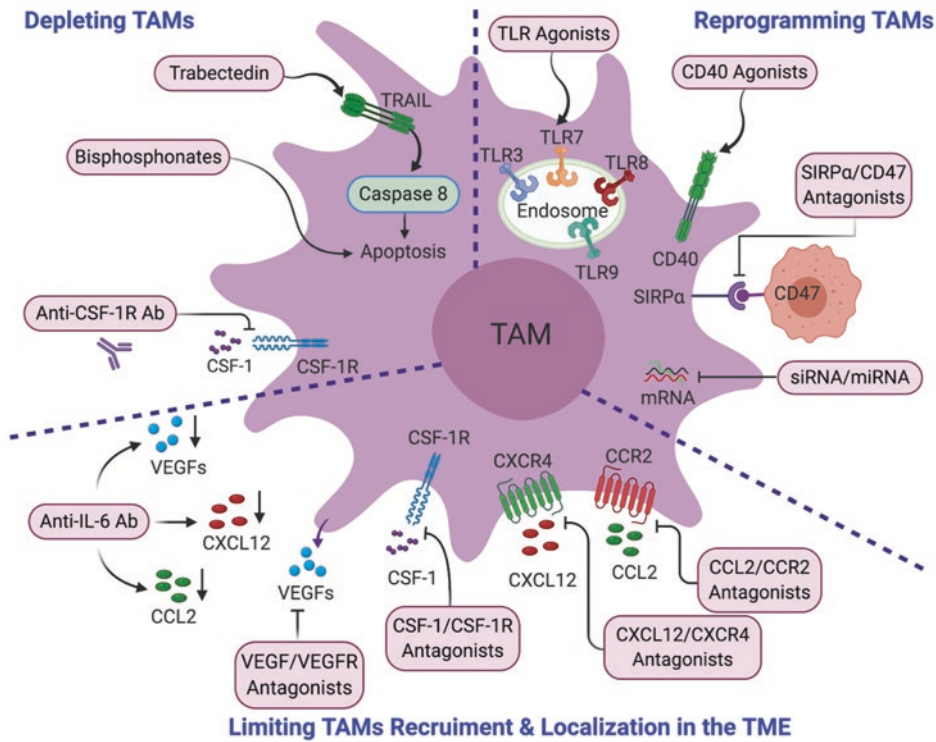
sues, especially accumulation of anti-inflammatory TAMs, is tightly associated with worse clinical outcome and resistance to therapy in various cancer types (La Fleur et al. 2020). Thus, manipulating TAMs in conjunction with chemotherapy, radiotherapy, or immunotherapy might improve standard cancer treatment response (Cheng et al. 2020). Moreover, the potential role of macrophages during cancer development has driven novel antitumor therapy development through targeting macrophages (Mantovani et al. 2017; Sawa-Wejksza and Kandefler-Szerszen 2018). Currently, several pharmacological strategies have been proposed either in preclinical study or in clinical trials through inhibiting TAMs recruitment, depleting TAMs, and reprogramming M2 TAMs to M1 TAMs in laboratory tumor models or clinical trials (Fig. 2) (Anfray et al. 2019). Besides, due to their intrinsic homing property, M1 macrophages or their derived components could also be used as drug delivery systems to actively carry payloads to the tumor sites (Guerra et al. 2017). Here the progress regarding TAMs as drug delivery systems, which mainly focusing on M1 macrophages, their derived exosomes, and membrane-coated nanoparticles will be summarized (Miller et al. 2015).

## 5.1 Target TAMs Recruitment and Localization for Cancer Therapy

Numerous evidences reveal that recruitment and localization of macrophage in tumors is driven by continuous accumulating monocytes from circulation due to some tumor-derived factors (Qian and Pollard 2010). These factors including colony-stimulating factor-1 (CSF-1), C-C motif chemokine ligand 2 (CCL-2), C-X-C motif chemokine ligand 12 (CXCL-12), and vascular endothelial growth factor (VEGF) have mediated interaction between monocytes and tumors cells; thus, suppressing the monocyte-chemotactic chemokines, cytokines, and their receptors could block the recruitment and localization of TAMs (Argyle and Kitamura 2018; Owen and Mohamadzadeh 2013; Zhang et al. 2020a).

### 5.1.1 Targeting CSF-1/CSF-1R Signaling

CSF-1/CSF-1 receptor (CSF-1R) signaling is one critical pathway related to TAMs recruitment, proliferation, differentiation, and survival and is also essential for stimulating chemotactic activity of monocytes and macrophages including the transition from M1 TAMs into M2 TAMs (Laoui et al. 2014; Dwyer et al. 2017). Studies have shown that CSF-1 enhances progression of mammary tumors to malignancy, high expression of CSF-1 in tumor tissues, or high expression of CSF-1R in TAMs which are associated with worse prognosis of tumor (Lin et al. 2001; Zhu et al. 2008; Koh et al. 2014). The absence of CSF-1R caused almost all macrophages to be dramatically depleted in mice (Erblich et al. 2011). Besides, G-CSF regulated macrophage phenotype, suppression of G-CSF enhanced circulating monocytes, and TAMs mutation and remarkably limited lung metastasis of triple-negative breast cancer, but if G-CSF was kept in high level, anti-CSF-1R therapy could strength anti-inflammatory TAMs and promote lung metastasis (Hollmen et al. 2016). Inhibition of M-CSF by both antibody and chemical inhibitor significantly suppressed tumor angiogenesis and lymphangiogenesis in mouse osteosarcoma model (Kubota et al. 2009). Antibody against CSF1-R depleted the resident subset of monocytes and TAMs without inhibiting inflammation, and macrophage blockade using a CSF-1R inhibitor also resulted in reduced infiltration of protumorigenic (M2) macrophages (MacDonald et al. 2010). Moreover, the mAb inhibiting the CSF-1R (RG7155) was proved both in vitro and in vivo to decrease F4/80+ TAMs accompanied by an increase in the CD8+/CD4+ T-cell ratio (Ries et al. 2014). Administration of RG7155 to patients led to striking reductions of CSF-1R + CD163+ macrophages in tumor tissues. Some clinical trials of RG7155 are underway in solid tumor treatment as monotherapy or combined with other therapies. Besides, targeting TAMs by CSF-1R blockade in breast cancer mice model could stimulate intratumoral type I interferon signaling, thus enhance the anticancer efficacy of platinum-based chemotherapeutics



**Fig. 2** Targeting TAMs for cancer therapy. Currently, three strategies, including 1) targeting TAMs recruitment and localization, 2) targeting TAMs inhibition, and 3) targeting TAMs reprogram, have been proposed in different stage of studies. For targeting TAMs recruitment and localization, four signaling axis, that is, CSF-1/CSF-1R signaling axis, CCL-2/CCR-2 axis, CXCL-12/CXCR-4

axis, and VEGF/VEGFR signaling axis, were highlighted. For targeting TAMs inhibition, two chemical agents Bisphosphonates and Trabectedin and other targets for TAMs-specific surface markers were discussed. Targeting TLR, SIRP $\alpha$ /CD47 axis, CD40, and other strategies were involved in TAMs reprogramming

