Targeting macrophages in cancer immunotherapy
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Immunotherapy is regarded as the most promising treatment for cancers. Various cancer immunotherapies, including adoptive cellular immunotherapy, tumor vaccines, antibodies, immune checkpoint inhibitors, and small-molecule inhibitors, have achieved certain successes. In this review, we summarize the role of macrophages in current immunotherapies and the advantages of targeting macrophages. To better understand and make better use of this type of cell, their development and differentiation characteristics, categories, typical markers, and functions were collated at the beginning of the review. Therapeutic strategies based on or combined with macrophages have the potential to improve the treatment efficacy of cancer therapies.

As a type of phagocytic cell that was initially identified as clearing foreign pathogens by Elie Metchnikoff, macrophages have gradually been considered for cancer immunotherapy in recent years. In light of their positive roles in current therapeutic strategies, they have become a promising target for improved cancer treatments. To facilitate the use of macrophages in cancer immunotherapy, we summarize their related characterization and research progress in this review.

CATEGORIES AND CHARACTERIZATION OF MACROPHAGES

Development and differentiation of macrophages

It is now widely accepted that macrophages in tissues, as well as monocytes in the peripheral blood, are classified as the mononuclear phagocytic system (MPS). This concept has developed over a long history, and its current version takes the origin, morphology, function, and kinetics of the cells into consideration. In MPS, macrophages originate from bone marrow stem cells, and their development goes through sequential stages as granulocyte–monocyte progenitor cells, pro-monocytes, and mature monocytes. After entering various tissues, monocytes differentiate into macrophages. However, in some lower multicellular organisms without circulating monocytes, such as Porifera, macrophages still exist. For patients with some lower multicellular organisms without circulating monocytes, extramedullary hematopoiesis, especially in the spleen. It has been reported that Ly6C+ patrolling monocytes are mainly responsible for detecting pathogens intravascularly and maintaining vascular integrity, while Ly6C- inflammatory monocytes are recruited to infectious sites and injuries, mediating extravascular inflammatory responses and then differentiating into macrophages. Some studies have also indicated that both Gr1+/Ly6Chigh and Gr1−/Ly6Clo monocytes have the potential to enter tissues and turn into macrophages, but the former are more likely to become M1 macrophages, while the latter are M2 phenotypes. Above all, macrophages in tissues are probably a mixture of embryo- and adult-derived cells.

Wherever the macrophages originated from, the macrophage colony-stimulating factor 1 receptor (CSF1R) is a key receptor that induces their differentiation. CSF1 and IL-34 are two ligands of CSF1R. These two factors function in different ways. It has been reported that macrophages in the liver, spleen, or bone marrow are typically regulated by CSF1-mediated signals, while IL-34 dominates the development of macrophages in the brain. Given the importance of CSF1R, its inhibitors are often used in scientific studies to deplete macrophages. In addition, the lack of Sfi1, which is a pioneering transcriptional regulator in myeloid lineage development, could result in a total depletion of CD11b+/Ly6C− macrophages. An expression disparity of Sfi1 determines the differentiation of Ly6C− monocytes into INOS+ macrophages or monocyte-derived dendritic cells. Id3 is indispensable for liver macrophages. PPARγ maintains the anti-inflammatory function of alveolar macrophages. Gata6 controls the proliferative renewal of peritoneal macrophages. LXR deficiency could cause a failure in the generation of splenic marginal zone macrophages and metallophilic macrophages. Epigenetic changes drive the differentiation of monocytes into macrophages. More factors participating in the differentiation of macrophages have been described in previous reviews.

Categories

Macrophages are widely distributed in various tissues. According to their histological locations, macrophages residing in specific
tissues can be categorized into Kupffer cells in the liver, microglial cells in the brain, osteoclasts in the osseous tissue, alveolar macrophages in the lung, mesangial cells in the kidney, subcapsular sinus macrophages in lymph, and so on.26,27 A summary of the ontogeny, functions, and markers of macrophages in different tissues is listed in Table 1. It has been shown that macrophages from different tissues possess diverse expression profiles for transcripts and proteins, which can have a profound impact on their phenotypes and functions.28,29

