



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

Meng-Qi Liu<sup>1#</sup> Jia-Wei Zhang<sup>1#</sup> Jian-Wei Zhu<sup>1,2\*</sup>

<sup>1</sup>Engineering Research Center of Cell & Therapeutic Antibody, Ministry of Education, School of Pharmacy, Shanghai Jiao Tong University, Shanghai, People's Republic of China

<sup>2</sup>Jeche Laboratories, Inc., Maryland, United States

Address for correspondence Jian-Wei Zhu, PhD, School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, People's Republic of China (e-mail: jianweiz@sjtu.edu.cn).

Pharmaceut Fronts 2023;5:e254–e273.

## Abstract

Tumor-associated macrophages (TAMs) constitute a significant component of the tumor microenvironment. This work reviewed the latest progress in comprehending the function of TAMs and their strategies for cancer therapy. TAMs are highly heterogeneous and plastic and exhibit different functional phenotypes in response to different signal stimuli. The emergence of single-cell technologies allows us to revisit their diversity in cancer. When their pro-inflammatory function is activated, antitumor TAMs support and activate adaptive immune cells to eliminate cancer cells through T cell-mediated killing. In the context of cancer, anti-inflammatory TAMs play a variety of pro-tumor functions, such as releasing cytokines to promote the recruitment of bone marrow cells, promoting tumor angiogenesis, and inhibiting cytotoxic T cell function. The plasticity of TAMs makes them a potential tumor therapeutic target, so finally, we updated strategies for targeting TAMs and the TAM-targeting agents currently being evaluated in clinical trials.

## Keywords

- ▶ tumor-associated macrophages
- ▶ inflammation
- ▶ cancer immunotherapy
- ▶ tumor microenvironment

## Introduction

In recent decades, significant advancements have been achieved in the realm of cancer research. We now understand that cancer is a complex ecosystem. The initiation and progression of cancer are influenced by both the intrinsic properties of cancer cells and their interactions with the many constituents of the tumor microenvironment (TME) in which they are situated. TME contains a wide diversity of immune cells, cancer-associated fibroblasts, extracellular matrix (ECM), and other secreted molecules.<sup>1</sup> Tumor-associated macrophages (TAMs), which are among the most numerous immune cell populations within TME, have garnered growing interest in recent times on account of

their complex interplay between the TME and tumor cells, as well as the subsequent progression of the tumor.

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

<sup>#</sup> These authors contributed to this work equally.

received  
September 10, 2023  
accepted  
November 17, 2023  
article published online  
December 13, 2023

DOI <https://doi.org/10.1055/s-0043-1777704>.  
ISSN 2628-5088.

© 2023. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution License, permitting unrestricted use, distribution, and reproduction so long as the original work is properly cited. (<https://creativecommons.org/licenses/by/4.0/>)  
Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

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

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

## Origin and Diversity of Macrophages

A great deal of research on macrophage cellular biology has been conducted since immunologist Metchnikoff proposed the concept of phagocytes in the 19th century, and substantial progress has been made in this area. We can now understand the origin and classification of macrophages<sup>11</sup> and here, in this review, we just briefly present it as an introductory background. Macrophages derive from two main sources in adult tissues. One originated from circulating monocytes generated by hematopoietic stem cells in the bone marrow (BM). After entering the bloodstream, monocytes migrate to various tissues, where they undergo differentiation into macrophages that are particular to the respective tissues. Examples of such tissue-specific macrophages are osteoclasts in bone, histiocytes in connective tissue, and Kupffer cells in the liver.<sup>12</sup> Macrophages originating from adult BM can be primarily categorized into “classical” inflammatory monocytes (Ly6C<sup>Hi</sup> CX3CR1<sup>Low</sup> in mouse, CD14<sup>2+</sup> CD16<sup>-</sup> in human) and “nonclassical” patrolling monocytes (Ly6C<sup>Low</sup> CX3CR1<sup>Hi</sup> CCR2<sup>Low</sup> in mouse, CD14<sup>+</sup> CD16<sup>2+</sup> in human). The recruitment of “Classical” inflammatory monocytes to the site of infection, tissue injury, and tumor is known to play a crucial role in the regulation of the inflammatory response. The “Nonclassical” patrol monocytes have a protective function, recognizing and detecting pathogens in the blood circulation and maintaining vascular integrity, rarely extravasating into tissues to differentiate into macrophages.<sup>13</sup> Additionally, they contribute to the removal of tumor debris in the hosts with tumors, as well as the recruitment and activation of natural killer (NK) cells.<sup>14</sup>

Macrophages are also derived from the fetal liver (FL) or yolk sac (YS) during embryonic development. During prenatal development, embryonic progenitors are responsible for initiating the formation of fetal tissue macrophages, which subsequently become tissue-resident macrophages (TRMs). These TRMs remain throughout an individual’s lifespan, existing independently of circulating monocytes.<sup>15,16</sup> Lineage-tracing studies have provided evidence indicating that microglia predominantly originate from YS macrophages,<sup>17</sup> while Langerhans cells are mixed from YS and FL monocytes.<sup>18</sup> Alveolar macrophages<sup>19</sup> and Kupffer cells<sup>20</sup> are mostly derived from FL monocytes, and BM monocytes can also undergo differentiation into Kupffer cells, with a minor contribution.<sup>21</sup> In other tissues, such as the intestine, dermis,

heart, and pancreas,<sup>22–25</sup> there is a coexistence of macrophages produced from BM monocytes and TRMs. Over time, the TRMs are gradually replaced by BM-derived macrophages. In addition to their common functions of pathogen defense, inflammatory response causing and fading, immune surveillance, and cell debris elimination, macrophage populations in these different organs have tissue-specific functions. For example, brain-resident macrophages, and microglia, participate in synaptic remodeling during development.<sup>26,27</sup> Kupffer cells in the liver participate in the elimination of microorganisms and cell debris from the blood and lipid metabolism.<sup>28</sup> Osteoclasts in bone fuse to form multinucleated cells that participate in bone resorption and support hematopoiesis.<sup>29</sup>

## Plasticity and Phenotype of Macrophages

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

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

M2 macrophages are primarily induced by cytokines, including IL-4 and IL-13, from Th2 cells as well as transforming growth factor- $\beta$  (TGF- $\beta$ ). These cytokines play a crucial role in immune modulation, tissue remodeling and angiogenesis, and the facilitation of tumor progression.<sup>32,33</sup> M2 macrophages regulate the TME by the secretion of chemokines, namely CC-chemokine ligand 2 (CCL2) and CCL17, which serve to attract Th2 cells and T regulatory cells.<sup>30</sup> In contrast to the functional role of M1 macrophages, M2 macrophages possess the ability to release cytokines with anti-inflammatory properties, including IL-4, IL-10, and TGF- $\beta$ , as well as pro-angiogenic molecules such as matrix

metalloproteinases and vascular endothelial growth factor (VEGF).<sup>32</sup> Besides, M2 macrophages also showed a notable upregulation of the mannose receptor CD206, alongside a downregulation of pro-inflammatory cytokines. The M2 macrophage population can be further categorized into distinct phenotypes, namely M2a, M2b, M2c, and M2d.<sup>34</sup>

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

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

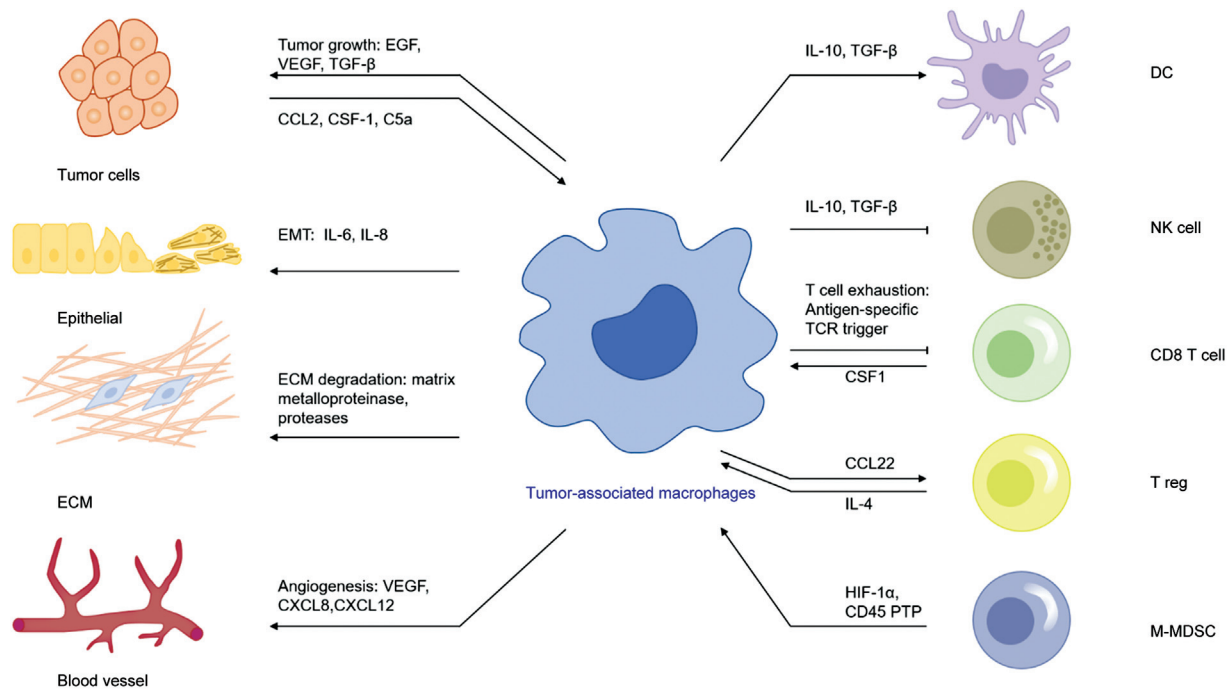
In a single-cell omics review of macrophage diversity published in 2022, the authors found that seven TAM subpopulations are retained in nearly all cancer types by reviewing recent cancer studies on scRNA-seq.<sup>41</sup> The authors suggested renaming these TAM subpopulations based on their anticipated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