(Salvagno et al. 2019). Local targeting of lung TAMs with pulmonary delivery of a CSF-1R inhibitor PLX-3397 could treat lung metastases of breast tumor by decreasing M2 macrophages and increasing M1 macrophages in the tumor microenvironment (Alhudaithi et al. 2020). Researchers also reported a potent, highly selective, and orally bioavailable CSF-1R inhibitor, IACS-9439, which could lead to a dose-dependent reduction in macrophages and lead to tumor growth inhibition in MC38 and PANC02 syngeneic tumor models (Czako et al. 2020). In some other studies, CSF-1R inhibitors could reduce TAMs number in tumor tissue, inhibit tumor growth, and obviously decrease tumor angiogenesis and metastasis. JNJ-28312141, an FMS-related receptor tyrosine kinase-3 (FLT3)

inhibitor, suppressed human nonsmall cell lung carcinoma growth and reduced tumor vasculature in a dose-dependent manner; these effects were highly correlated with marked reductions in F4/80+ TAMs. Another CSF-1R inhibitor, BLZ945, showed potentials as a potent antitumor drug in breast cancer, glioblastoma, and cervical carcinoma in preclinical studies. It was confirmed existing potent ability converting tumorigenic M2 macrophages into the antitumor M1 macrophages. Numerous studies revealed that targeting of TAMs through inhibiting CSF-1R could suppress tumor growth and metastasis, and currently massive clinical trials are launched to evaluate bio-safety and bio-activity of antibodies/small molecules targeting CSF-1/CSF-1R signaling alone or combined with other therapies in differ-

ent types of tumors (Table 1). While researchers also found that anti-CSF-1R and anti-CSF-1 antibodies or CSF-1R small-molecule inhibitors treatment increased breast cancer metastasis, thus the CSF-1/CSF-1R signaling serving as the therapeutic targets for breast cancer still needs further investigation (Swierczak et al. 2014).

### 5.1.2 Targeting CCL-2/CCR-2 Axis

CCL-2, also widely known as monocyte chemoattractant protein-1(MCP-1), is a C-C motif chemokine overexpressed in solid tumors and tightly associated with increased tumor adhesion, migration, invasion, progression, and angiogenesis (Hao et al. 2020). Besides, CCL-2 could also recruit monocytes into an immunosuppressive tumor microenvironment: Tumor cells recruited inflammatory monocytes to tumors sites through CCL-2/CCR-2 axis, and the recruited monocytes were polarized to TAMs to promote tumor survival and facilitate tumor metastasis (Qian et al. 2011; McClellan et al. 2012). As its main receptor, the upregulation of CCR-2 was also reported correlated with cancer metastasis and relapse (Nagarsheth et al. 2017) and recruitment of CCR-2 positive TAMs to sites of liver metastasis conferred a poor prognosis in human colorectal cancer (Grossman et al. 2018). Recent work suggested that during esophageal carcinogenesis, CCL-2/CCR-2 axis-mediated TAMs recruitment could induce immune evasion through PD-1 signaling (Yang et al. 2020). Blocking of the CCL-2/CCR-2 axis decreased macrophage infiltration and reduced tumor growth (Lim et al. 2016). Besides, disruption of CCL-2/CCR-2 axis could obviously reduce TAMs number in tumors, thereby suppressing tumor growth and dissemination in some certain cancer types (Fujimoto et al. 2009).

Anti-CCL-2 antibody or CCL-2 inhibitors were proved to block glioma, colon, prostate, and melanoma cancers proliferation in animal models (Loberg et al. 2007). Li et al. reported that blockade of CCL-2/CCR-2 axis by using CCR-2 antagonist or CCR-2 knockout could inhibit liver tumor malignant growth and metastasis, reduce postsurgical recurrence, and enhance survival through suppressing inflammatory monocytes

recruitment, infiltration, and TAMs M2 polarization (Li et al. 2017). In a preclinical study, a CCL-2 neutralizing antibody led to a remarkable tumor growth suppression in hepatocellular cancer (Teng et al. 2017). Apart from small molecule inhibitors and antibodies, RNA aptamer CCL-2 inhibitor could also reduce M1 TAMs liver infiltration and pathogenic angiogenesis (Bartneck et al. 2019). In clinical trial, Carlumab (CNTO888), an anti-CCL-2 human monoclonal antibody, is being used either alone or in combination with standard chemotherapies for solid tumors patients' treatment (Sandhu et al. 2013). The other approach to decrease the action of CCL-2 is to block CCR-2. Pharmacological CCR-2 inhibitors and humanized antibody that recognizes CCR-2 were examined. BMS-813160, a potent and selective CCR-2/CCR-5 antagonist, alone or combined with chemotherapy or Nivolumab in patients with advanced solid tumors such as colorectal cancer and pancreatic cancer, is launched in clinical trials (NCT03184870). A CCR-2 inhibitor RS 504393 could inhibit xenograft prostate tumor growth in SCID mice fed with high fat diet (Hao et al. 2020). An orally active, high-affinity CCR-2 antagonist CCX872 could also enhance anti-PD-1 effect to slow progression of gliomas and improve survival and is currently in the clinical trial for patients with advanced and metastatic pancreatic cancer (Flores-Toro et al. 2020). PF-04136309, small-molecule inhibitors of CCR-2 showed efficacy in preclinical study: In an orthotopic model of murine pancreatic cancer, CCR-2 blockade by PF-04136309 depleted inflammatory monocytes and macrophages from the primary tumor and premetastatic liver resulting in tumor growth inhibition, antitumor immunity enhancement, and metastasis reduction (Sanford et al. 2013); Currently, PF-04136309 has moved forward to clinical trials, and the inhibitor combined with chemotherapeutic regimen FOLRIRINOX presented favorable response in pancreatic cancer patients during phase 1b clinical trial (Nywening et al. 2016), while another clinical trial in PF-04136309 combined with nab-paclitaxel and gemcitabine for metastatic pancreatic patients was terminated in May