Based on phenotypes and functions, macrophages can be typically divided into M1 (proinflammatory, classically activated macrophages) and M2 (anti-inflammatory, alternatively activated macrophages) types (Fig. 2). In brief, M1 macrophages can be induced by IFN-γ, lipopolysaccharide (LPS), TNF-α or granulocyte–macrophage colony-stimulating factor (GM-CSF), followed by activation of Toll-like receptor signaling pathways. They play a positive role in the removal of pathogens and tumor cells. On the one hand, M1 macrophages express high levels of antigen-presenting MHC complexes, which accelerate the activation of adaptive immune responses. On the other hand, they act directly on target cells by generating nitric oxide, reactive oxygen species, and reactive nitrogen species. Moreover, M1 macrophages promote inflammatory responses by secreting cytokines such as TNF-α, IL-1α, IL-1β, IL-6, IL-12, IL-18, and IL-23.30,31 Excessive M1 macrophage-mediated responses may lead to tissue damage, which is the main cause of atherosclerosis and other chronic inflammation.32–34 M2 macrophages can be induced by cytokines, such as IL-4, IL-13, glucocorticoids, M-CSF/CSF1, IL-10, IL-33, IL-21, and TGF-β.35–37 Accompanied by increased production of polyamines and ornithine through the arginase pathway, high secretion of IL-10, PGE2, TGF-β, but low IL-12, they are major participants in the clearance of parasites and homeostasis, such as tissue remodeling and regeneration, wound healing and anti-inflammation.38,39 When M2 macrophages develop further, they are refined into M2a, M2b, M2c, and M2d subgroups.40 Their specific characterizations have been reviewed by Abbas Shapouri Moghaddam et al.41 Macrophages have strong plasticity. It has been shown that different phenotypes could possibly transform mutually under certain conditions.

Tumor-associated macrophages (TAMs) generally represent a major component of myeloid cells present in tumors. For some solid tumors, TAMs can arise from several origins: as residual macrophages derived from the yolk sac, infiltrating macrophages as the major replenishment recruited from bone marrow/Ly6C+–circulating monocytes, and a minority from the spleen.8,42–47 It has been demonstrated that TAMs with different origins may act differently than anti-macrophage oncotherapies.41 In most established tumors, TAMs tend to be considered M2-skewed macrophages because they possess the majority of the representative properties of M2 macrophages, usually including but not limited to high expression levels of arginase-1, mannose receptor, and a low MHC class II complex.48 Transcriptome profile analysis revealed that TAMs are more similar to fetal macrophages but not inflammatory macrophages.41 However, as macrophages are plastic, there is also evidence suggesting that TAMs actually have both M1 and M2 expression patterns or expression patterns distinct from those of M1 and M2 macrophages.49 Since 90–95% of neoplasms are closely associated with a chronic inflammatory status, it has been suggested that M1 macrophages may induce tumor initiation by creating a mutagenic microenvironment, while M2 macrophages promote malignancy progression.46,49 It is also believed that TAMs may exert both tumor-promoting and tumor-inhibiting functions,50,51 which make TAMs a potential target for cancer therapies.

Typical markers
To be distinguished from other immunocytes, macrophages can be characterized by phagocytosis and the expression of CD11b, F4/80, and CSF1R in mice or CD79, CD163, CD16, CD312, and CD115 in humans.43 Specifically, to present antigens and activate adaptive immune responses, M1 macrophages often express high levels of MHC class II molecules and costimulatory molecules, such as CD40, CD80, and CD86, while M2 macrophages contain upregulated levels of endocytosis-related receptors, such as the human scavenger receptors CD163 and Stabilin-1 and C-type lectin receptors, including CD206, CD301, with CD1 and CD209.43 In addition to the proinflammatory or anti-inflammatory cytokines mentioned above, polarized macrophages generate different types of chemokines. CXCL9, CXCL10, CXCL11, and CCL5 are usually secreted by M1 macrophages to recruit Th1, Th17, and cytotoxic T cells, while CCL2, CCL17, CCL18, CCL22, and CCL24 are produced by M2 macrophages in most cases.31,38,40