## The Role of TAMs in Tumor Progression

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

### Tumorigenesis and Metastasis

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



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

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

Tumor metastasis is a phenomenon in which tumor cells depart from the primary tumor site and establish colonies in other organs via circulatory or lymphatic systems. This process is widely recognized as a leading contributor to the death of cancer patients.<sup>45</sup> TAMs induce epithelial-mesenchymal transition (EMT) of tumor cells by the secretion of a variety of cytokines and inflammatory mediators, including IL-6, IL-8, and TGF- $\beta$ . This secretion process serves to augment the invasive capabilities of tumor cells throughout the metastatic phase. Studies in pancreatic ductal adenocarcinoma (PDAC) and NSCLC have demonstrated that intratumoral macrophage density, EMT markers, and intra-

epithelial TGF- $\beta$  levels are positively correlated with tumor grade. TAMs effectively induce EMT through TGF- $\beta$  and activation of  $\beta$ -catenin pathways in intratumoral cancer cells, thereby promoting tumor metastasis.<sup>46,47</sup> In addition, the ECM plays a crucial role as a tissue barrier against tumor metastasis.<sup>48</sup> TAMs facilitate the degradation of the ECM and the connections between cells and ECM by secreting matrix metalloproteinases (MMP9, MMP-12), serine proteases, and cathepsin, thereby promoting the spread and metastasis of tumor cells.<sup>49–51</sup> In colorectal cancer (CRC), TAMs play a role in promoting EMT in tumor cells by regulating the JAK2/STAT3/miR-506–3p/FoxQ1 axis to enhance tumor cell migration, invasion, and metastasis of tumor cells. Additionally, this regulatory axis leads to the production of CCL2 through a positive feedback loop, which contributes to macrophage recruitment and affects tumor progression.<sup>52</sup>

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



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

### TAM-Mediated Immune Suppression

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

### T Cell

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

### Myeloid-Derived Suppressor Cells

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

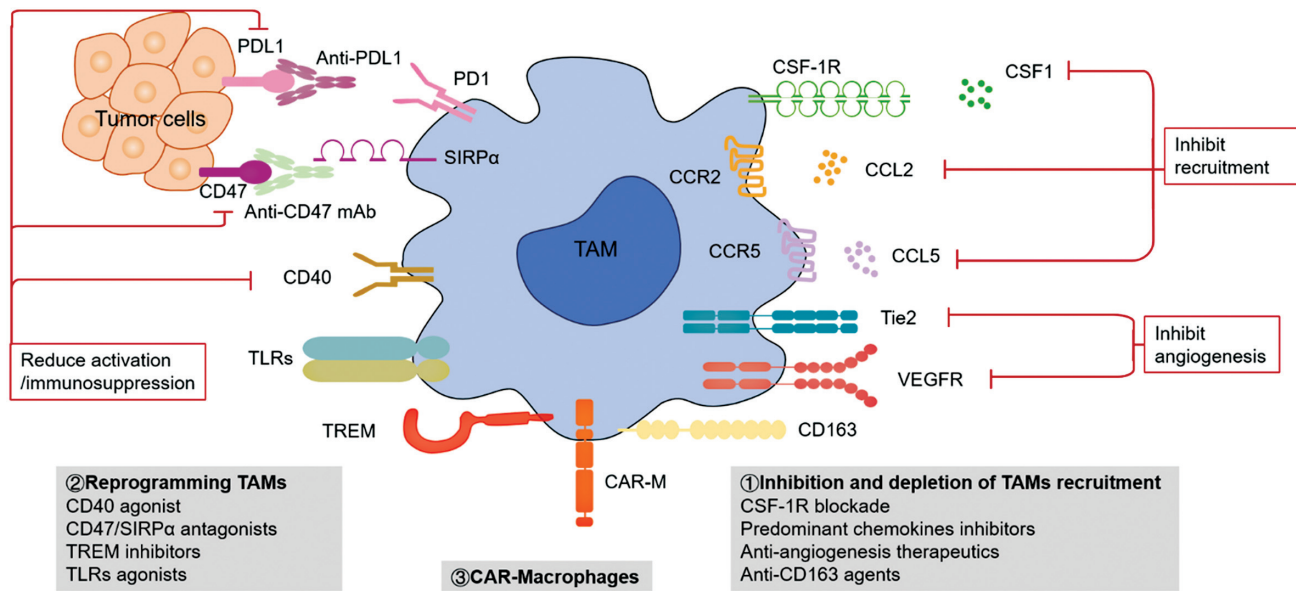
### Therapeutics Targeting TAMs

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

### Inhibition and Depletion of TAM Recruitment

#### CSF-1R Blockade

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



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

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

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

Antibodies represent another effective strategy to inhibit CSF-1R signaling. An anti-mouse CSF-1R antibody depletes most of the TAM and shows good tumor-suppressive efficacy

when combined with a PD-1 inhibitor.<sup>97</sup> Anti-CSF-1R antibody combined with cisplatin induces class I IFN in breast lobular cancer in another study.<sup>98</sup> It could also cooperate with GM-CSF-secreting tumor vaccine and anti-PD-1 agent to suppress PDAC when treated before and after the therapy schedule.<sup>99</sup> This may be because GM-CSF recruits monocytes, which are polarized to M1 macrophages by anti-CSF-1R antibody. As previously reported, GM-CSF protects macrophages from being killed by CSF-1R inhibitors and mediates the re-polarization.<sup>83,94</sup>

Although CSF-1R antibodies seem to be potential immune modulators, they perform poorly clinically. In a phase I clinical trial evaluating AMG-820, patented by Amgen, 32% of patients with mixed types of solid tumors had stable disease.<sup>100</sup> The investigators attribute the poor response to the high proportion of CRCs and the single treatment with AMG-820. However, even when combined with nivolumab after radiotherapy, half of the patients treated with cabiralizumab (BMS) still showed progressive disease.<sup>101</sup> Similar results have been seen with other anti-CSF-1R antibodies. Roche developed emactuzumab (RG7155), which blocks CSF-1R signaling through its binding to the extracellular domains 4 and 5 of CSF-1R and prevents the receptor from dimerizing the interface.<sup>102</sup> Preclinically, emactuzumab efficiently induces apoptosis of macrophages both *in vitro* and *in vivo*. Clinically, like PLX3397, emactuzumab exhibited potential in treating diffuse-type giant cell tumor (dT-GCT) patients.<sup>103</sup> Disappointingly, however, when combined with paclitaxel, emactuzumab showed no further benefit in patients with advanced solid tumors,<sup>104</sup> leading Roche to discontinue the trial in 2017. At the 2019 American Society of Clinical Oncology, Eli Lilly announced the results of a phase I study, which aimed to evaluate the safety and preliminary efficacy of LY3022855 in patients with metastatic breast

cancer or castration-resistant prostate cancer (CRPC). LY3022855 shows limited activity with no complete or partial responses observed in cohorts. Cabiralizumab, patented by Five Prime, failed to meet its primary endpoints in a phase II trial (NCT03336216) in advanced PC. Despite the disappointing clinical news from anti-CSF-1R antibodies, small molecules pexidartinib combined with paclitaxel demonstrated favorable tolerability and exhibited preliminary encouraging efficacy in patients with advanced solid tumors.<sup>105</sup>

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

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

### Predominant Chemokines

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

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

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

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

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

### Antiangiogenesis Therapeutics

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

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

VEGF could be downstream of the Ang2 signal.<sup>151</sup> Bevacizumab treatment reduces IL-10-expressing circulating macrophage,<sup>152</sup> which could be a result of Ang2-VEGF blockade. However, Ang2 impedes the normalization of blood vessels in the U87 orthotopic model with DC101 (anti-VEGFR2 antibody) treatment,<sup>153</sup> suggesting there may be a feedback loop between Ang2 and VEGF signals. Thus, blocking VEGF and Ang2 simultaneously seems more reliable.<sup>150,154,155</sup> As pointed out, cediranib (a VEGFR tyrosine kinase inhibitor) could further suppress the growth of GI261 and U87 xenografts combined with MEDI3617 (anti-Ang2 antibody).<sup>150</sup> However, the combination does not suppress TAM infiltration nor enhance the M1/M2 ratio compared with single-agent treatment. To our surprise, blocking CSF-1 partially neutralizes OS benefits provided by combined therapy, suggesting that the benefits depend on macrophages. Another group treats GI261 (poorly vascular-

ized) and MGG8 (highly vascularized) tumor models with anti-Ang2-VEGF bispecific antibodies (BsAbs) showing similar tumor-inhibitory activity but a different macrophage state.<sup>154</sup> Targeting VEGFA with bevacizumab unexpectedly increases the M2/M1 ratio,<sup>156</sup> and simultaneous blockade of both two targets significantly polarizes macrophages toward an M1-like phenotype with a higher M1/M2 ratio.<sup>154</sup>