**Table 1** List of clinical trials targeting CSF-1/CSF-1R signaling

Identifier	Agent	Description	Phase	Condition
NCT02371369	PLX-3397	Small molecule	Phase III	Pigmented villonodular synovitis or giant cell tumor of the tendon sheath
NCT02071940	PLX-3397	Small molecule	Phase II	Melanoma
NCT02390752	PLX-3397	Small molecule	Phase I/II	Refractory leukemias and refractory solid tumors
NCT01349049	PLX-3397	Small molecule	Phase I/II	Acute myeloid leukemia
NCT02452424	PLX-3397	Small molecule	Phase I/II	Advanced melanoma and other solid tumors
NCT01004861	PLX-3397	Small molecule	Phase I	Solid tumors
NCT01525602	PLX-3397	Small molecule	Phase I	Advanced solid tumors
NCT01042379	PLX-3397	Small molecule	Phase I	Breast cancer
NCT02777710	PLX-3397	Small molecule	Phase I/II	Glioblastoma and gliosarcoma
NCT02584647	PLX-3397	Small molecule	Phase I/II	Unresectable sarcoma and malignant peripheral nerve sheath tumors
NCT01596751	PLX-3397	Small molecule	Phase Ib/II	Metastatic breast cancer
NCT02472275	PLX-3397	Small molecule	Phase I	Intermediate- or high-risk prostate cancer
NCT01790503	PLX-3397	Small molecule	Phase Ib/II	Newly diagnosed glioblastoma
NCT01804530	PLX-7486	Small molecule	Phase I	Advanced solid tumors
NCT02880371	ARRY-382	Small molecule	Phase Ib/II	Advanced solid tumors
NCT02829723	BLZ-945	Small molecule	Phase I/II	Advanced solid tumors
NCT03069469	DCC-3014	Small molecule	Phase I	Hematological tumors and solid tumors
NCT03238027	SNDX-6352	Small molecule	Phase I	Solid tumors
NCT03502330	Cabiralizumab	mAb	Phase II	Advanced pancreatic cancer
NCT03697564	Cabiralizumab	mAb	Phase II	Stage IC pancreatic cancer
NCT03768531	Cabiralizumab	mAb	Phase II	Resectable biliary tract cancer
NCT02526017	Cabiralizumab	mAb	Phase I	Selected advanced tumors
NCT03502330	Cabiralizumab	mAb	Phase I	Advanced melanoma, nonsmall cell lung cancer, and renal cell carcinoma
NCT02471716	Cabiralizumab	mAb	Phase I/II	Tenosynovial giant cell tumor
NCT02265536	LY-3022855	mAb	Phase I	Breast or prostate cancer
NCT01346358	IMC-CS4 (LY-3022855)	mAb	Phase I	Advanced solid tumors
NCT03101254	LY-3022855	mAb	Phase I/II	Melanoma
NCT03153410	LY-3022855	mAb	Phase I	Pancreatic cancer
NCT02923739	Emactuzumab	mAb	Phase II	Platinum-resistant ovarian cancer
NCT02323191	Emactuzumab	mAb	Phase I	Advanced solid tumors

Identifier	Agent	Description	Phase	Condition
NCT01494688	Emactuzumab	mAb	Phase I	Advanced solid tumors
NCT01444404	AMG-820	mAb	Phase I	Advanced solid tumors
NCT02713529	AMG-820	mAb	Phase Ib/II	Pancreatic cancer, colorectal cancer, and nonsmall cell lung cancer
NCT02807844	Laenotuzumab	mAb	Phase Ib/II	Advanced malignancies
NCT02948101	PD-0360324	mAb	Phase II	Platinum-resistant epithelial ovarian cancer

2017 (Jahchan et al. 2019). Plozalizumab (MLN1202), a humanized mAb to CCR-2, was conducted in phase 2 clinical trial for bone metastasis of unspecified tumors, while Plozalizumab combined with nivolumab (immune-checkpoint inhibitor) for patients with advanced melanoma in phase 1 clinical trial was terminated in May 2018 because of serious adverse events (Vela et al. 2015; Yumimoto et al. 2019). Thus, it should be noted that durable CCL2/CCR2 axis inhibition might lead to the compensation of other chemokine-dependent pathways, which facilitates the recruitment of TAMs in the tumor microenvironment.

### 5.1.3 Targeting CXCL-12/CXCR-4 Axis

CXCL-12, also known as stromal cell-derived factor-1 (SDF-1), is widely expressed in many different types of tissues. CXCL-12 is the ligand for the chemokine receptor CXCR-4 (Liekens et al. 2010). Recent work also demonstrated that CXCL-12 could bind to another seven-transmembrane span receptor CXCR-7 with high affinity (Sun et al. 2010). Both CXCR-4 and CXCR-7 played critical roles on tumor growth and metastasis, and a lot researches have suggested that the CXCR-4 and its ligand CXCL-12 are potential targets for cancer therapy (Zhou et al. 2019). CXCL-12/CXCR-4 axis could directly or indirectly activate massive signaling pathways including PI3 kinase, stress-activated protein kinase, c-Jun N-terminal kinase, and mitogen-activated protein kinase (MAPK) to promote tumor growth, adhesion, migration, and survival (Dewan et al. 2006). Because of expressing CXCR-4, macrophages could migrate along CXCL-12 gradient. It was shown that CXCL-12 production by breast cancer cells results in increased macrophage number in breast tumor (Bonapace et al. 2014). Similar responses were also showed in monocytes: CXCL-12 released by multiple myeloma cells could trigger monocyte migration and anti-CXCR-4 antibodies could obviously suppress monocyte recruitment. BMS-936564/MDX-1338, a human mAb recognizing human CXCR-4, could significantly induce apoptosis *in vitro* and inhibit tumor growth in several established experimental tumor models

(Kuhne et al. 2013). Plerixafor (AMD3100), CXCR-4 inhibitor, could suppress the postsepsis-induced melanoma progression, TAMs accumulation, and TAMs *in situ* proliferation in mice model. Plerixafor could also selectively reduce the number of M2 TAMs and suppress tumor revascularization and re-growth (Zhou et al. 2018). More specifically, cancer-associated fibroblast-derived CXCL12 attracted CXCR-4 expressing M2-like macrophages to infiltrate into tumors, which promoted cancer stem cells-like transition, proliferation, and migration of cancer cells in oral squamous cell carcinoma (Li et al. 2019b). Progranulin presents carcinogenic roles in breast cancer. Recent studies revealed that Progranulin KO TAMs inhibited invasion, migration, and EMT of breast cancer cells through their exosomes. miR-5100 in Progranulin KO TAMs-derived exosomes was upregulated, which might contribute to CXCL-12 regulation, thereby suppressing CXCL-12/CXCR-4 axis and finally inhibiting the invasion, migration, and EMT of breast cancer cells (Yue et al. 2021). Similar results were also shown in liver metastasis of colorectal cancer: Several miRNAs upregulated in colorectal cancer cells by suppressing CXCL-12/CXCR-4 axis activation could be transferred to TAMs via exosomes and mediate crosstalk between CXCR-4 overexpressing cancer cells and TAMs (Wang et al. 2020). Apart from antibodies, small-molecules, and microRNAs, small modified peptides designed as CXCR-4 antagonists were also widely tested: A novel CXCR-4 antagonist BKT140 showed potent therapeutic efficacy against human nonsmall cell lung cancer *in vitro* and *in vivo*. BKT140 could also augment therapeutic effects of chemotherapy and radiotherapy (Fahham et al. 2012). Similarly, a peptidic CXCR-4 inhibitor TF14016 also suppressed metastases of small cell lung cancer cells in mice (Otani et al. 2012). Similarly, as CXCR-4 antagonists, T140 analogs were also known as antimetastatic agents for breast cancer therapy (Tamamura et al. 2003). Targeting CXCL-12/CXCR-4 axis could also be in combination with standard cancer therapies: CXCL-12 upregulation in hepatocellular carcinoma models triggered hypoxia, immunosuppressive cells recruitment,



T-regulatory cells, and M2 TAMs accumulation after sorafenib treatment. CXCR-4 inhibitor AMD3100 and Sorafenib enhanced PD-1 blockade-induced hepatocellular carcinoma suppression through inhibiting the polarization toward an immunosuppressive microenvironment (Chen et al. 2015).