Basic functions of macrophages
One of the basic functions of macrophages is phagocytosis. Through phagocytosis, macrophages can clear erythrocytes, apoptotic cells, and effete cells to maintain homeostasis. Neutropenia and splenomegaly may occur when neutrophils and erythrocytes in the spleen and liver cannot be phagocytized.52 This type of clearance process is independent of immune
| Tissue                        | Macrophage            | Ontogeny                      | Function                                                                                           | Identifying markers                                                                 | Refs.          |
|------------------------------|-----------------------|-------------------------------|----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|----------------|
| Liver                        | Kupffer cells         | Yolk sac derived             | Clearance of bacteria, aged erythrocytes, and cellular debris from the blood; regulation of the immune response; involvement in liver injury repair | F4/80<sup>hi</sup> CD11b<sup>lo</sup> Siglec-1<sup>+</sup> CD68<sup>+</sup> Galectin-3<sup>+</sup> CD80<sup>lo</sup>/<sup>−</sup> PPARγ<sup>+</sup> Ly6C<sup>−</sup> CX3CR1<sup>−</sup> Clec4F<sup>+</sup> TIM-4<sup>+</sup> | 27,62,223–225 |
| Monocyte-derived liver macrophage (MoMFs) | Monocyte derived     | Rapid accumulation and involvement in immune responses after organ damage |                                                                                                    |                                                                                       | 226–229        |
| Liver capsular macrophages   | Monocyte derived      |                               | Sensing bacteria reaching the hepatic capsule; inhibition of the hepatic spread of peritoneal pathogens; recruiting neutrophils | F4/80<sup>+</sup> MHC-II<sup>+</sup> CD11b<sup>+</sup> CD64<sup>+</sup> CD103<sup>+</sup> CX3CR1<sup>+</sup> TIM-4<sup>+</sup> CD207<sup>+</sup> | 230            |
| Lung                         | Alveolar macrophages  | Yolk sac and fetal liver progenitors | Immune surveillance; phagocytosis of inhaled particles                                                | F4/80<sup>lo</sup> CD11b<sup>lo</sup> CD11c<sup>+</sup> CD14<sup>lo</sup> DEC205<sup>+</sup> MHC-II<sup>lo</sup> CD68<sup>+</sup> Siglec F<sup>+</sup> MARCO<sup>+</sup> CD206<sup>+</sup> Dectin-1<sup>+</sup> Galectin-3<sup>+</sup> Merk<sup>+</sup> CD64<sup>+</sup> Siglec-1<sup>+</sup> | 27,223,231–233 |
| Interstitial macrophages     | Fetal liver and bone marrow-derived monocytes |                               | Immune surveillance                                                                                  |                                                                                       | 233–236        |
| Central nervous system       | Microglial cells      | Yolk sac derived             | Functioning as immune surveillance; promote neuronal survival and remove dead neurons; synaptic remodeling | F4/80<sup>+</sup> CD11b<sup>+</sup> CD45<sup>+</sup> CX3CR1<sup>hi</sup> Iba-1<sup>+</sup> P2RY12<sup>+</sup> | 26,27,236,237 |
| Perivascular macrophages     | Yolk sac or fetal liver progenitors |                               | Blood–brain barrier integrity; phagocytosis; antigen presentation; lymphatic clearance                |                                                                                       | 237–248        |
| Tissue                | Macrophage                  | Ontogeny                        | Function                                                                 | Identifying markers                                                                 | Refs.         |
|----------------------|-----------------------------|---------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------|
| Meningeal macrophages | Yolk sac derived            | Immune surveillance              |                                                                          | F4/80<sup>+</sup>, CD11b<sup>+</sup>, CD45<sup>lo</sup>, Iba-1<sup>+</sup>, CD209b<sup>+</sup>, CX3CR1<sup>hi</sup>, Chnrb4<sup>+</sup> | 27, 237, 249  |
| Bone                 | Osteoclast                   | Monocyte derived                | Resorption of organic matter and minerals from the bone matrix           | Calcitonin receptor<sup>+</sup>, ER-HR3<sup>+</sup>, F4/80<sup>+</sup>, Tartrate-resistant acid phosphatase (TRAP)<sup>-</sup> | 26, 27, 250–252 |
| Bone marrow          | Yolk sac derived or fetal liver-derived monocytes | Promoting erythropoiesis; maintenance of the hematopoietic stem cells niche |                                                                          | Siglec-1<sup>+</sup>, F4/80<sup>+</sup> | 250, 253      |
| Spleen               | Marginal zone macrophages    | Bone marrow-derived monocytes    | Clearance of pathogens present in the circulation; retention of marginal zone B cells | CD68<sup>+</sup>, Dectin-2<sup>+</sup>, F4/80<sup>+</sup>, LXRx<sup>-</sup>, MARCO<sup>+</sup>, TIM-4<sup>-</sup>, SIGN-R1<sup>+</sup> | 22, 254–256   |
| Spleen               | Marginal metallophilic macrophages | Bone marrow-derived monocytes    | Clearance of pathogens present in the circulation                       | CD68<sup>+</sup>, F4/80<sup>+</sup>, LXRx<sup>-</sup>, Siglec-1<sup>+</sup> | 257           |
| Spleen               | White pulp (tingible body) macrophages | Not clear                        | Clearance of apoptotic B cells                                          | CD68<sup>+</sup>, MFG-EB<sup>+</sup>, MerTk<sup>-</sup>, TIM-4<sup>-</sup>, CD36<sup>-</sup> | 257–259        |
| Spleen               | Red pulp macrophages         | Yolk sac-derived or fetal liver-derived monocytes | Clearance of effete red blood cells; immunosurveillance; detoxification; iron recycling; antigen delivery to DCs | F4/80<sup>hi</sup>, CD11b<sup>lo</sup>, Siglec-1<sup>lo</sup>, CD68<sup>+</sup>, MHC-II<sup>+</sup>, CSF1R<sup>+</sup>, SIRPa<sup>-</sup>, Siglec F<sup>-</sup>, CD163<sup>+</sup>, Dectin-2<sup>+</sup>, VCAM1<sup>+</sup>, Spi-C<sup>-</sup>, Heme Oxigenase<sup>+</sup>, Ferroportin<sup>+</sup> | 223, 255, 259–261 |
| Kidney               | Mesangial cell               | Monocyte derived                | Intraglomerular mesangial cells; regulation of glomerular filtration; mesangial matrix formation; phagocytosis; monitoring of glucose concentrations | F4/80<sup>+</sup>, CD11b<sup>+</sup>, CD103<sup>-</sup>, CX3CR1<sup>+</sup>, SIRPa<sup>-</sup>, Siglec F<sup>-</sup> | 223           |
| Lymph node           | Subcapsular sinus macrophages | Yolk sac-derived or bone marrow-derived monocyte | Limiting the systemic dissemination of pathogens and bacterial infections; promote the presentation of antigens | F4/80<sup>+</sup>, MARCO<sup>+</sup>, Siglec-1<sup>hi</sup>, CD11b<sup>hi</sup>, Ligands for the cysteine-rich domain of the mannose receptor<sup>-</sup> | 223, 262, 263  |
| Medullary macrophages | Bone marrow-derived monocytes | Highly phagocytic and rapidly clear pathogens |                                                                          | CD11b<sup>+</sup>, Siglec-1<sup>+</sup>, F4/80<sup>+</sup>, MARCO<sup>+</sup>, SIGN-R1<sup>+</sup> | 223, 263, 264 |
| Serosal Tissues       | Pleural macrophages          | Immune surveillance              |                                                                          | CD11b<sup>hi</sup>, F4/80<sup>hi</sup> | 265–267        |
responses and is regarded as the fundamental function of macrophages. When pathogens or aberrant cells, such as tumor cells, are recognized by macrophages, they can be phagocytized and processed into antigen peptides. Macrophages present these peptides to MHC class II molecules on their surface and stimulate T-cell proliferation and activation with the synergistic effect of costimulatory molecules. It has been reported that adult macrophages are primarily responsible for host defense, while