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

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

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

### Scavenger Receptor CD163

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



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

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

## Reprogramming TAMs

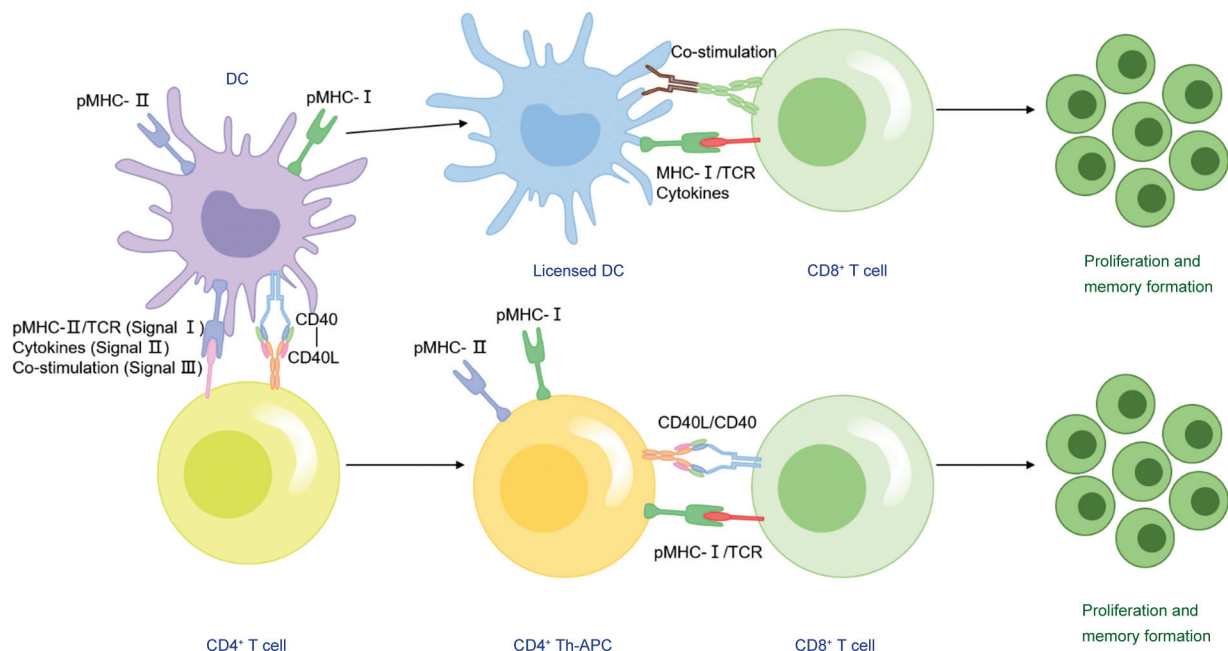
### CD40 Agonist

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

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

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

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



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

shows higher efficacy only when the tumor reaches a larger size with more TAM infiltration. Moreover, the efficacy of combined therapy is abrogated when the anti-CSF-1R antibody is administered in advance, indicating the dependence of macrophages. It echoes another report, in which macrophages produce more pro-inflammatory cytokines like IL-12, and TNF- $\alpha$  while less IL-10.<sup>180</sup>

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

Similar to other TNFRs, CD40 activates downstream signals only when the molecules are trimerized.<sup>182–185</sup> Thus, an effective agonist should trigger the trimerization. Antibodies that could engage with Fc $\gamma$ RIIB gain better agonistic activity because of cross-linking.<sup>186–189</sup> However, it is the cross-linking, but not Fc $\gamma$ RIIB, that is necessary for CD40 agonistic therapeutics. For example, Fabs multimerized by PEG ligation still show promising suppressive efficacy against BCL1 lymphoma. Moreover, multi-fused CD40L could fully activate CD40-expressing cells without further cross-linking.<sup>183,190,191</sup> In addition to cross-linking, the rigid hinge structure imparts considerable agonistic activity to human IgG2 antibodies even when the Fc domain is depleted.<sup>171,189,192</sup> CD40 agonists with the murine IgG2 format have been argued to have poor tumor suppressive activity.<sup>186</sup> Surprisingly, however, mIgG2 FGK4.5 shows encouraging efficacy in PDA, B16-OVA, and MMTV-PyMT mouse tumor models,<sup>172,178</sup> indicating that binding epitopes also influence the effect of CD40 agonistic antibodies.<sup>187,188</sup>

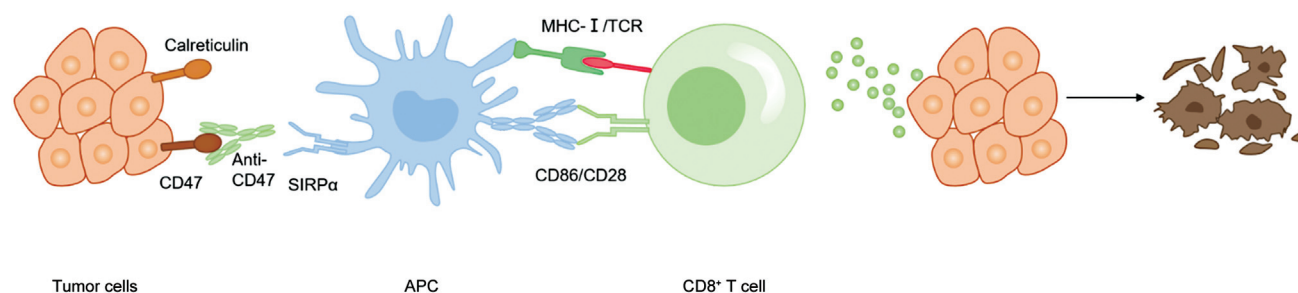
Clinically, the CD40 agonistic antibody shows a preliminary positive effect. Although SGN-40 with the IgG1 format is not effective enough against B cell lymphoma,<sup>193,194</sup> hIgG2 selicrelumab (CP-870,893) in combination with gemcitabine improves clinical outcomes in patients diagnosed with met-

astatic melanoma.<sup>176</sup> In an additional phase I trial involving patients diagnosed with resectable PDAC, selicrelumab elevates infiltration of mature DCs, M1 macrophages, and T cells into the tumor.<sup>195</sup> In addition, both CD4<sup>+</sup> and CD8<sup>+</sup> T cells express more PD-1, which is a sign of activation. Systematic adverse effects of CD40 agonistic antibody could be avoided by intratumoral injection.<sup>196</sup> Patients with treatment-naïve melanoma show good responses to intratumoral administration of sotigalimab (APX005M) plus pembrolizumab (NCT02706353). The treatment is well tolerated. There are no dose-limiting toxicities and no discontinuations or deaths due to occurrence of treatment-related events. The overall response rate in this trial reached 50%. Sotigalimab is also showing potential in another trial enrolling patients diagnosed with anti-PD(L)1 refractory melanoma (NCT03123783). Partial responses were seen in 5/33 patients and the disease control rate was 48%. The data collected from PDAC and melanoma, which are thought to be highly immune-suppressed, are really attractive. However, antibodies like SGN-40 seem to be less clinically effective. This may result from different antibody epitopes or the different tumor types they treat. Also, the IgG1 format of antibodies like SGN-40 may kill macrophages or DCs by the ADCC effect. It is necessary to optimize antibody structure before initiating clinical trials.

#### CD47/SIRP $\alpha$ Antagonists

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

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



**Fig. 4** Blockade of CD47–SIRP $\alpha$  interaction promotes phagocytosis and antigen presentation. CD47 antibody and prephagocytic molecules such as calreticulin work synergistically to enhance the phagocytosis of APC on tumor cells and activate CD8<sup>+</sup> T cell-mediated immune responses by presenting tumor-specific antigens, thereby causing tumor killing. APC, antigen-presenting cell.

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

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

Currently, a considerable number of therapeutic interventions that block the CD47/SIRP $\alpha$  axis are being actively studied in clinical trials. Based on preliminary results, CD47-blocking peptides show attractive efficacy against hematologic malignancies. In a study enrolling patients with non-Hodgkin's lymphoma refractory to rituximab, 36 and 14% of patients administered with magrolimab (Hu5F9-G4) in combination with rituximab (NCT02953509) show complete and partial response, respectively.<sup>215</sup> In another trial, individuals diagnosed with relapsed/refractory hematologic malignancies exhibited favorable tolerance to treatment and initial indications of antitumor efficacy were observed with TTI-621.<sup>216</sup> Patients with advanced solid tumors may also benefit from anti-CD47 therapy. The administration of magrolimab in conjunction with cetuximab (NCT02953782) prolongs median OS in patients with KRAS-mutant advanced CRC compared with historical controls.<sup>217</sup> In addition, treatment-related increases in macrophage immune cell infiltrates in patients with stable disease and baseline T cell infiltration were associated with longer OS. Attractive results are also reported from a phase I trial of AO-176. Among 27 patients with diverse advanced solid tumors, one patient had a confirmed partial response, and seven experienced stable disease. The drug was well tolerated.<sup>218</sup> An additional trial evaluating AO-176 in combination with paclitaxel (NCT03834948) in patients with solid tumors is ongoing. In addition, drugs targeting CD47 can also be combined with drugs targeting PD-1 or PD-L1 to improve antitumor efficacy. In phase I research including patients with advanced solid tumors, the BsAb IBI322, which

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

### TREM Inhibitors

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

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

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

### TLRs Agonists

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

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

tumors. BO-112 is a double-stranded synthetic RNA consisting of poly-IC and polyethyleneimine. In both preclinical animal models and an initial clinical trial involving human subjects, administration of anti-PD-1 mAb in combination with other treatments resulted in an augmented local IFN activity and CD8 T cell infiltration, achieving partial responses in 3 of 28 patients and stable disease in 10.<sup>243</sup> The phase II clinical trial evaluated the combination of BO-112 with pembrolizumab in patients with advanced or metastatic melanoma (NCT04570332). Of 40 patients with evaluable responses, 10 achieved responses, 3 achieved complete response, 7 achieved partial response, and 17 achieved stable disease, showing a clear trend of clinical benefit.

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

### CAR-Macrophages

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

A study conducted by Klichinsky and colleagues involved the genetic modification of human macrophages using HER2-targeting CARs. The researchers then proceeded to assess the efficacy of these modified CAR-M cells in terms of their ability to eliminate tumors in xenografted mouse



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

## Conclusion

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

plasticity of TAMs and the interaction between TAMs, tumor cells, and tumor-infiltrating T cells. The clustering of TAM subsets through distinctive molecular features will help us deeply understand the heterogeneity of macrophages, and thus more accurately target TAMs in clinical practice.

## Prospect

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

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

## Supporting Information

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

## Conflict of Interest

None declared.