#### 5.1.4 Targeting VEGF/VEGFR Signaling

Tumor angiogenesis is a critical process for necessary nutrients and oxygen supply to support quickly tumor tissues growing (Nishida et al. 2006). Among them, the family of vascular endothelial growth factors (VEGFs) plays critical roles as regulators of angiogenesis. VEGF/VEGFR signaling pathway is upregulated in numerous cancer types and contributes to out-of-controlled angiogenesis and metastatic spreading (Ceci et al. 2020). VEGFs promote tumor angiogenesis via regulating endothelial cells proliferation and survival (Lee et al. 2015) and might promote tumor cell proliferation through activating VEGFR1 signaling (Bhattacharya et al. 2016). Tumor cells could also recruit and reprogram macrophages derived from circulating monocytes; these recruited and reprogrammed macrophages could also work as a main source of angiogenic factors (Cassetta et al. 2019). For example, angiopoietin-2 produced by tumor endothelium could enhance TIE2 receptor expressing monocytes recruiting in tumor cells. TAMs could also release angiogenic factors such as VEGFA as the response of hypoxia in tumor avascular areas; the released VEGFA could further stimulate macrophages and endothelial cells conversely (Mazzone and Bergers 2019). TAMs were able to release cytokines that indirectly contribute to tumor angiogenesis by the induction of a pro-angiogenic program in tumor cells. Tumor cells and recruited TAMs cooperated in the TME to amplify the production of pro-angiogenic factors resulting in an angiogenic switch. Apart from pro-angiogenesis, TAMs also tightly involved in lymph angiogenesis under inflammatory conditions or carcinogenetic conditions (Yang et al. 2018).

VEGFs can recruit macrophages to the tumor and promote TAMs development (Lapeyre-Prost et al. 2017; Linde et al. 2012). Thus, targeting VEGF/VEGFR has potential suppressing macrophage recruit and localize to the tumor (Yang et al. 2018). The potent proangiogenic activities of TAMs in the TME and the existing of angiogenesis-relevant macrophage subpopulation Tie2 expressing monocytes (TEMs) demonstrate novel antitumor strategies through targeting angiogenesis (Johansson-Percival et al. 2018; Chen et al. 2016). Dampening TME recruitment might be an effective method to block tumor angiogenesis. Inhibiting Ang2-Tie2 crosstalk by using Ang2-CovX-Bodies prevented TEMs infiltration (Huang et al. 2011). Anti-ANG2 monoclonal antibody (mAb) could also disrupt connection between TEMs and endothelial cells, leading to suppressed angiogenesis in pancreatic insulinomas and mammary carcinomas (Mazzieri et al. 2011). As ANG2 overexpression could induce anti-VEGF therapy resistance, antibodies targeting both ANG2 and VEGF could abrogate angiogenesis and promote antitumor efficacy (Klopper et al. 2016; Scheuer et al. 2016). YKL-40, pro-angiogenic factor, could mediate angiogenesis both in vitro and in mice tumor models. Neutralizing mAbs targeting YKL-40 could suppress angiogenesis and progression of tumor (Faibish et al. 2011).

Besides, macrophages could mediate anti-VEGF therapy resistance through changing VEGFR expression level (Dalton et al. 2017), and TAMs might play critical roles during these processes (Itatani et al. 2018): TAMs have been shown to help escape from antiangiogenic therapy of glioblastoma both in preclinical models and in clinic and could work as a potential biomarker and therapeutic target (Lu-Emerson et al. 2013). Similarly, macrophages could be activated and recruited to the TME and further contribute to anti-VEGF resistance in regular ovarian cancer mice model, but there is no resistance anti-VEGF antibody when macrophages were depleted in a macrophage-deficient mouse model, while this resistance could still be triggered by macrophages injection (Dalton et al. 2017). Targeting TAMs might restore or enhance antiangiogenic

therapeutic effects. As one of bisphosphonate drugs, Zoledronic was widely used for treating osteoporosis and bone metastases in clinic, while Zoledronic also showed ability to deplete macrophages. Researchers have investigated the role of macrophages depletion in anti-VEGF antibody therapy resistance in mice bearing ovarian tumor models: Macrophages inhibited by Zoledronic could overcome anti-VEGF antibody therapy resistance, enhance tumor growth suppression, and obviously extend survival when compared with vehicle and only anti-VEGF therapy groups (Dalton et al. 2017). Recent study also presented that expression of CCL-2 could promote antiangiogenic therapy resistance; mNOX-E36, a CCL-2 inhibitor inhibiting TAMs recruitment and angiogenesis, could enhance the efficacy of bevacizumab in glioma models (Cho et al. 2019). Similar strategies were also used by targeting CXCL-12/CXCR-4 axis: NOX-A12, a novel CXCL-12 inhibitor, could reverse TAMs recruitment and potentiate the antitumor effects of anti-VEGF therapy (Deng et al. 2017). Combination of CXCR-4 inhibitors and anti-VEGF therapy was also reported to slow GBM xenografts progression both in preclinic and in clinical trial: CXCR-4 antagonist PRX177561 increased the antitumor effects of bevacizumab in preclinical models of human glioblastoma (Gravina et al. 2017); another CXCR-4 antagonist POL5551 combined with VEGF inhibition could also improve survival in an intracranial mouse model of glioblastoma (Barone et al. 2014). AMD3100 against CXCR-4 was applied with a combination of bevacizumab in patients with recurrent high-grade glioma in clinical trial. Thus, suppressing VEGF/VEGFR signaling combined with other inhibiting TAMs strategies will bring more benefits for cancer therapies.

## 5.2 Target TAMs Inhibition for Cancer Therapy

There are two approaches to decrease the population of TAMs for cancer therapy: One is to reduce the monocytes number in the circulating system and the other is to suppress the population of

macrophages already accumulated in the tumor tissues (Sawa-Wejksza and Kandefer-Szerszen 2018).