| Tissue                      | Macrophage                                    | Ontogeny                          | Function                                                                 | Identifying markers                          | Refs.         |
|-----------------------------|-----------------------------------------------|-----------------------------------|-------------------------------------------------------------------------|---------------------------------------------|---------------|
| Bone marrow-derived         | Bone marrow-derived                           | Regulation of IgA production in   | Siglec F<sup>+</sup> RELM<sup>+</sup> TIM-4<sup>-</sup> CD11b<sup>+</sup>  | 268–270                                     |
| Large peritoneal            | or fetal liver-derived                       | the gut                             | CD11c<sup>+</sup> SIGN-R1<sup>+</sup> F4/80<sup>+</sup> GATA-6<sup>-</sup> |                                            |
| Small peritoneal            | Bone marrow-derived                           | Immune surveillance                | CD11b<sup>+</sup> CD11c<sup>-</sup>   | 265,269,270                                  |
| peritoneal macrophages      | monocytes                                    |                                   | F4/80<sup>+</sup> Id2<sup>-</sup> Langerin<sup>+</sup> RUNX3<sup>+</sup> |                                            |
| Skin                        | Langerhans cells                              | Interaction with T lymphocytes;    | CD11b<sup>+</sup> CD11c<sup>-</sup> F4/80<sup>+</sup>                   | 27,271,272                                  |
| Dermal macrophages          | Bone marrow-derived                           | Immune surveillance                | Dectin-1<sup>+</sup> Dectin-2<sup>-</sup> F4/80<sup>-</sup> CD64<sup>+</sup>| 27,223,255,273 |
| Adipose tissue              | Adipose tissue-associated macrophages        | Not clear                          | Adipogenesis; adaptive thermogenesis; regulation of insulin sensitivity | CD45<sup>-</sup> F4/80<sup>-</sup> PPARγ<sup>-</sup> | 274,275      |
| Gastrointestinal Tract      | Intestinal lamina propria macrophages         | Bone marrow-derived                | Maintenance of intestinal homeostasis; recognition and removal of       | CD11b<sup>+</sup> CD11c<sup>-</sup> CX3CR1<sup>+</sup> F4/80<sup>-</sup> CD64<sup>-</sup> | 27,276       |
| Blood                       | Ly6C<sup>lo</sup> monocytes                  | Bone marrow-derived                | Immune surveillance; maintenance of vascular integrity                 | CD11b<sup>+</sup> CD43<sup>-</sup> CX3CR1<sup>+</sup> Ly6C<sup>lo</sup>| 27,277,278 |
| Tumor                       | Tumor-associated macrophage                   | Yolk sac derived or monocyte      | Promote tumor growth; inhibit tumoricidal immune response; initiate    | Murine: Ly6C<sup>+</sup> MHC-II<sup>-</sup> | 42,279–281 |
|                            |                                               | derived                           | angiogenesis; activate matrix remodeling; aid invasion and intravasation| CX3CR1<sup>+</sup> CCR2<sup>+</sup> CD62L<sup>+</sup> TIE2<sup>+</sup> Human: |              |

Table 1. continued
fetal macrophages are involved in tissue remodeling.40 Macrophages play an important role in the development and homeostasis. For example, microglia are required in almost every precise developmental stage of the central nervous system.57 Cardiac macrophages help maintain homeostasis in the steady-state heart by facilitating myocardial conduction.58 CCR2–macrophages are instrumental in cardiac recovery, coronary development, and postnatal coronary growth.59,60 Impaired activation or depletion of Kupffer cells leads to hepatic steatosis and insulin resistance.61–63 Defects in perivascular macrophages can give rise to the unsuccessful establishment of the blood–brain barrier.64 It is well known that macrophages are related to many diseases. Here, we will focus on its role in tumors in the following sections.