## References

- 1 Bejarano L, Jordão MJC, Joyce JA. Therapeutic targeting of the tumor microenvironment. *Cancer Discov* 2021;11(04):933–959
- 2 Mantovani A, Allavena P, Marchesi F, Garlanda C. Macrophages as tools and targets in cancer therapy. *Nat Rev Drug Discov* 2022;21(11):799–820
- 3 Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25(12):677–686
- 4 Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011;331(6024):1565–1570
- 5 Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. *Annu Rev Pathol* 2020;15:123–147
- 6 Cassetta L, Pollard JW. Targeting macrophages: therapeutic approaches in cancer. *Nat Rev Drug Discov* 2018;17(12):887–904
- 7 Yin S, Huang J, Li Z, et al. The prognostic and clinicopathological significance of tumor-associated macrophages in patients with gastric cancer: a meta-analysis. *PLoS One* 2017;12(01):e0170042
- 8 Mei J, Xiao Z, Guo C, et al. Prognostic impact of tumor-associated macrophage infiltration in non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* 2016;7(23):34217–34228
- 9 Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol* 2017;14(07):399–416
- 10 Pittet MJ, Michielin O, Migliorini D. Clinical relevance of tumour-associated macrophages. *Nat Rev Clin Oncol* 2022;19(06):402–421
- 11 Tauber AI. Metchnikoff and the phagocytosis theory. *Nat Rev Mol Cell Biol* 2003;4(11):897–901
- 12 Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8(12):958–969
- 13 Auffray C, Fogg D, Garfa M, et al. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 2007;317(5838):666–670
- 14 Kubo H, Mensurado S, Gonçalves-Sousa N, Serre K, Silva-Santos B. Primary tumors limit metastasis formation through induction of IL15-mediated cross-talk between patrolling monocytes and NK cells. *Cancer Immunol Res* 2017;5(09):812–820
- 15 Schulz C, Gomez Perdiguero E, Chorro L, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* 2012;336(6077):86–90
- 16 Ginhoux F, Guilliams M. Tissue-resident macrophage ontogeny and homeostasis. *Immunity* 2016;44(03):439–449
- 17 Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010;330(6005):841–845
- 18 Merad M, Manz MG, Karsunky H, et al. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol* 2002;3(12):1135–1141
- 19 Guilliams M, De Kleer I, Henri S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J Exp Med* 2013;210(10):1977–1992
- 20 Yona S, Kim KW, Wolf Y, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013;38(01):79–91
- 21 Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* 2015;518(7540):547–551
- 22 Bain CC, Bravo-Blas A, Scott CL, et al. Constant replenishment from circulating monocytes maintains the macrophage pool in the intestine of adult mice. *Nat Immunol* 2014;15(10):929–937
- 23 Tamoutounour S, Guilliams M, Montanana Sanchis F, et al. Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin. *Immunity* 2013;39(05):925–938
- 24 Epelman S, Lavine KJ, Beaudin AE, et al. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. *Immunity* 2014;40(01):91–104
- 25 Calderon B, Carrero JA, Ferris ST, et al. The pancreas anatomy conditions the origin and properties of resident macrophages. *J Exp Med* 2015;212(10):1497–1512
- 26 Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science* 2011;333(6048):1456–1458
- 27 Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 2012;74(04):691–705
- 28 Klein I, Cornejo JC, Polakos NK, et al. Kupffer cell heterogeneity: functional properties of bone marrow derived and sessile hepatic macrophages. *Blood* 2007;110(12):4077–4085
- 29 Pollard JW. Trophic macrophages in development and disease. *Nat Rev Immunol* 2009;9(04):259–270
- 30 Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;11(10):889–896
- 31 Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. *Blood* 2008;112(04):935–945
- 32 Spiller KL, Anfang RR, Spiller KJ, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds. *Biomaterials* 2014;35(15):4477–4488
- 33 Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol Regul Integr Comp Physiol* 2010;298(05):R1173–R1187
- 34 Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008;13:453–461
- 35 Leader AM, Grout JA, Maier BB, et al. Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification. *Cancer Cell* 2021;39(12):1594–1609.e12
- 36 Zilionis R, Engblom C, Pfirschke C, et al. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity* 2019;50(05):1317–1334.e10
- 37 Braun DA, Street K, Burke KP, et al. Progressive immune dysfunction with advancing disease stage in renal cell carcinoma. *Cancer Cell* 2021;39(05):632.e8–648.e8
- 38 Wu SZ, Al-Eryani G, Roden DL, et al. A single-cell and spatially resolved atlas of human breast cancers. *Nat Genet* 2021;53(09):1334–1347
- 39 Pombo Antunes AR, Scheyltjens I, Lodi F, et al. Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization. *Nat Neurosci* 2021;24(04):595–610
- 40 Mulder K, Patel AA, Kong WT, et al. Cross-tissue single-cell landscape of human monocytes and macrophages in health and disease. *Immunity* 2021;54(08):1883–1900.e5
- 41 Ma RY, Black A, Qian BZ. Macrophage diversity in cancer revisited in the era of single-cell omics. *Trends Immunol* 2022;43(07):546–563
- 42 Loyher PL, Hamon P, Laviron M, et al. Macrophages of distinct origins contribute to tumor development in the lung. *J Exp Med* 2018;215(10):2536–2553
- 43 Sharma SK, Chintala NK, Vadrevu SK, Patel J, Karbowiczek M, Markiewski MM. Pulmonary alveolar macrophages contribute to the premetastatic niche by suppressing antitumor T cell responses in the lungs. *J Immunol* 2015;194(11):5529–5538
- 44 Li D, Ji H, Niu X, et al. Tumor-associated macrophages secrete CC-chemokine ligand 2 and induce tamoxifen resistance by activating PI3K/Akt/mTOR in breast cancer. *Cancer Sci* 2020;111(01):47–58

- 45 Steeg PS. Targeting metastasis. *Nat Rev Cancer* 2016;16(04):201–218
- 46 Helm O, Held-Feindt J, Grage-Griebenow E, et al. Tumor-associated macrophages exhibit pro- and anti-inflammatory properties by which they impact on pancreatic tumorigenesis. *Int J Cancer* 2014;135(04):843–861
- 47 Bonde AK, Tischler V, Kumar S, Soltermann A, Schwendener RA. Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. *BMC Cancer* 2012;12:35
- 48 Barkan D, Green JE, Chambers AF. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. *Eur J Cancer* 2010;46(07):1181–1188
- 49 Kessenbrock K, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010;141(01):52–67
- 50 Tekin C, Aberson HL, Waasdorp C, et al. Macrophage-secreted MMP9 induces mesenchymal transition in pancreatic cancer cells via PAR1 activation. *Cell Oncol (Dordr)* 2020;43(06):1161–1174
- 51 Gocheva V, Wang HW, Gadea BB, et al. IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev* 2010;24(03):241–255
- 52 Wei C, Yang C, Wang S, et al. Crosstalk between cancer cells and tumor associated macrophages is required for mesenchymal circulating tumor cell-mediated colorectal cancer metastasis. *Mol Cancer* 2019;18(01):64
- 53 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(05):646–674
- 54 Lin EY, Pollard JW. Tumor-associated macrophages press the angiogenic switch in breast cancer. *Cancer Res* 2007;67(11):5064–5066
- 55 Hughes R, Qian BZ, Rowan C, et al. Perivascular M2 macrophages stimulate tumor relapse after chemotherapy. *Cancer Res* 2015;75(17):3479–3491
- 56 De Palma M, Venneri MA, Galli R, et al. Tie2 identifies a hematopoietic monocytes required for tumor lineage of proangiogenic vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell* 2005;8(03):211–226
- 57 Harney AS, Karagiannis GS, Pignatelli J, et al. The selective Tie2 inhibitor rebastinib blocks recruitment and function of Tie2<sup>Hi</sup> macrophages in breast cancer and pancreatic neuroendocrine tumors. *Mol Cancer Ther* 2017;16(11):2486–2501
- 58 Mazzeri R, Pucci F, Moi D, et al. Targeting the ANG2/TIE2 axis inhibits tumor growth and metastasis by impairing angiogenesis and disabling rebounds of proangiogenic myeloid cells. *Cancer Cell* 2011;19(04):512–526
- 59 Cheng S, Li Z, Gao R, et al. A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. *Cell* 2021;184(03):792–809.e23
- 60 Kloosterman DJ, Akkari L. Macrophages at the interface of the co-evolving cancer ecosystem. *Cell* 2023;186(08):1627–1651
- 61 Komohara Y, Fujiwara Y, Ohnishi K, Takeya M. Tumor-associated macrophages: potential therapeutic targets for anti-cancer therapy. *Adv Drug Deliv Rev* 2016;99(Pt B):180–185
- 62 Noman MZ, Desantis G, Janji B, et al. PD-L1 is a novel direct target of HIF-1 $\alpha$ , and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med* 2014;211(05):781–790
- 63 Kuang DM, Zhao Q, Peng C, et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. *J Exp Med* 2009;206(06):1327–1337
- 64 Bloch O, Crane CA, Kaur R, Safaei M, Rutkowski MJ, Parsa AT. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. *Clin Cancer Res* 2013;19(12):3165–3175
- 65 Wang L, Rubinstein R, Lines JL, et al. VISTA, a novel mouse Ig superfamily ligand that negatively regulates T cell responses. *J Exp Med* 2011;208(03):577–592
- 66 Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004;10(09):942–949
- 67 Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. *Cell Res* 2017;27(01):109–118
- 68 Liu C, Chikina M, Deshpande R, et al. Treg cells promote the SREBP1-dependent metabolic fitness of tumor-promoting macrophages via repression of CD8<sup>+</sup> T cell-derived interferon- $\gamma$ . *Immunity* 2019;51(02):381.e6–397.e6
- 69 Kersten K, Hu KH, Combes AJ, et al. Spatiotemporal co-dependency between macrophages and exhausted CD8<sup>+</sup> T cells in cancer. *Cancer Cell* 2022;40(06):624–638.e9
- 70 Bi K, He MX, Bakouny Z, et al. Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma. *Cancer Cell* 2021;39(05):649–661.e5
- 71 Gabrilovich DI. Myeloid-derived suppressor cells. *Cancer Immunol Res* 2017;5(01):3–8
- 72 Corzo CA, Condamine T, Lu L, et al. HIF-1 $\alpha$  regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* 2010;207(11):2439–2453
- 73 Kumar V, Cheng P, Condamine T, et al. CD45 phosphatase inhibits STAT3 transcription factor activity in myeloid cells and promotes tumor-associated macrophage differentiation. *Immunity* 2016;44(02):303–315
- 74 Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004;104(08):2224–2234
- 75 Kumar V, Patel S, Tcyganov E, Gabrilovich DI. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends Immunol* 2016;37(03):208–220
- 76 Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012;12(04):253–268
- 77 Han N, Baghdadi M, Ishikawa K, et al. Enhanced IL-34 expression in Nivolumab-resistant metastatic melanoma. *Inflamm Regen* 2018;38:3
- 78 Kumar V, Donthireddy L, Marvel D, et al. Cancer-associated fibroblasts neutralize the anti-tumor effect of CSF1 receptor blockade by inducing PMN-MDSC infiltration of tumors. *Cancer Cell* 2017;32(05):654.e5–668.e5
- 79 Kai K, Iwamoto T, Zhang D, et al. CSF-1/CSF-1R axis is associated with epithelial/mesenchymal hybrid phenotype in epithelial-like inflammatory breast cancer. *Sci Rep* 2018;8(01):9427
- 80 Baghdadi M, Endo H, Takano A, et al. High co-expression of IL-34 and M-CSF correlates with tumor progression and poor survival in lung cancers. *Sci Rep* 2018;8(01):418
- 81 Boulakirba S, Pfeifer A, Mhaidly R, et al. IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. *Sci Rep* 2018;8(01):256
- 82 Van Overmeire E, Stijlemans B, Heymann F, et al. M-CSF and GM-CSF receptor signaling differentially regulate monocyte maturation and macrophage polarization in the tumor microenvironment. *Cancer Res* 2016;76(01):35–42
- 83 Ao JY, Zhu XD, Chai ZT, et al. Colony-stimulating factor 1 receptor blockade inhibits tumor growth by altering the polarization of tumor-associated macrophages in hepatocellular carcinoma. *Mol Cancer Ther* 2017;16(08):1544–1554
- 84 Li M, Li M, Yang Y, et al. Remodeling tumor immune microenvironment via targeted blockade of PI3K- $\gamma$  and CSF-1/CSF-1R pathways in tumor associated macrophages for pancreatic cancer therapy. *J Control Release* 2020;321:23–35
- 85 Neubert NJ, Schmittnaegel M, Bordry N, et al. T cell-induced CSF1 promotes melanoma resistance to PD1 blockade. *Sci Transl Med* 2018;10(436):eaan3311
- 86 Niehus SE, Tran DDH, Mischak M, Koch A. Colony-stimulating factor-1 receptor provides a growth advantage in epithelial cancer cell line A431 in the presence of epidermal growth factor receptor inhibitor gefitinib. *Cell Signal* 2018;51:191–198