Bisphosphonates, including alendronate, ibandronate, risedronate, and zoledronic acid, are primary agents in the current pharmacological arsenal to prevent the loss of bone density and treat osteoporosis and related diseases (Drake et al. 2008). The use of bisphosphonates has been related to inhibit proliferation, migration, and invasion of macrophages, which share the same lineage with osteoclasts, causing apoptosis (Rogers and Holen 2011). As chemotherapeutics, no matter traditional free bisphosphonates or nanoparticles-captured bisphosphonates, both showed cytotoxicity to monocytes/macrophages and have been used to decrease their population in tumors (Farrell et al. 2018). In preclinical models, bisphosphonates could effectively inhibit tumor growth through suppressing TAMs infiltration in breast tumors (Holen and Coleman 2010). Some research also presented that Trabectedin, a registered antineoplastic agent that suppresses cancer cell growth, could also trigger specific death of circulating monocytes/macrophages through TNF-related apoptosis-inducing ligand (TRAIL)-dependent apoptosis, as circulating monocytes/macrophages expressed functional TRAIL receptors 1 and 2, which are susceptible to the cytotoxicity of trabectedin (Germano et al. 2013). Trabectedin-treated pancreatic ductal adenocarcinoma showed reduced TAMs, decreased angiogenesis, activated antitumoral CTLs, with promising clinical outcomes (Borgoni et al. 2018). Similarly, in cutaneous melanoma mice model, trabectedin showed significant activity of reducing TAMs and tumor blood vessel density and inhibited lung metastasis of melanoma (Carminati et al. 2019). Besides, a combination of checkpoint blockade and angiogenesis inhibitors could be an effective strategy to promote the curative effect of Trabectedin (Seliger 2019). Trabectedin also revealed a strategy of immunomodulation in chronic lymphocytic leukemia: Trabectedin depleted myeloid-derived suppressor cells and TAMs and increased memory T-cells in xenograft and immunocompetent chronic lymphocytic leukemia mouse models and could also

suppress PD-1/PD-L1 axis through targeting PD-L1-positive chronic lymphocytic leukemia cells, monocytes/macrophages, and T-cells (Banerjee et al. 2019). Similar results were also shown in skeletal prostate cancer models: Trabectedin reduced prostate cancer bone resident tumor size, which was associated with trabectedin-reduced M2 macrophages (Jones et al. 2019). Depletion of TAMs by trabectedin could also switch the epigenetic profile of pancreatic cancer infiltrating T-cells and restore their antitumor phenotype (Borgoni et al. 2018).

There also have other strategies to depleting TAMs through targeting their specific surface markers. Sialic acid receptors in the Siglec family are highly expressed on the surface of TAMs and most have immunosuppressive effects. Targeted delivery of zoledronic acid through the sialic acid-Siglec axis could kill and reversal M2-like TAMs and inhibit S180 tumor growth (Tang et al. 2020). Similarly, Folate receptor beta (FR beta) was found expressed on macrophages in human glioma and rat C6 glioma. A recombinant immunotoxin consisting of anti-FR beta mAbs and *Pseudomonas* exotoxin A could significantly reduce TAMs numbers and suppress tumor growth (Nagai et al. 2009). Apart from sialic acid receptors and FR beta, activated macrophages also expressed the pattern recognition receptor scavenger receptor A (SR-A), a small peptide SR-A ligand could compete with physiological SR-A ligand *in vitro*, and deficiency of SR-A suppressed progression and metastasis of ovarian and pancreatic cancer *in vivo* (Neyen et al. 2013). M2 macrophage-targeting peptide (M2pep), which could bind to murine M2 macrophages and M2-like TAMs, was fused with proapoptotic peptide KLA. This fusion peptide could also reduce TAM population *in vivo* but need high concentrations (Ngambenjawong et al. 2016).

Although targeting CSF-1/CSF-1R axis or CXCL-12/CXCR-4 axis could suppress TAMs through blocking the recruitment and localization of TAM, researchers also find that in some other studies, the use of these axis inhibitors decreased TAMs number in tumor tissue, inhibited tumor proliferation, and significantly decreased angio-

genesis and metastasis (Anfray et al. 2019). Besides, the use of the anti-IL-6 antibody had a strong anticancer effect by decreasing CCL-2, VEGF, and CXCL-12. There was a significant decline in CCL-2, CXCL-12, and VEGF as well as the number of TAMs in tumor tissue in patients treated with anti-IL-6 antibody for 6 months (Chen and Chen 2015).

### 5.3 Reprogramming TAMs for Cancer Therapy

As the most abundant tumor-infiltrating immune cells in TME, TAMs play a crucial role in tumor development, invasion, and metastasis (Pathria et al. 2019). Emerging evidences demonstrate that the plasticity and diversity of TAMs allow them to be classified along the M1-M2 polarization axis (Genard et al. 2017). M2 macrophages, also known as alternatively activated macrophages, can be induced by the TH<sub>2</sub> cytokines such as IL-4, IL-10, and IL-13 and promote tumor angiogenesis, immunosuppression, and drug resistance (Gordon and Taylor 2005). On the contrary, the TH<sub>1</sub> cytokines such as IL12, IL-18, or activated TLRs stimulate macrophages to M1 macrophages, also known as classically activated macrophages (Biswas and Mantovani 2010). M1 macrophages present the ability to eliminate tumor cells by releasing reactive oxygen/nitrogen intermediates, together with pro-inflammatory cytokines such as TNF, IFN- $\gamma$ . However, in TME of solid tumors, most TAMs are devoid of cytotoxic activity and closely related to the M2 phenotype with poor clinical outcome of patients. Thus, reprogramming the TAMs through chemotherapy, radiotherapy, and immunotherapy could be a promising strategy to eradicate tumors.

#### 5.3.1 TLR Agonists to Reprogram TAMs

Currently, several options including TLRs agonists and compounds or mAb targeting inhibitory proteins of M1 phenotype are used to select M1 phenotype or reprogram TAMs from M2 to M1 phenotype (Mantovani et al. 2017). As pattern-

recognition receptors in innate immunity, TLRs stimulate and activate M1-phenotype polarization via their engagement with the ligands. Thus, various agonists targeting TLRs, such as TLR3, TLR7, and TLR9, have been developed to evaluate their capacity to reprogram TAMs into antitumor effectors (Anfray et al. 2019).

Stimulation of TLR3 by poly I:C upregulated costimulatory molecules CD40, CD80, CD86, and M1-specific markers MHC-II on M2 macrophage. Simultaneously, the expression of pro-inflammatory cytokines and M2-specific markers Tim-3, CD206 was reduced (Shime et al. 2012). In MC38 tumor model, poly I:C reverted the M2 phenotype to M1 macrophages and eradicated the tumor in an IFN- $\alpha/\beta$ -dependent signaling pathway. Recently, Liu et al. developed novel polypeptide micelles to target TAM. Poly I:C-loaded polypeptide micelles showed property for targeting TAM, efficiently reeducated TAMs into M1 macrophages, substantially activated T lymphocytes and NK cells in melanoma models, indicating repolarizing TAMs into M1 phenotype in situ for effective immunotherapy of cancer (Liu et al. 2018). Ferumoxitol, a clinically approved nanoparticle, in combination with TLR3 agonist poly I:C induced pro-inflammatory macrophage polarization for regression of primary and metastatic murine melanoma (Zhao et al. 2018a; Zanganeh et al. 2016). In 2018, a phase II trial has been performed to evaluate the safety and therapeutic effect of combination of Poly I:C and immune checkpoint blockade in unresectable hepatocellular carcinoma, while its analog, poly-ICLC, has been tested as a tumor vaccine to boost antitumor immunity (Anfray et al. 2019).