FUNCTIONS OF MACROPHAGES IN CANCERS

By secreting various factors and affecting other immune cells, macrophages not only play a role in chronic inflammation but also initiate, promote, or suppress the development of cancer. Ornithine, VEGF, EGF, and TGF-β are examples of tumor-promoting factors derived from macrophages, while nitric oxide generated by inducible nitric oxide synthase in macrophages can inhibit tumor growth.32,33,65,66 Macrophages have been demonstrated to be involved directly or indirectly in several key features of malignant tumors, including angiogenesis, invasiveness, metastasis, regulation of the tumor microenvironment, and therapeutic resistance (Fig. 3).

Angiogenesis

By expressing WNT7B, WNT5A, WNT11, VEGF-C, VEGF-D, and other factors, macrophages are deeply involved in vasculogenesis and lymphogenesis.57–59 In addition, TAMs can enhance tumor hypoxia and glycolysis,71 two important causes of angiogenesis.72,73 HIF-1α is a protein induced in hypoxia conditions. It has been demonstrated that HIF-1α is an important transcriptional factor regulating the transcription of angiogenesis-associated genes, such as VEGF, bFGF, PDGF, and PGE2 in TAMs.74,75 Through the synthesis of WNT7B, macrophages also stimulate vascular endothelial cells to produce VEGF.76 Other TAM-produced proangiogenic molecules that recruit or activate endothelial cells include CXCL12, TNF-α, IL-1β, IL-8, Sema4d, adrenomedullin, and thymidine phosphorylase.41,77,78 Studies on liver diseases have revealed that in addition to producing proangiogenic molecules, macrophages can benefit the formation of complex vascular networks by interacting with the sprouting vasculature.79 Live imaging showed that macrophages drive sprouting angiogenesis via VEGF signaling and coordinate blood vessel regression in wound healing by clearing apoptotic endothelial cells.80 Preventing macrophages from entering avascular areas by blocking the Sem3A3/Nrp1 signaling pathway could inhibit angiogenesis.81 It has been reported that angiogenic macrophages are similar to fetal counterparts based on their characteristic expression of Tie2.77,82 Targeting Tie2 or its ligand ANG2 inhibits angiogenesis in certain tumor models, such as those for breast and pancreatic cancers.82 Depletion of TAMs inhibits angiogenesis.74,83 A close relationship between macrophages and angiogenesis has been discussed in previous reviews.84,85

Invasiveness and metastasis

Macrophages can not only increase the density of blood vessels but also promote the invasiveness and metastasis of tumor cells. By expressing matrix metalloproteinases, cathepsin, urokinase plasminogen activator, and matrix remodeling enzymes, such as lysyl oxidase and osteonectin, macrophages dissolve the extracellular matrix to pave the way for tumor cell escape.86 TAMs upregulate cytokines, such as IL-1ra, to promote metastasis by enhancing tumor cell stemness.87 Secretion of TGF-β and growth factors, such as EGF analogs, promotes epithelial–mesenchymal transition and invasiveness of tumor cells.88,89 Exosomes released from M2 macrophages are responsible for cancer metastasis by transferring certain miRNAs into cancer cells, such as colorectal cancer and pancreatic ductal adenocarcinoma cells.90,91

In addition to macrophages in primary tumors, macrophages can also assist in tumor survival and colonization at premetastatic lesions. It has been demonstrated that macrophages are required for the early dissemination of breast cancer, and early disseminated macrophages contribute to late metastasis.92 Tumor exosomes are crucial in tumor organotropic metastasis. It has been observed that pancreatic cancer cell-derived exosomes preferentially colocalize with macrophages in liver metastasis sites.93 Exosome-educated macrophages facilitate premetastatic niche formation via secretion of TGF-β.95 In addition, the interplay between integrin a4 on macrophages and VECA1 on tumor cells promotes cancer cell survival.96 Results from other studies support the indispensable role of monocytes/macrophages recruited to premetastatic niches in promoting circulating tumor cell survival and colonization in metastatic lesions.97,98 At lung metastasis nodules of breast cancer, CCL2 produced by tumor cells is an important chemokine for the recruitment and retention of inflammatory monocytes/macrophages.99 By recruiting Ly6C+ monocytes via CCL2, fibrocytes prepare a premetastatic niche in the lung for melanoma cells.100 After differentiating of CCR2+Ly6C+ inflammatory monocytes into Ly6C- macrophages, these cells accelerate tumor cell extravasation by generating VEGF.101
Tissue-resident macrophages have also been demonstrated to promote or restrict metastasis. Alveolar macrophages promote hepatocellular carcinoma lung metastasis by producing an inflammatory mediator, leukotriene B4. By suppressing Th1 responses, alveolar macrophages facilitate breast tumor cells to metastasize. Kupffer cells engulf cancer cells in a Dectin-2-dependent manner to limit liver metastasis.