- 87 Ide H, Seligson DB, Memarzadeh S, et al. Expression of colony-stimulating factor 1 receptor during prostate development and prostate cancer progression. *Proc Natl Acad Sci U S A* 2002;99(22):14404–14409
- 88 Richardsen E, Uglehus RD, Due J, Busch C, Busund LT. The prognostic impact of M-CSF, CSF-1 receptor, CD68 and CD3 in prostatic carcinoma. *Histopathology* 2008;53(01):30–38
- 89 Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001;193(06):727–740
- 90 Kluger HM, Dolled-Filhart M, Rodov S, Kacinski BM, Camp RL, Rimm DL. Macrophage colony-stimulating factor-1 receptor expression is associated with poor outcome in breast cancer by large cohort tissue microarray analysis. *Clin Cancer Res* 2004;10(1, Pt 1):173–177
- 91 Webb MW, Sun J, Sheard MA, et al. Colony stimulating factor 1 receptor blockade improves the efficacy of chemotherapy against human neuroblastoma in the absence of T lymphocytes. *Int J Cancer* 2018;143(06):1483–1493
- 92 Dammeyer F, Lievense LA, Kaijen-Lambers ME, et al. Depletion of tumor-associated macrophages with a CSF-1R kinase inhibitor enhances antitumor immunity and survival induced by DC immunotherapy. *Cancer Immunol Res* 2017;5(07):535–546
- 93 Shi G, Yang Q, Zhang Y, et al. Modulating the tumor microenvironment via oncolytic viruses and CSF-1R inhibition synergistically enhances anti-PD-1 immunotherapy. *Mol Ther* 2019;27(01):244–260
- 94 Pyonteck SM, Akkari L, Schuhmacher AJ, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat Med* 2013;19(10):1264–1272
- 95 Yan D, Kowal J, Akkari L, et al. Inhibition of colony stimulating factor-1 receptor abrogates microenvironment-mediated therapeutic resistance in gliomas. *Oncogene* 2017;36(43):6049–6058
- 96 Peng X, Hou P, Chen Y, et al. Preclinical evaluation of 3D185, a novel potent inhibitor of FGFR1/2/3 and CSF-1R, in FGFR-dependent and macrophage-dominant cancer models. *J Exp Clin Cancer Res* 2019;38(01):372
- 97 Schaer D, Li YX, Dobkin J, et al. Modulating the intra-tumor immune balance through combinatorial blockade of CSF-1R and PD-L1 enhances anti-tumor efficacy. Paper presented at: 31st Annual Meeting and Associated Programs of the Society for Immunotherapy of Cancer (SITC 2016): part two. *J Immunother Cancer* 2016;4 (Suppl 1):73
- 98 Salvagno C, Ciampicotti M, Tuit S, et al. Therapeutic targeting of macrophages enhances chemotherapy efficacy by unleashing type I interferon response. *Nat Cell Biol* 2019;21(04):511–521
- 99 Saung MT, Muth S, Ding D, et al. Targeting myeloid-inflamed tumor with anti-CSF-1R antibody expands CD137+ effector T-cells in the murine model of pancreatic cancer. *J Immunother Cancer* 2018;6(01):118
- 100 Papadopoulos KP, Gluck L, Martin LP, et al. First-in-human study of AMG 820, a monoclonal anti-colony-stimulating factor 1 receptor antibody, in patients with advanced solid tumors. *Clin Cancer Res* 2017;23(19):5703–5710
- 101 Wainberg Z, Piha-Paul S, Luke J, et al. First-in-human phase 1 dose escalation and expansion of a novel combination, anti-CSF-1 receptor (cabiralizumab) plus anti-PD-1 (nivolumab), in patients with advanced solid tumors. Paper presented at: 32nd Annual Meeting and Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2017): Late-Breaking Abstracts. *J Immunother Cancer* 2017;5(Suppl 3):89
- 102 Ries CH, Cannarile MA, Hoves S, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* 2014;25(06):846–859
- 103 Lamb YN. Pexidartinib: first approval. *Drugs* 2019;79(16):1805–1812
- 104 Gomez-Roca CA, Italiano A, Le Tourneau C, et al. Phase I study of emactuzumab single agent or in combination with paclitaxel in patients with advanced/metastatic solid tumors reveals depletion of immunosuppressive M2-like macrophages. *Ann Oncol* 2019;30(08):1381–1392
- 105 Wesolowski R, Sharma N, Reebel L, et al. Phase Ib study of the combination of pexidartinib (PLX3397), a CSF-1R inhibitor, and paclitaxel in patients with advanced solid tumors. *Ther Adv Med Oncol* 2019;11:1758835919854238
- 106 Pradel LP, Ooi CH, Romagnoli S, et al. Macrophage susceptibility to emactuzumab (RG7155) treatment. *Mol Cancer Ther* 2016;15(12):3077–3086
- 107 Quail DF, Bowman RL, Akkari L, et al. The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. *Science* 2016;352(6288):aad3018
- 108 Draghiciu O, Lubbers J, Nijman HW, Daemen T. Myeloid derived suppressor cells—an overview of combat strategies to increase immunotherapy efficacy. *Oncol Immunology* 2015;4(01):e954829
- 109 Gyori D, Lim EL, Grant FM, et al. Compensation between CSF1R+ macrophages and Foxp3+ Treg cells drives resistance to tumor immunotherapy. *JCI Insight* 2018;3(11):e120631
- 110 Beffinger M, Tallón de Lara P, Tugues S, et al. CSF1R-dependent myeloid cells are required for NK-mediated control of metastasis. *JCI Insight* 2018;3(10):e97792
- 111 MacDonald KP, Rowe V, Bofinger HM, et al. The colony-stimulating factor 1 receptor is expressed on dendritic cells during differentiation and regulates their expansion. *J Immunol* 2005;175(03):1399–1405
- 112 Percin GI, Eitler J, Kranz A, et al. CSF1R regulates the dendritic cell pool size in adult mice via embryo-derived tissue-resident macrophages. *Nat Commun* 2018;9(01):5279
- 113 Argyle D, Kitamura T. Targeting macrophage-recruiting chemokines as a novel therapeutic strategy to prevent the progression of solid tumors. *Front Immunol* 2018;9:2629
- 114 van Deventer HW, Palmieri DA, Wu QP, McCook EC, Serody JS. Circulating fibrocytes prepare the lung for cancer metastasis by recruiting Ly-6C+ monocytes via CCL2. *J Immunol* 2013;190(09):4861–4867
- 115 Ren G, Zhao X, Wang Y, et al. CCR2-dependent recruitment of macrophages by tumor-educated mesenchymal stromal cells promotes tumor development and is mimicked by TNF $\alpha$ . *Cell Stem Cell* 2012;11(06):812–824
- 116 Ren G, Liu Y, Zhao X, et al. Tumor resident mesenchymal stromal cells endow naïve stromal cells with tumor-promoting properties. *Oncogene* 2014;33(30):4016–4020
- 117 Szekely B, Bossuyt V, Li X, et al. Immunological differences between primary and metastatic breast cancer. *Ann Oncol* 2018;29(11):2232–2239
- 118 Brummer G, Fang W, Smart C, et al. CCR2 signaling in breast carcinoma cells promotes tumor growth and invasion by promoting CCL2 and suppressing CD154 effects on the angiogenic and immune microenvironments. *Oncogene* 2020;39(11):2275–2289
- 119 Fujita S, Ikeda T. The CCL2-CCR2 axis in lymph node metastasis from oral squamous cell carcinoma: an immunohistochemical study. *J Oral Maxillofac Surg* 2017;75(04):742–749
- 120 Wang Z, Xie H, Zhou L, et al. CCL2/CCR2 axis is associated with postoperative survival and recurrence of patients with non-metastatic clear-cell renal cell carcinoma. *Oncotarget* 2016;7(32):51525–51534
- 121 Grossman JG, Nywening TM, Belt BA, et al. Recruitment of CCR2+ tumor associated macrophage to sites of liver metastasis confers a poor prognosis in human colorectal cancer. *Oncol Immunology* 2018;7(09):e1470729
- 122 Yang H, Zhang Q, Xu M, et al. CCL2-CCR2 axis recruits tumor associated macrophages to induce immune evasion through PD-