TLR7 agonist was demonstrated to induce the nuclear translocation of NF- $\kappa$ B in macrophages with the production of pro-inflammatory cytokines (De Meyer et al. 2012). Topical administration of TLR7 agonist imiquimod with radiotherapy has been approved to synergistically inhibit tumor growth and cyclophosphamide further increased the therapeutic effect and induced immunologic memory in cutaneous breast cancer metastases. Currently, imiquimod is the only TLR agonist approved by Food and Drug

Administration for topical injection in intraepidermal carcinoma and basal cell carcinoma.

In the past years, resiquimod has attracted more attention for its role to reeducate TAMs into M1 macrophages (Thauvin et al. 2019). Resiquimod, also known as R848, is a dual agonist of the toll-like receptors TLR7/8 (Chi et al. 2017). Similar to imiquimod, R488 showed more powerful as a TLR agonist, with more ability to elicit antitumor immune immunity. Despite several studies have showed promising results based on the topical injection in melanoma patients, there are no ongoing clinical trials due to that systemic administration of R848 is burdened with toxicities: anemia, inflammation, and flu-like symptoms (Perkins et al. 2012; Hasham et al. 2017). To overcome this issue, various methods have been introduced. MEDI9197, a formulation of R848 was designed with a lipid tail to increase lipid solubility and be retained at the injection sites, thus limiting systemic toxicity. As an alternative, R848 was covalently linked with vitamin E and modified by hyaluronic acid into a prodrug nanopreparation (CDNP-R848), providing a sustained release of R848 by subcutaneous injection (Rodell et al. 2018). The prodrug nanopreparation successfully delivered R848 to TAMs in vivo and altered the functional orientation of the TAMs toward M1 macrophage, leading to inhibited tumor growth and protecting the mice against tumor re-challenge. When CDNP-R848 was combined with anti-PD-1 blockade, improved immunotherapy response rates were observed, including an anti-PD-1 therapy-resistant tumor model. In addition, Rodell et al. identified R848 as a potent driver of M1 macrophage polarization and developed R848-loaded  $\beta$ -cyclodextrin nanoparticles to efficiently deliver R848 to TAM in MC38 colorectal tumors (Rodell et al. 2018). These strategies were able to trigger the IL-12 production of TAMs in TME, demonstrating that engineered R848-nanoparticle combinations could efficiently modulate TAMs for cancer immunotherapy.

As to the TLR9, preclinical data of the agonists singly or in combination with other agents showed efficacy, which led to clinical trials in patients with advanced malignancy (Karapetyan

et al. 2020). In the preclinical models, intratumoral, subcutaneous, and intravenous routes of injection have been performed with potent antitumor responses in treated and metastatic sites with the repolarization of TAMs (Hofmann et al. 2008). In the clinic, despite TLR9 agonist monotherapy or combined with chemotherapy and targeted therapy showed no obvious efficacy in advanced solid tumors, especially with an intravenous injection, the greatest excitement has been reserved for TLR9 agonists in combination with immune checkpoint inhibitors. Currently, a clinical trial has been registered to evaluate the antitumor efficacy of TLR9 agonist CpG in combination with nivolumab in metastatic pancreatic cancer patients (NCT04612530).

Despite these *in vitro* data are promising, there is limited evidence for TLRs agonists as adjuvants in the field of cancer vaccination. In this regard, nanoparticles are considered as a potential strategy to load TLRs agonists to the target tumor sites. For example, nanoparticles loaded with poly I:C or R848 presented the power to elicit potent innate immune responses in lymph nodes, resulting in therapeutic effect without systemic release of inflammatory cytokines (Bocanegra Gondan et al. 2018; Da Silva et al. 2019).

### 5.3.2 Reprogramming TAMs by mAb and Fusion Protein

In the TME, reprogramming M2 macrophages into M1 proinflammatory phenotype is a potential antitumor immunotherapy which is involved in increased activity of macrophage phagocytosis. The macrophage phagocytosis is balanced by activating and inhibitory signals. CD47 is an immune checkpoint that confers a “do-not-eat-me” signal to host immune surveillance via binding to its ligand, SIRP $\alpha$  on the surface of phagocytic cells, such as macrophages and dendritic cells (Zhang et al. 2017). SIRP $\alpha$  binds to CD47 and transmits intracellular signals through its cytoplasmic domain. The cytoplasmic tail of SIRP $\alpha$  contains four immunoreceptor tyrosine-based inhibition motifs (ITIMs) and becomes phosphorylated after binding of CD47, leading to prevention of myosin-IIA accumulation at the

phagocytic synapse and inhibition of phagocytosis (Tsai and Discher 2008; Barclay and Brown 2006; van Beek et al. 2005). The “do-not-eat-me” signals play key roles for tissue homeostasis. However, CD47 overexpression has been adopted by various cancer cells to escape the innate immune surveillance. In this sense, pharmacological blockade of CD47-SIRP $\alpha$  axis by monoclonal antibody or fusion protein could reprogram macrophage phagocytosis in multiple preclinical models (Galli et al. 2015; Weiskopf et al. 2016; Zhang et al. 2018a). For instance, reprogramming of TAMs into M1 macrophages has been achieved by the combination of anti-SIRP $\alpha$  antibody with CSF-1R inhibitor (Kulkarni et al. 2018). TTI-621, a CD47-blocking SIRP $\alpha$ Fc fusion protein, triggers tumor cell phagocytosis by M1 macrophages *in vitro* and exhibits antitumor activity *in vivo*. Combinational therapies have the potential to increase the effect (Petrova et al. 2017). Data from a phase 1b trial showed that the macrophage checkpoint inhibitor Hu5F9-G4 combined with anti-CD20 antibody rituximab demonstrated promising activity in advanced lymphoma patients (Advani et al. 2018). Based on these promising evidences, several clinical trials are performed to evaluate the safety and activity of anti-CD47/SIRP $\alpha$  monotherapy, also in combination with other agents (Table 2).

As an alternative target to favor cytotoxic functions of TAMs, CD40 is a costimulatory protein found on antigen-presenting cells including macrophages and dendritic cells. CD40L on TH cells binding to CD40 activates antigen presenting cells and triggers various downstream effects, including upregulation of the MHC molecule expression, secretion of proinflammatory cytokines, and finally cytotoxic T-cell activation (Zippelius et al. 2015; Zhang et al. 2018b). Re-educating macrophages using CD40 agonistic antibodies could recover tumor immune surveillance and reprogram TAMs toward M1-polarization in various tumor models (Vonderheide 2020). However, the antitumor activities have been moderate. Since anti-CD40 therapy induced PD-L1 upregulation in TAM, combining CD40 agonists with anti-PD-1 ther-