Effects of macrophages on tumor microenvironment

Many factors, such as CSF1, VEGF-A, CXCL12, ANG2, CCL5, and CCL2, in solid tumors, can recruit angiogenic macrophages. This enrichment allows macrophages to play a major role in the construction of the tumor immune microenvironment. Granulin generated by TAMs can induce fibrosis, which prevents T cells from infiltrating. Attenuation of the TAM antigen presentation ability results in a reduction in T-cell activation and proliferation.

Exosomes consisting of various miRNAs derived from TAMs orchestrate an immunosuppressive tumor microenvironment by causing Treg/Th17 imbalance. It has been summarized that tumor-associated macrophages support a suppressive tumor microenvironment in three ways: (1) by consuming the metabolites, e.g., L-arginine, which is essential for T-cell activation, can be metabolized by TAMs with high expression of ARG1. (2) by producing the cytokines and chemokines, IL-10, TGF-β, and PGE2, which are primarily secreted by TAMs, to inhibit the activation and function of various immune cells, including cytotoxic T cells, but induce and maintain regulatory T cells, (3) by expressing inhibitory molecules. TAMs elicit immune suppression through the expression of inhibitory receptors or immune checkpoint ligands (e.g., MHC-I molecules, PD-L1, PD-L2, CD80, CD86, B7-H4 and VISTA). These molecules deliver an inhibitory signal to ligand- or receptor-expressing immune cells.

Therapeutic resistance

Macrophages are also an important cell-extrinsic factor that mediates the resistance of tumor cells to chemotherapy or radiotherapy. By expressing IL-6, TNF-α, cathepsin B and S, or inducing other cells to produce IL-6, macrophages activate STAT3.
in tumor cells, which enhances the proliferation and survival of malignant cells under treatment with several chemotherapeutics. The epithelial to mesenchymal transition, which can be elicited by macrophages, has been demonstrated to be another mechanism behind chemoresistance. Exosomal miR-223 from macrophages has been reported to cause a chemoresistant phenotype after being delivered into epithelial ovarian cancer cells. miR-21 derived from macrophages is responsible for cisplatin resistance in gastric cancer cells. Macrophages exacerbate fatty acid beta-oxidation of gastric cancer cells by generating growth differentiation factor 15 so that the cancer cells are more resistant to 5-fluorouracil treatment. Metabolites, including deoxycytidine, from macrophages, weakened the therapeutic effect of gemcitabine in pancreatic ductal adenocarcinoma. Murine pancreatic ductal adenocarcinoma models showed an enhanced therapeutic response toward gemcitabine after depleting macrophages with liposomal clodronate. As summarized by Marek Nowak et al., TAMs contribute to chemoresistance by inducing prosurvival and antiapoptotic signals in cancer cells, as well as their protumoral polarization.

It has been reported that irradiation promotes the accumulation and M2 polarization of macrophages. Heparin-binding epidermal growth factor, which is primarily secreted by macrophages, could reduce the radiosensitivity of head and neck cancer cells by activating the nonhomologous end-joining pathway. TNF-α has a radioprotective function in a TAM-produced VEGF-dependent manner. Carcinoembryonic antigen has been identified as a radioresistance marker in colorectal cancer because it induces M2 polarization of macrophages. Inhibition of differentiation of M2 macrophages showed enhanced responses to radiotherapy in breast cancer. Of note, dying cancer cells after treatment with chemotherapeutics or radiation might also initiate antitumor immune responses. Whether the function of macrophages leads to sensitization or resistance to traditional therapy is complex. Better understanding of the mechanisms can improve the efficacy of traditional oncotherapy.

**INvolvement of macrophages in current immunotherapy**

Due to the limitations and shortages of traditional cancer treatments, immunotherapy has become the most promising cancer treatment. Various cancer immunotherapy strategies have emerged. These include adoptive cellular immunotherapy, tumor vaccines, antibodies, immune checkpoint inhibitors, and small-molecule inhibitors. Although most of these strategies are not meant to target macrophages directly or originally, macrophages contribute significantly to the final outcomes.