- 1 signaling in esophageal carcinogenesis. *Mol Cancer* 2020;19(01):41
- 123 Brummer G, Acevedo DS, Hu Q, et al. Chemokine signaling facilitates early-stage breast cancer survival and invasion through fibroblast-dependent mechanisms. *Mol Cancer Res* 2018;16(02):296–308
- 124 Schmall A, Al-Tamari HM, Herold S, et al. Macrophage and cancer cell cross-talk via CCR2 and CX3CR1 is a fundamental mechanism driving lung cancer. *Am J Respir Crit Care Med* 2015;191(04):437–447
- 125 Wu X, Singh R, Hsu DK, et al. A small molecule CCR2 antagonist depletes tumor macrophages and synergizes with anti-PD-1 in a murine model of cutaneous T-cell lymphoma (CTCL). *J Invest Dermatol* 2020;140(07):1390–1400.e4
- 126 Moisan F, Francisco EB, Brozovic A, et al. Enhancement of paclitaxel and carboplatin therapies by CCL2 blockade in ovarian cancers. *Mol Oncol* 2014;8(07):1231–1239
- 127 Kalbasi A, Komar C, Tooker GM, et al. Tumor-derived CCL2 mediates resistance to radiotherapy in pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2017;23(01):137–148
- 128 Noel M, O'Reilly EM, Wolpin BM, et al. Phase 1b study of a small molecule antagonist of human chemokine (C-C motif) receptor 2 (PF-04136309) in combination with nab-paclitaxel/gemcitabine in first-line treatment of metastatic pancreatic ductal adenocarcinoma. *Invest New Drugs* 2020;38(03):800–811
- 129 Nywening TM, Wang-Gillam A, Sanford DE, et al. Targeting tumour-associated macrophages with CCR2 inhibition in combination with FOLFIRINOX in patients with borderline resectable and locally advanced pancreatic cancer: a single-centre, open-label, dose-finding, non-randomised, phase 1b trial. *Lancet Oncol* 2016;17(05):651–662
- 130 Sanford DE, Belt BA, Panni RZ, et al. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancer: a role for targeting the CCL2/CCR2 axis. *Clin Cancer Res* 2013;19(13):3404–3415
- 131 Sandhu SK, Papadopoulos K, Fong PC, et al. A first-in-human, first-in-class, phase I study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 in patients with solid tumors. *Cancer Chemother Pharmacol* 2013;71(04):1041–1050
- 132 Pienta KJ, Machiels JP, Schrijvers D, et al. Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer. *Invest New Drugs* 2013;31(03):760–768
- 133 Brana I, Calles A, LoRusso PM, et al. Carlumab, an anti-C-C chemokine ligand 2 monoclonal antibody, in combination with four chemotherapy regimens for the treatment of patients with solid tumors: an open-label, multicenter phase 1b study. *Target Oncol* 2015;10(01):111–123
- 134 Yao M, Smart C, Hu Q, Cheng N. Continuous delivery of neutralizing antibodies elevate CCL2 levels in mice bearing MCF10CA1d breast tumor xenografts. *Transl Oncol* 2017;10(05):734–743
- 135 Nywening TM, Belt BA, Cullinan DR, et al. Targeting both tumour-associated CXCR2<sup>+</sup> neutrophils and CCR2<sup>+</sup> macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. *Gut* 2018;67(06):1112–1123
- 136 Long KB, Gladney WL, Tooker GM, Graham K, Fraietta JA, Beatty GL. IFN $\gamma$  and CCL2 cooperate to redirect tumor-infiltrating monocytes to degrade fibrosis and enhance chemotherapy efficacy in pancreatic carcinoma. *Cancer Discov* 2016;6(04):400–413
- 137 Wang SW, Liu SC, Sun HL, et al. CCL5/CCR5 axis induces vascular endothelial growth factor-mediated tumor angiogenesis in human osteosarcoma microenvironment. *Carcinogenesis* 2015;36(01):104–114
- 138 Tang S, Xiang T, Huang S, et al. Ovarian cancer stem-like cells differentiate into endothelial cells and participate in tumor angiogenesis through autocrine CCL5 signaling. *Cancer Lett* 2016;376(01):137–147
- 139 Casagrande N, Borghese C, Visser L, Mongiat M, Colombatti A, Aldinucci D. CCR5 antagonism by maraviroc inhibits Hodgkin lymphoma microenvironment interactions and xenograft growth. *Haematologica* 2019;104(03):564–575
- 140 Kranjc MK, Novak M, Pestell RG, Lah TT. Cytokine CCL5 and receptor CCR5 axis in glioblastoma multiforme. *Radiol Oncol* 2019;53(04):397–406
- 141 Kaplon H, Muralidharan M, Schneider Z, Reichert JM. Antibodies to watch in 2020. *MAbs* 2020;12(01):1703531
- 142 Lapeyre-Prost A, Terme M, Pernot S, et al. Immunomodulatory activity of VEGF in cancer. *Int Rev Cell Mol Biol* 2017;330:295–342
- 143 Kim I, Kim HG, So JN, Kim JH, Kwak HJ, Koh GY. Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-Kinase/Akt signal transduction pathway. *Circ Res* 2000;86(01):24–29
- 144 Huang H, Bhat A, Woodnutt G, Lappe R. Targeting the ANGPT-TIE2 pathway in malignancy. *Nat Rev Cancer* 2010;10(08):575–585
- 145 Turrini R, Pabois A, Xenarios I, Coukos G, Delaloye JF, Doucey MA. TIE-2 expressing monocytes in human cancers. *Oncol Immunology* 2017;6(04):e1303585
- 146 Steinberger KJ, Forget MA, Bobko AA, et al. Hypoxia-inducible factor  $\alpha$  subunits regulate Tie2-expressing macrophages that influence tumor oxygen and perfusion in murine breast cancer. *J Immunol* 2020;205(08):2301–2311
- 147 Chen L, Li J, Wang F, et al. Tie2 expression on macrophages is required for blood vessel reconstruction and tumor relapse after chemotherapy. *Cancer Res* 2016;76(23):6828–6838
- 148 Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 2008;8(08):592–603
- 149 Coffelt SB, Tal AO, Scholz A, et al. Angiopoietin-2 regulates gene expression in TIE2-expressing monocytes and augments their inherent proangiogenic functions. *Cancer Res* 2010;70(13):5270–5280
- 150 Peterson TE, Kirkpatrick ND, Huang Y, et al. Dual inhibition of Ang-2 and VEGF receptors normalizes tumor vasculature and prolongs survival in glioblastoma by altering macrophages. *Proc Natl Acad Sci U S A* 2016;113(16):4470–4475
- 151 Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. *Proc Natl Acad Sci U S A* 2002;99(17):11205–11210
- 152 Cattin S, Fellay B, Pradervand S, et al. Bevacizumab specifically decreases elevated levels of circulating KIT<sup>+</sup>CD11b<sup>+</sup> cells and IL-10 in metastatic breast cancer patients. *Oncotarget* 2016;7(10):11137–11150
- 153 Chae SS, Kamoun WS, Farrar CT, et al. Angiopoietin-2 interferes with anti-VEGFR2-induced vessel normalization and survival benefit in mice bearing gliomas. *Clin Cancer Res* 2010;16(14):3618–3627
- 154 Kloeppe J, Riedemann L, Amoozgar Z, et al. Ang-2/VEGF bispecific antibody reprograms macrophages and resident microglia to anti-tumor phenotype and prolongs glioblastoma survival. *Proc Natl Acad Sci U S A* 2016;113(16):4476–4481
- 155 Hidalgo M, Martinez-Garcia M, Le Tourneau C, et al. First-in-human phase I study of single-agent vanucizumab, a first-in-class bispecific anti-angiopoietin-2/anti-VEGF-A antibody, in adult patients with advanced solid tumors. *Clin Cancer Res* 2018;24(07):1536–1545
- 156 Scholz A, Harter PN, Cremer S, et al. Endothelial cell-derived angiopoietin-2 is a therapeutic target in treatment-naive and bevacizumab-resistant glioblastoma. *EMBO Mol Med* 2016;8(01):39–57