**Table 2** List of clinical trials reprogramming TAMs

Identifier	Agent	Description	Strategy	Phase	Condition
NCT03717103	IBI188	Humanized anti-CD47 mAb	Single agent; combination with rituximab	Phase I	Advanced malignancies
NCT03763149	IBI188	Humanized anti-CD47 mAb	Single agent	Phase I	Advanced malignancies
NCT03834948	AO-176	Humanized anti-CD47 mAb	Single agent; combination with paclitaxel	Phase I/II	Solid tumor
NCT04257617	ZL1201	Humanized anti-CD47 antibody	Single agent	Phase I	Locally advanced solid tumor
NCT04349969	AK117	Humanized anti-CD47 antibody	Single agent	Phase I	Neoplasms malignant
NCT02367196	CC-90002	Humanized CD47-blocking antibody	Single agent; combination with rituximab	Phase I	Hematologic neoplasms
NCT02663518	TTI-621	SIRP $\alpha$ -Fc fusion protein, human IgG1 subclass	Single agent; combination with rituximab	Phase I	Hematologic malignancies, solid tumor
NCT03248479	Magrolimab	Humanized anti-CD47 mAb	Single agent; combination with azacitidine	Phase I	Hematological malignancies
NCT04599634	Magrolimab	Humanized anti-CD47 mAb	Combination with obinutuzumab and venetoclax	Phase I	Relapsed and refractory indolent B-cell malignancies
NCT04445701	AO-176	Humanized anti-CD47 mAb	Single agent; combination with bortezomib and/or dexamethasone	Phase I/II	Relapsed/refractory multiple myeloma
NCT03013218	ALX148	High-affinity SIRP $\alpha$ variant	Single agent; combination with pembrolizumab; combination with trastuzumab; combination with rituximab	Phase I	Advanced solid tumors, lymphoma
NCT04306224	IMC-002	Humanized anti-CD47 mAb	Single agent	Phase I	Solid tumor, lymphoma
NCT04417517	ALX148	High-affinity SIRP $\alpha$ variant	Combination with azacitidine	Phase I/II	Higher risk myelodysplastic syndrome
NCT03527147	Hu5F9-G4	Humanized anti-CD47 mAb	Combination with acalabrutinib	Phase I	Relapsed or refractory aggressive non-Hodgkin's lymphoma
NCT04406623	SL-172154	Fusion protein consisting of human SIRP $\alpha$ and CD40L	Single agent	Phase I	Ovarian cancer

apy resulted in significantly lower tumor burden than either monotherapy alone (Diggs et al. 2020). Concurrent CSF-1R blockade and CD40 agonists lead to significant changes in the composition of immune infiltrates, causing an increased differentiation of proinflammatory M1 macrophages and also priming potent cytotoxic T-cell response in the draining lymph nodes of “cold” tumor models (Wiehagen et al. 2017).

### 5.3.3 Other Strategies to Reprogram TAMs

With the progress of oligonucleotide delivery, gene therapies, including microRNA (miRNA), small interfering RNA (siRNA), and messenger RNA (mRNA), are promising strategies to re-educate macrophages for cancer treatment. MiRNAs are tiny, single-stranded noncoding RNA molecule containing 19–24 nucleotides. Substantial research data depict that miRNAs are capable of silencing the gene expression either by degrading mRNA or repressing the gene transcription (Chatterjee et al. 2020). By performing miRNA profiling studies on macrophages, Graff and colleagues identified the miRNAs were associated with macrophage responses to inflammatory stimuli and demonstrated that miR-125a-3p and miR-26a-2 promote M1-like phenotype (Graff et al. 2012). Cai et al. presented the first evidence that miRNA-155 was a key molecule re-educating TAMs into pro-inflammatory M1 macrophages. In a mouse sarcoma model, delivering miRNA-155 by lipid-coated calcium phosphate nanoparticles to TAMs successfully increased IL-12 expression with controlled tumor growth and extended survival (Cai et al. 2012). SiRNA has been employed to block the expression of immunosuppressive genes in TAMs. Shi et al. developed a robust nanoparticle platform for efficient siRNA delivery and gene silencing in macrophages (Liang et al. 2018). Silencing CCL-18 in macrophages remarkably suppressed breast cancer cells migration via regulation of the TAMs behavior.

In addition, researchers also exploited new strategies and carriers to increase the target gene expression in TAMs, such as charge-altering releasable transporters. This method has been

used to deliver a combination of CD80, CD86, and OX40L mRNAs in several tumor models and induced systemic antitumor responses in vivo (Haabeth et al. 2019). As far as we know, no RNA delivery systems have been approved to reprogram TAMs, and more potent and promising strategies are still needed to initiate RNA delivery technology for TAMs reprogramming.

## 5.4 TAM as Drug Delivery Systems

As a novel strategy to deliver payloads, the biomimetic delivery system recently showed its superiority on cancer targeting drug delivery (Yoo et al. 2011). Neutrophils, red blood cells, and macrophages are three common drug carriers (Fang et al. 2018). Through binding to IL-6 and TNF- $\alpha$ , surface adhesion molecules expressed on neutrophils lead to the intrinsic target to inflammation. Red blood cells can increase the long-circulation time of cargo nanoparticles in blood circulation without tumor-targeting ability. Macrophages not only circulate in the blood circulation like neutrophils and red blood cells, but also specifically target tumors through integrin on macrophages binding to VCAM-1 of tumor cells. Herein, macrophage-based drug deliveries are more versatile (Xia et al. 2020).

Given the intrinsic homing property, M1 macrophages can be employed as a biomimetic delivery system to actively carry payloads to the tumor sites (Guerra et al. 2017). Up to now, researchers have mainly designed strategies to utilize M1 macrophage and M1 macrophage-derived exosomes as tumor-targeted drug carriers. Furthermore, membrane fragments have also been extracted to coat nanoparticles to increase the targeting ability. Here, these three strategies will be detailed analyzed: M1 macrophages, M1 macrophages-derived exosomes, and M1 macrophage membrane-coated nanoparticles (Fig. 3).

### 5.4.1 M1 Macrophages as Drug Carriers

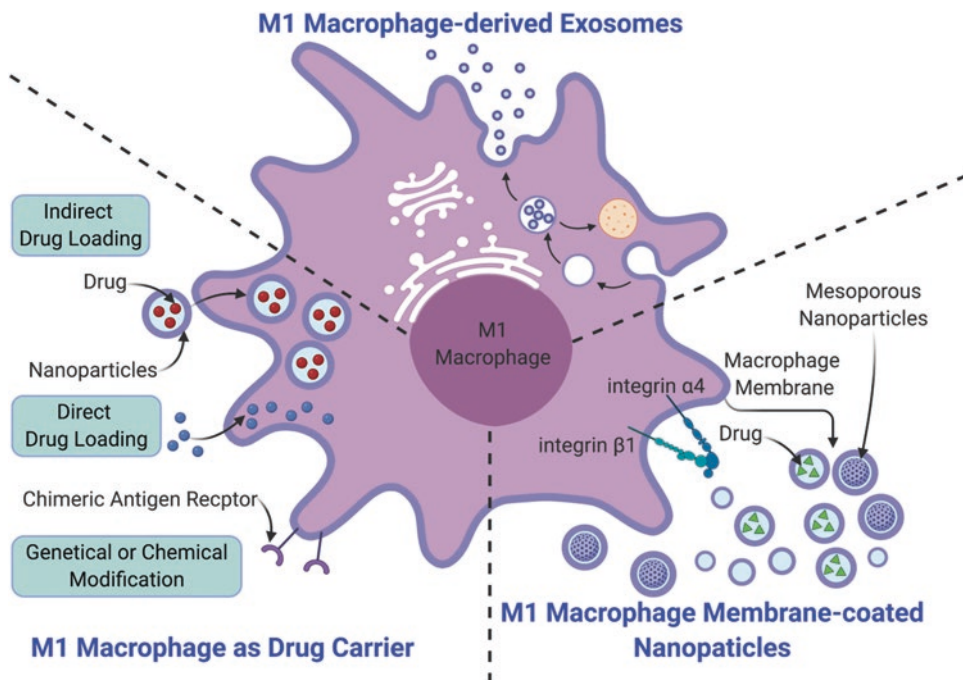
M1 macrophages including RAW264.7 cells and bone-marrow-derived macrophages (BMDMs) are extensively used for tumor-targeted delivery