Immune checkpoint inhibitors

To date, many immune checkpoint blockade therapies have been reported, but the most commonly used therapies in the clinic are anti-PD-1 and anti-PD-L1 therapies. Cancer immunotherapy based on inhibiting the immune checkpoints CTLA-4 and PD-1 aim at relieving immune suppression rather than simply reinforcing immune responses. Blocking the PD-1/PD-L1 pathways with inhibitors to enhance the cytotoxic function of T cells has made certain achievements in the resolution of malignancies. However, even if the adaptive immune system is compromised or the function of T cells cannot be fully recovered by PD-1 inhibitors under specific circumstances, PD-1/PD-L1 antagonisms can still increase antitumor efficacy. Therefore, more immune cell types should be involved in PD-1/PD-L1 inhibitor treatment. Additional studies revealed that both PD-L1 and PD-1 are expressed in TAMs, promoting immune suppression and escape. PD-1+ TAM phagocytosis can be rescued by PD-L1 blockade, which leads to a direct decrease in tumor burden. Furthermore, anti-PD-1 or PD-L1 immune checkpoint blockade induced an M1 macrophage polarization. M1 macrophage polarization or repolarization has been linked to an enhanced antineoplastic effect by numerous studies. Of note, macrophages might play a negative role in anti-PD-1 treatment, such as by preventing cytotoxic T cells from reaching tumor cells. In addition, in vivo imaging showed the transfer of an anti-PD-1 antibody from CD8+ T cells to TAMs through the binding of Fc-Fcy receptors shortly after its administration. Blocking such binding reduced the accumulation of anti-PD-1 antibody in TAMs and prolonged its retention time in CD8+ T cells, leading to the regression of tumors.

Along with the concept of immune checkpoints on T cells, several checkpoints that are mainly associated with macrophages have also been discovered. CD47 is a poor prognostic factor in tumor cells, and its interaction with SRPs on macrophages helps tumor cells evade phagocytic clearance by macrophages. Blocking CD47 has resulted in macrophage-mediated tumor inhibition. The inhibitory receptor LILRB1 expressed on macrophages prevents tumor cells from being phagocytosed by interacting with the beta-2 microglobulin (β2M) subunit of the MHC class I complex. The CD24-Siglec-10 axis promotes immune evasion by downregulating macrophage phagocytosis. Inhibition of these immune checkpoints has significantly increased cancer immunotherapy efficacy.

**Tumor vaccines**

Vaccines can be divided into two categories: preventive vaccines and therapeutic vaccines. Preventive vaccines are often designed to induce specific adaptive immunity, chiefly humoral immunity, before the occurrence of disease, which is normally caused by infection with a virus or bacteria. Thus, it can be used to reduce the incidence of viral or bacterial infection-induced carcinoma. Typical examples of preventive vaccines are those for HBV or HPV. Although a proper adaptive immune response is believed to be the primary reason for the effectiveness of these vaccines, it has been reported that immediate innate immunity other than time-consuming adaptive immunity is principally responsible for the spontaneous regression of cancer.

Therapeutic vaccines are usually designed to elicit protective T cells. However, Maxime Thoreau et al. demonstrated that cooperation between T cells and macrophages is required to achieve the effects of a therapeutic vaccine. A denser presence of macrophages along with tumor regression has shown to precede the infiltration of CD8+ T cells. Numerous approaches choose synthetic peptides, recombinant proteins, whole tumor cells, viral vectors, bacteria or nucleic acids as vaccination candidates to activate T cells via antigen-presenting cells, which are mostly dendritic cells. Among these, some regimens that used GM-CSF as an adjuvant generated obvious immune responses. Sipuleucel-T was the first therapeutic vaccine approved by the FDA to be used in a particular group of prostate cancer patients. A fusion protein combining a targeting tumor antigen prostate acid phosphatase with GM-CSF was used to induce antigen-specific T cells. It prolonged the survival of patients in a few clinical trials. A STING agonist formulated with GM-CSF showed remarkable antitumor efficacy in multiple established tumors. Some tumor cells used as whole-cell vaccines can secrete GM-CSF. In addition, oncolytic virotherapy, which increases the targeting of cancer cells through virus infection, could induce antitumor immune responses, especially in cells that had been engineered to express GM-CSF. GM-CSF is combined for the purpose of enhancing DC functions and limiting Treg regulation. However, GM-CSF could also induce M1 macrophage polarization and activate macrophages to exert an antitumor function.

In another virus-related tumor immunotherapy study, Danyang Wang et al. used an NF-kB-activating gene expression adenovirus-associated virus system to express an artificial neoantigen on the tumor cell surface, which could be targeted by specific immune
cells. When they chose calreticulin, a signal to promote phagocytic uptake, the cancer cells could be engulfed by macrophages.\textsuperscript{161} In addition, exosomes derived from M1- but not M2-polarized macrophages boosted the antitumor vaccine by eliciting a release of Th1 cytokines and a stronger antigen-specific cytotoxic T-cell response.\textsuperscript{162} Xu et al. reported that a listeria-based tumor vaccine benefited anti-PD-1 therapy against hepatocellular carcinoma by skewing macrophage polarization.\textsuperscript{163}