- 157 Bendell JC, Sauri T, Gracián AC, et al; McCaVE Study Group. The McCaVE trial: vanucizumab plus mFOLFOX-6 versus bevacizumab plus mFOLFOX-6 in patients with previously untreated metastatic colorectal carcinoma (mCRC). *Oncologist* 2020;25(03):e451–e459
- 158 Martin-Liberal J, Hollebecque A, Aftimos P, et al. First-in-human, dose-escalation, phase 1 study of anti-angiopoietin-2 LY3127804 as monotherapy and in combination with ramucirumab in patients with advanced solid tumours. *Br J Cancer* 2020;123(08):1235–1243
- 159 Hyman DM, Rizvi N, Natale R, et al. Phase I study of MEDI3617, a selective angiopoietin-2 inhibitor alone and combined with carboplatin/paclitaxel, paclitaxel, or bevacizumab for advanced solid tumors. *Clin Cancer Res* 2018;24(12):2749–2757
- 160 Han S, Lee SJ, Kim KE, et al. Amelioration of sepsis by TIE2 activation-induced vascular protection. *Sci Transl Med* 2016;8(335):335ra55
- 161 Park JS, Kim IK, Han S, et al. Normalization of tumor vessels by Tie2 activation and Ang2 inhibition enhances drug delivery and produces a favorable tumor microenvironment. *Cancer Cell* 2016;30(06):953–967
- 162 Fabriek BO, van Bruggen R, Deng DM, et al. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. *Blood* 2009;113(04):887–892
- 163 Graversen JH, Madsen M, Moestrup SK. CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. *Int J Biochem Cell Biol* 2002;34(04):309–314
- 164 Foks M, Wągrowiska-Danilewicz M, Danilewicz M, Bonczysta M, Olborski B, Stasikowska-Kanicka O. The number of CD163 positive macrophages is associated with more advanced skin melanomas, microvessels density and patient prognosis. *Pol J Pathol* 2019;70(03):217–222
- 165 Chen T, Chen J, Zhu Y, et al. CD163, a novel therapeutic target, regulates the proliferation and stemness of glioma cells via casein kinase 2. *Oncogene* 2019;38(08):1183–1199
- 166 Maniecki MB, Etzerodt A, Ullhøi BP, et al. Tumor-promoting macrophages induce the expression of the macrophage-specific receptor CD163 in malignant cells. *Int J Cancer* 2012;131(10):2320–2331
- 167 Graversen JH, Svendsen P, Dagnæs-Hansen F, et al. Targeting the hemoglobin scavenger receptor CD163 in macrophages highly increases the anti-inflammatory potency of dexamethasone. *Mol Ther* 2012;20(08):1550–1558
- 168 Etzerodt A, Maniecki MB, Graversen JH, Møller HJ, Torchilin VP, Moestrup SK. Efficient intracellular drug-targeting of macrophages using stealth liposomes directed to the hemoglobin scavenger receptor CD163. *J Control Release* 2012;160(01):72–80
- 169 Jackaman C, Yeoh TL, Acuil ML, Gardner JK, Nelson DJ. Murine mesothelioma induces locally-proliferating IL-10(+)/TNF- $\alpha$ (+) CD206(-)/CX3CR1(+) M3 macrophages that can be selectively depleted by chemotherapy or immunotherapy. *Oncol Immunology* 2016;5(06):e1173299
- 170 de Silva S, Fromm G, Shuptrine CW, et al. CD40 enhances type I interferon responses downstream of CD47 blockade, bridging innate and adaptive immunity. *Cancer Immunol Res* 2020;8(02):230–245
- 171 Vitale LA, Thomas LJ, He LZ, et al. Development of CDX-1140, an agonist CD40 antibody for cancer immunotherapy. *Cancer Immunol Immunother* 2019;68(02):233–245
- 172 Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* 2011;331(6024):1612–1616
- 173 Ngiow SF, Young A, Blake SJ, et al. Agonistic CD40 mAb-driven IL12 reverses resistance to anti-PD1 in a T-cell-rich tumor. *Cancer Res* 2016;76(21):6266–6277
- 174 Ma HS, Poudel B, Torres ER, et al. A CD40 agonist and PD-1 antagonist antibody reprogram the microenvironment of non-immunogenic tumors to allow T-cell-mediated anticancer activity. *Cancer Immunol Res* 2019;7(03):428–442
- 175 Zippelius A, Schreiner J, Herzig P, Müller P. Induced PD-L1 expression mediates acquired resistance to agonistic anti-CD40 treatment. *Cancer Immunol Res* 2015;3(03):236–244
- 176 Bajor DL, Mick R, Riese MJ, et al. Long-term outcomes of a phase I study of agonist CD40 antibody and CTLA-4 blockade in patients with metastatic melanoma. *Oncol Immunology* 2018;7(10):e1468956
- 177 Buhtoiarov IN, Lum H, Berke G, Paulnock DM, Sondel PM, Rakhmievich AL. CD40 ligation activates murine macrophages via an IFN-gamma-dependent mechanism resulting in tumor cell destruction in vitro. *J Immunol* 2005;174(10):6013–6022
- 178 Kashyap AS, Schmittnaegel M, Rigamonti N, et al. Optimized antiangiogenic reprogramming of the tumor microenvironment potentiates CD40 immunotherapy. *Proc Natl Acad Sci U S A* 2020;117(01):541–551
- 179 Hoves S, Ooi CH, Wolter C, et al. Rapid activation of tumor-associated macrophages boosts preexisting tumor immunity. *J Exp Med* 2018;215(03):859–876
- 180 Wiehagen KR, Girgis NM, Yamada DH, et al. Combination of CD40 agonism and CSF-1R blockade reconditions tumor-associated macrophages and drives potent antitumor immunity. *Cancer Immunol Res* 2017;5(12):1109–1121
- 181 Rakhmievich AL, Buhtoiarov IN, Malkovsky M, Sondel PM. CD40 ligation in vivo can induce T cell independent antitumor effects even against immunogenic tumors. *Cancer Immunol Immunother* 2008;57(08):1151–1160
- 182 Richards DM, Seifrin JP, Gieffers C, Hill O, Merz C. Concepts for agonistic targeting of CD40 in immuno-oncology. *Hum Vaccin Immunother* 2020;16(02):377–387
- 183 Wyzgol A, Müller N, Fick A, et al. Trimer stabilization, oligomerization, and antibody-mediated cell surface immobilization improve the activity of soluble trimers of CD27L, CD40L, 41BBL, and glucocorticoid-induced TNF receptor ligand. *J Immunol* 2009;183(03):1851–1861
- 184 An HJ, Kim YJ, Song DH, et al. Crystallographic and mutational analysis of the CD40-CD154 complex and its implications for receptor activation. *J Biol Chem* 2011;286(13):11226–11235
- 185 Wajant H. Principles of antibody-mediated TNF receptor activation. *Cell Death Differ* 2015;22(11):1727–1741
- 186 Li F, Ravetch JV. Inhibitory Fc $\gamma$  receptor engagement drives adjuvant and anti-tumor activities of agonistic CD40 antibodies. *Science* 2011;333(6045):1030–1034
- 187 Richman LP, Vonderheide RH. Role of crosslinking for agonistic CD40 monoclonal antibodies as immune therapy of cancer. *Cancer Immunol Res* 2014;2(01):19–26
- 188 Dahan R, Barnhart BC, Li F, Yamniuk AP, Korman AJ, Ravetch JV. Therapeutic activity of agonistic, human anti-CD40 monoclonal antibodies requires selective Fc $\gamma$ R engagement. *Cancer Cell* 2016;29(06):820–831
- 189 Liu X, Zhao Y, Shi H, et al. Human immunoglobulin G hinge regulates agonistic anti-CD40 immunostimulatory and antitumor activities through biophysical flexibility. *Nat Commun* 2019;10(01):4206
- 190 Merz C, Sykora J, Marschall V, et al. The hexavalent CD40 agonist HERA-CD40L induces T-cell-mediated antitumor immune response through activation of antigen-presenting cells. *J Immunother* 2018;41(09):385–398
- 191 Eriksson E, Milenova I, Wenthe J, Moreno R, Alemany R, Loskog A. IL-6 signaling blockade during CD40-mediated immune activation favors antitumor factors by reducing TGF- $\beta$ , collagen type I, and PD-L1/PD-1. *J Immunol* 2019;202(03):787–798
- 192 White AL, Chan HT, French RR, et al. Conformation of the human immunoglobulin G2 hinge imparts superagonistic properties to