(Xia et al. 2020). Up to now, there are three M1 macrophage-based drug delivery systems. The first system is using macrophage as a direct anti-tumor drug carrier. Drug-loaded M1 macrophages were developed by simply incubation. For example, Fu and colleagues constructed a doxorubicin-loaded RAW264.7 cells to treat 4 T1 tumors. Data showed that doxorubicin did not significantly affect and alter the tumor-tropic capacity of M1 macrophages to tumor cells in vitro and in vivo. Importantly, the doxorubicin-loaded M1 macrophages potently inhibited the tumor metastasis and prolonged the life span, with reduced toxicity (Fu et al. 2015). The second one is using macrophage as an indirect anti-tumor drug carrier. Instead of directly loading drug, macrophages were employed to load nanoparticles containing drugs. This strategy could reduce the cytotoxicity of drugs on macrophages and further increase the drug load. Tao et al. used BMDMs to load nano/paclitaxel (paclitaxel-loaded nano-formulations) and found

that nano/paclitaxel-loaded M1 macrophages showed better efficacy in malignant glioma (Tao et al. 2013). The third one is to modify macrophage as a drug carrier. To further potentiate the property of these carriers, macrophages can be further modified. M1 macrophages can be genetically or chemically modified to enhance immunological activity. Zhang et al. developed induced pluripotent stem cells-derived engineered chimeric antigen receptor-expressing macrophage and conferred the macrophage antigen-dependent functions such as secretion of cytokines, polarization toward the pro-inflammatory state (Zhang et al. 2020b).

#### 5.4.2 M1 Macrophages-Derived Exosomes as Drug Carriers

Exosomes are membrane-bound extracellular vesicles that originated from the endosomal compartment of cells (Bunggulawa et al. 2018). Compared to M1 macrophage, M1 macrophage-derived exosomes present similar membrane



**Fig. 3** TAMs as drug delivery systems. Three strategies were highlighted for TAMs as drug delivery systems: 1) M1 macrophages as drug carriers through direct drug loading, indirect drug loading, or genetical/chemical

modification; 2) M1 macrophages-derived exosomes as drug carriers; and 3) M1 macrophage membrane-coated nanoparticles as drug carriers



properties and can be utilized to load drugs for cancer treatment. Kim and colleagues used M1 macrophage-derived exosomes to load paclitaxel via a sonication method. This delivery system increased the cytotoxicity more than 50 times in multidrug-resistant cancer cells (Kim et al. 2016). M1 macrophage-derived exosomes also could be a cancer vaccine adjuvant owing to the specific proinflammatory function (Wang et al. 2019). M1 macrophage-derived exosomes not only potentiated tumor-targeted drug delivery, but also could be engineered with liposomes including different components (Rayamajhi et al. 2019). In short, M1 macrophages and M1 macrophage-derived exosomes retained the targeting ability and proinflammatory function. However, the yield of M1 macrophage-derived exosomes is still low, and proper engineering of M1 macrophages and constructing exosome-mimetic vesicles may be the potential strategies to overcome this issue.

#### 5.4.3 Nanoparticles Coated with M1 Macrophage Membrane as Drug Carriers

Considering the excellent tumor-targeting abilities and tumor infiltration of M1 macrophages, cell membranes of M1 macrophages can be extracted to coat nanoparticles and increase the targeting ability of nanoparticles in tumors. M1 macrophage membrane-coated nanoparticles could be constructed by coextruding the isolated macrophage membranes with nanoparticles and exhibited more persistent circulation and higher tumor accumulation (Cao et al. 2016; Zhao et al. 2018b). Cao et al. reported drug-loaded liposomes with isolated macrophage membranes to increase the drug delivery to metastasis sites. The macrophage membranes potentiated the cellular uptake of liposomes in cancer cells and presented potent suppression on metastatic breast cancer (Cao et al. 2016). This is due to the high expression of integrin  $\alpha 4$  and  $\beta 1$  on macrophages, which could bind to VCAM-1 on breast cancer cells. Herein, M1 macrophage-derived membrane has the targeting property for tumors. Xuan and colleagues engineered macrophage cell membrane-coated mesoporous silica nano-capsules

(MSNCs) by a biomimetic drug-delivery system. The macrophage membrane reduced the uptake percentage of MSNCs by immune cells and tissues, effectively prolong the half-life in blood circulation. Compared to the totally cleared non-coated MSNCs, the macrophage membrane-coated MSNCs showed 32% retention in blood circulation and the targeting accumulation of macrophage membrane-coated MSNCs led to complete tumor eradication (Xuan et al. 2015). In summary, membranes from M1 macrophage could also be used to modify various nanoparticles for drug delivery.

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## 6 Conclusions

Pro-tumoral functions of TAMs make them attractive targets for cancer immunotherapy. As described in this book chapter, several preclinical and clinical studies revealed that depletion of TAMs significantly augments the response of immunotherapy and chemo-/radiotherapy and suppresses the cancer metastasis. However, as we know, TAMs represent a heterogeneous population with distinct functions that vary according to cancer types and the stages of cancer progression. One of the future works need to be done to define the subsets of TAMs involving tumorigenesis, which can be targeted for effective therapies, while saving the macrophages with antitumor functions (Guerriero 2018; Cassetta and Pollard 2018). One big challenge is to investigate metastasis-associated macrophage (MAM) phenotypes involved in cancer metastasis, which is major cause of death of cancer patients. To define those subsets of macrophages, new technologies, such as single cell sequencing, digital spatial profiling, multiplex immunofluorescence, and metabolomics, can be applied (Pathria et al. 2019). Despite multiple early stages of clinical trials are going on, scarce knowledge about TAMs in clinic human cancers is available since much of these data were generated from *in vitro* cellular models or mouse models. TAMs may possess more potential for cancer target therapy than previously thought. More studies should emphasize on the regulation of TAMs, such as

reprogramming TAMs, targeting inhibitory molecules, which will help improve the efficacy of current cancer therapeutics, such as immunotherapy, chemotherapy, and radiotherapy, and ultimately open a new door for targeting TAMs immunotherapy (Cassetta and Pollard 2018).

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