- immunostimulatory anticancer antibodies. *Cancer Cell* 2015;27(01):138–148
- 193 de Vos S, Forero-Torres A, Ansell SM, et al. A phase II study of dacetuzumab (SGN-40) in patients with relapsed diffuse large B-cell lymphoma (DLBCL) and correlative analyses of patient-specific factors. *J Hematol Oncol* 2014;7:44
- 194 Fayad L, Ansell SM, Advani R, et al. Dacetuzumab plus rituximab, ifosfamide, carboplatin and etoposide as salvage therapy for patients with diffuse large B-cell lymphoma relapsing after rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone: a randomized, double-blind, placebo-controlled phase 2b trial. *Leuk Lymphoma* 2015;56(09):2569–2578
- 195 Byrne KT, Betts CB, Mick R, et al. Neoadjuvant selicrelumab, an agonist CD40 antibody, induces changes in the tumor microenvironment in patients with resectable pancreatic cancer. *Clin Cancer Res* 2021;27(16):4574–4586
- 196 Knorr DA, Dahan R, Ravetch JV. Toxicity of an Fc-engineered anti-CD40 antibody is abrogated by intratumoral injection and results in durable antitumor immunity. *Proc Natl Acad Sci U S A* 2018;115(43):11048–11053
- 197 Weiskopf K. Cancer immunotherapy targeting the CD47/SIRP $\alpha$  axis. *Eur J Cancer* 2017;76:100–109
- 198 Zhang W, Huang Q, Xiao W, et al. Advances in anti-tumor treatments targeting the CD47/SIRP $\alpha$  axis. *Front Immunol* 2020;11:18
- 199 Vonderheide RH. CD47 blockade as another immune checkpoint therapy for cancer. *Nat Med* 2015;21(10):1122–1123
- 200 Nigro A, Ricciardi L, Salvato I, et al. Enhanced expression of CD47 is associated with off-target resistance to tyrosine kinase inhibitor gefitinib in NSCLC. *Front Immunol* 2020;10:3135
- 201 Zhang X, Wang Y, Fan J, et al. Blocking CD47 efficiently potentiated therapeutic effects of anti-angiogenic therapy in non-small cell lung cancer. *J Immunother Cancer* 2019;7(01):346
- 202 Pai S, Bamodu OA, Lin YK, et al. CD47-SIRP $\alpha$  signaling induces epithelial-mesenchymal transition and cancer stemness and links to a poor prognosis in patients with oral squamous cell carcinoma. *Cells* 2019;8(12):1658
- 203 Arrieta O, Aviles-Salas A, Orozco-Morales M, et al. Association between CD47 expression, clinical characteristics and prognosis in patients with advanced non-small cell lung cancer. *Cancer Med* 2020;9(07):2390–2402
- 204 Weiskopf K, Jahchan NS, Schnorr PJ, et al. CD47-blocking immunotherapies stimulate macrophage-mediated destruction of small-cell lung cancer. *J Clin Invest* 2016;126(07):2610–2620
- 205 Puro RJ, Bouchlaka MN, Hiebsch RR, et al. Development of AO-176, a next-generation humanized anti-CD47 antibody with novel anticancer properties and negligible red blood cell binding. *Mol Cancer Ther* 2020;19(03):835–846
- 206 Ma L, Zhu M, Gai J, et al. Preclinical development of a novel CD47 nanobody with less toxicity and enhanced anti-cancer therapeutic potential. *J Nanobiotechnology* 2020;18(01):12
- 207 Tsao LC, Crosby EJ, Trotter TN, et al. CD47 blockade augmentation of trastuzumab antitumor efficacy dependent on antibody-dependent cellular phagocytosis. *JCI Insight* 2019;4(24):e131882
- 208 Petrova PS, Viller NN, Wong M, et al. TTI-621 (SIRP $\alpha$ Fc): a CD47-blocking innate immune checkpoint inhibitor with broad antitumor activity and minimal erythrocyte binding. *Clin Cancer Res* 2017;23(04):1068–1079
- 209 Sikic BI, Lakhani N, Patnaik A, et al. First-in-human, first-in-class phase I trial of the anti-CD47 antibody Hu5F9-G4 in patients with advanced cancers. *J Clin Oncol* 2019;37(12):946–953
- 210 Weiskopf K, Ring AM, Ho CC, et al. Engineered SIRP $\alpha$  variants as immunotherapeutic adjuvants to anticancer antibodies. *Science* 2013;341(6141):88–91
- 211 Voets E, Paradé M, Lutje Hulsik D, et al. Functional characterization of the selective pan-allele anti-SIRP $\alpha$  antibody ADU-1805 that blocks the SIRP $\alpha$ -CD47 innate immune checkpoint. *J Immunother Cancer* 2019;7(01):340
- 212 Kauder SE, Kuo TC, Harrabi O, et al. ALX148 blocks CD47 and enhances innate and adaptive antitumor immunity with a favorable safety profile. *PLoS One* 2018;13(08):e0201832
- 213 Adams S, van der Laan LJ, Vernon-Wilson E, et al. Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. *J Immunol* 1998;161(04):1853–1859
- 214 Saito Y, Iwamura H, Kaneko T, et al. Regulation by SIRP $\alpha$  of dendritic cell homeostasis in lymphoid tissues. *Blood* 2010;116(18):3517–3525
- 215 Advani R, Flinn I, Popplewell L, et al. CD47 blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N Engl J Med* 2018;379(18):1711–1721
- 216 Ansell SM, Maris MB, Lesokhin AM, et al. Phase I study of the CD47 blocker TTI-621 in patients with relapsed or refractory hematologic malignancies. *Clin Cancer Res* 2021;27(08):2190–2199
- 217 Fisher GA, Lakhani NJ, Eng C, et al. A phase Ib/II study of the anti-CD47 antibody magrolimab with cetuximab in solid tumor and colorectal cancer patients. *J Clin Oncol* 2020;38(04):114
- 218 Burris HA III, Spira AI, Taylor MH, et al. A first-in-human study of AO-176, a highly differentiated anti-CD47 antibody, in patients with advanced solid tumors. *J Clin Oncol* 2021;39(15):2516
- 219 Wang J, Sun Y, Chu Q, et al. Phase I study of IB1322 (anti-CD47/PD-L1 bispecific antibody) monotherapy therapy in patients with advanced solid tumors in China. *Cancer Res* 2022;82(12\_Supplement):CT513
- 220 Liao R, Sun TW, Yi Y, et al. Expression of TREM-1 in hepatic stellate cells and prognostic value in hepatitis B-related hepatocellular carcinoma. *Cancer Sci* 2012;103(06):984–992
- 221 Zhou J, Chai F, Lu G, et al. TREM-1 inhibition attenuates inflammation and tumor within the colon. *Int Immunopharmacol* 2013;17(02):155–161
- 222 Ho CC, Liao WY, Wang CY, et al. TREM-1 expression in tumor-associated macrophages and clinical outcome in lung cancer. *Am J Respir Crit Care Med* 2008;177(07):763–770
- 223 Sigalov AB. A novel ligand-independent peptide inhibitor of TREM-1 suppresses tumor growth in human lung cancer xenografts and prolongs survival of mice with lipopolysaccharide-induced septic shock. *Int Immunopharmacol* 2014;21(01):208–219
- 224 Wu J, Li J, Salcedo R, Mivechi NF, Trinchieri G, Horuzsko A. The proinflammatory myeloid cell receptor TREM-1 controls Kupffer cell activation and development of hepatocellular carcinoma. *Cancer Res* 2012;72(16):3977–3986
- 225 Wu Q, Zhou W, Yin S, et al. Blocking triggering receptor expressed on myeloid cells-1-positive tumor-associated macrophages induced by hypoxia reverses immunosuppression and anti-programmed cell death ligand 1 resistance in liver cancer. *Hepatology* 2019;70(01):198–214
- 226 Ford JW, Gonzalez-Cotto M, MacFarlane AW IV, et al. Tumor-infiltrating myeloid cells co-express TREM1 and TREM2 and elevated TREM-1 associates with disease progression in renal cell carcinoma. *Front Oncol* 2022;11:662723
- 227 Shen ZT, Sigalov AB. Novel TREM-1 inhibitors attenuate tumor growth and prolong survival in experimental pancreatic cancer. *Mol Pharm* 2017;14(12):4572–4582
- 228 Mayes E, Juric V, Binnewies M, et al. Therapeutic targeting of TREM1 with PY159 promotes myeloid cell reprogramming and unleashes anti-tumor immunity. *Mol Cancer Ther* 2021;20(12\_Supplement):104
- 229 Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. *Curr Opin Immunol* 2009;21(01):38–46
- 230 Turnbull IR, Gilfillan S, Cella M, et al. Cutting edge: TREM-2 attenuates macrophage activation. *J Immunol* 2006;177(06):3520–3524

- 231 Yao Y, Li H, Chen J, et al. TREM-2 serves as a negative immune regulator through Syk pathway in an IL-10 dependent manner in lung cancer. *Oncotarget* 2016;7(20):29620–29634
- 232 Molgora M, Esaulova E, Vermi W, et al. TREM2 modulation remodels the tumor myeloid landscape enhancing anti-PD-1 immunotherapy. *Cell* 2020;182(04):886–900.e17
- 233 Timperi E, Gueguen P, Molgora M, et al. Lipid-associated macrophages are induced by cancer-associated fibroblasts and mediate immune suppression in breast cancer. *Cancer Res* 2022;82(18):3291–3306
- 234 Zhang H, Liu Z, Wen H, et al. Immunosuppressive TREM2(+) macrophages are associated with undesirable prognosis and responses to anti-PD-1 immunotherapy in non-small cell lung cancer. *Cancer Immunol Immunother* 2022;71(10):2511–2522
- 235 Wang XQ, Tao BB, Li B, et al. Overexpression of TREM2 enhances glioma cell proliferation and invasion: a therapeutic target in human glioma. *Oncotarget* 2016;7(03):2354–2366
- 236 Tang W, Lv B, Yang B, et al. TREM2 acts as a tumor suppressor in hepatocellular carcinoma by targeting the PI3K/Akt/ $\beta$ -catenin pathway. *Oncogenesis* 2019;8(02):9
- 237 Patnaik A, Hamilton EP, Winer IS, Tan W. A phase 1a dose-escalation study of PY314, a TREM2 (Triggering Receptor Expressed on Macrophages 2) targeting monoclonal antibody. *J Clin Oncol* 2022;40(16):2678–2648
- 238 Medzhitov R, Janeway C Jr. The Toll receptor family and microbial recognition. *Trends Microbiol* 2000;8(10):452–456
- 239 Kaur A, Baldwin J, Brar D, Salunke DB, Petrovsky N. Toll-like receptor (TLR) agonists as a driving force behind next-generation vaccine adjuvants and cancer therapeutics. *Curr Opin Chem Biol* 2022;70:102172
- 240 Pradere JP, Dapito DH, Schwabe RF. The Yin and Yang of Toll-like receptors in cancer. *Oncogene* 2014;33(27):3485–3495
- 241 Radolec M, Orr B, Taylor S, et al. Systemic immune checkpoint blockade and intraperitoneal chemo-immunotherapy in recurrent ovarian cancer: an interim analysis. *Gynecol Oncol* 2022;166(Suppl 1):S165–S166
- 242 Kyi C, Roudko V, Sabado R, et al. Therapeutic immune modulation against solid cancers with intratumoral poly-ICLC: a pilot trial. *Clin Cancer Res* 2018;24(20):4937–4948
- 243 Márquez-Rodas I, Longo F, Rodríguez-Ruiz ME, et al. Intratumoral nanoplexed poly I:C BO-112 in combination with systemic anti-PD-1 for patients with anti-PD-1-refractory tumors. *Sci Transl Med* 2020;12(565):eabb0391
- 244 Sun L, Kees T, Almeida AS, et al. Activating a collaborative innate-adaptive immune response to control metastasis. *Cancer Cell* 2021;39(10):1361–1374.e9
- 245 Vaccelli E, Galluzzi L, Eggermont A, et al. Trial watch: FDA-approved Toll-like receptor agonists for cancer therapy. *OncoImmunology* 2012;1(06):894–907
- 246 Maalej KM, Merhi M, Inchakalody VP, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. *Mol Cancer* 2023;22(01):20
- 247 Chen Y, Yu Z, Tan X, et al. CAR-macrophage: a new immunotherapy candidate against solid tumors. *Biomed Pharmacother* 2021;139:111605
- 248 Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 2020;38(08):947–953
- 249 Anderson N, Klichinsky M, Ciccaglione K, et al. Pre-clinical development of CT-1119, a mesothelin targeting chimeric antigen receptor macrophage (CAR-M), for solid tumor immunotherapy. *J Immunother Cancer* 2022;10(Suppl 2):A1–A1603
- 250 Zhang W, Liu L, Su H, et al. Chimeric antigen receptor macrophage therapy for breast tumours mediated by targeting the tumour extracellular matrix. *Br J Cancer* 2019;121(10):837–845
- 251 Labrijn AF, Janmaat ML, Reichert JM, Parren PW. Bispecific antibodies: a mechanistic review of the pipeline. *Nat Rev Drug Discov* 2019;18(08):585–608
- 252 Han L, Chen J, Ding K, et al. Efficient generation of bispecific IgG antibodies by split intein mediated protein trans-splicing system. *Sci Rep* 2017;7(01):8360