Antibodies
Checkpoint inhibitors, such as nivolumab (Opdivo) and pembrolizumab (Keytruda), are monoclonal antibodies. In addition, many other monoclonal antibodies have been approved for clinical cancer immunotherapy by the FDA. Rituximab and trastuzumab are examples of these monoclonal antibodies. Rituximab is used in B-cell lymphoma by targeting CD20. B lymphoma cells are more sensitive to macrophages in the presence of rituximab.\textsuperscript{164} Its combination with cyclophosphamide induced nearly complete tumor elimination in resistant bone marrow by activating macrophages.\textsuperscript{165} After blocking the CD47-SIRPα axis, rituximab-induced macrophage phagocytosis was augmented in nongenital B diffuse large B-cell lymphoma patients.\textsuperscript{166} Trastuzumab is an HER2-targeting antibody that has shown promising efficacy in breast cancer therapy. It has been reported that antibody-dependent cell phagocytosis mediated by macrophages is the main cause of the effectiveness of trastuzumab plus CD47 blockade.\textsuperscript{167} By binding with Fcy receptors on macrophages, trastuzumab triggered macrophage phagocytic killing, and this function was augmented after increasing the expression of Fcy receptors on macrophages.\textsuperscript{168} In addition, trastuzumab resistance was overcome by shifting macrophages from the M2 to M1 phenotype.\textsuperscript{169}

Adoptive cell therapy
Adoptive cell therapy is also a very promising therapy that induces tumor regression by transferring specific immune cells to the tumor-bearing host. These cells may come from the host itself or some other donors. They are commonly manipulated to possess better effector functions and proliferate to a sufficient number in vitro before administration.\textsuperscript{170} Typical examples include T cells with engineered chimeric antigen receptors (CAR-Ts) or gene-modified T-cell receptors (TCR-Ts). In 2006, the adoptive transfer of TCR-engineered lymphocytes, which recognize an antigen named MART-1, caused tumor regression in metastatic melanoma patients.\textsuperscript{171} In 2010, administration of CAR-T cells against CD19 efficiently eliminated B cells in a patient with follicular lymphoma.\textsuperscript{172} However, insufficient infiltration into solid tumors is a major limitation for these T-cell-based immunotherapies. Local low-dose irradiation increased T-cell recruitment by inducing M1-phenotype macrophage differentiation.\textsuperscript{173} Cytokine release syndrome is considered to be closely related to the efficacy of adoptive cell therapy, but serious cytokine release syndrome may lead to death. It has been reported that cytokine release syndrome induced by CAR-T-cell transfer is mediated by macrophages.\textsuperscript{174} Inhibiting or neutralizing GM-CSF abolished macrophage-derived cytokines, which released syndrome-related cytokines and enhanced CAR-T-cell functions.\textsuperscript{175,176} Therefore, taking the response of macrophages into account may benefit adoptive modified T-cell therapy. Modified macrophages with the chimeric antigen receptor (CARMA) have also been tested by Klichinsky et al. The first generation of chimeric antigen receptors, which combine the scFv of anti-CD19, anti-mesothelin, or anti-HER2 antibodies with a CD3 intracellular domain, has been constructed. This CARMA displayed a strong tumoricidal function in preclinical models.\textsuperscript{177}

Small-molecule inhibitors
Because of several advantages, such as oral bioavailability, the relatively low cost, ease of crossing physiological barriers or access to intracellular targets, small-molecule drugs are complementary and synergistic with other immune-oncology therapies.\textsuperscript{178} Numerous small-molecule inhibitors have been proven to suppress tumors by targeting macrophage-associated molecules. For example, IDO is a poor prognosis indicator that is often highly expressed in macrophages, dendritic cells, and tumor cells. Small-molecule inhibitors targeting IDO have been tested in clinical trials to reestablish positive immune responses.\textsuperscript{179,180} ARG1 is a cytosolic enzyme that plays a key role in the immunosuppressive function of TAMs. Compounds inhibiting arginase have shown potential in tumor suppression.\textsuperscript{181} RRX-001, a small-molecule inhibitor, downregulated not only CD47 on cancer cells but also SIRPα on macrophages and showed hypotoxicity but strong antitumor activity in clinical trials.\textsuperscript{182} In addition, small-molecule inhibitors have great potential in combination with other oncotherapy strategies. Inhibition of Bcl-2 family members improved the efficacy of CAR-T therapy in B-cell malignancy.\textsuperscript{183} PI3K-γ inhibitors, such as IPI-549, overcome immune checkpoint resistance by reshaping the tumor microenvironment, including switching macrophage polarization from the M2 to M1 phenotype.\textsuperscript{184} Small-molecule inhibitors targeting CXCR2 on neutrophils and CCR2 on macrophages improve the chemotherapeutic effects in pancreatic ductal adenocarcinoma models.\textsuperscript{185} PLX-3397, a small-molecule inhibitor of CSF1R, cKIT, and FLT3 has been demonstrated to decrease tumor burden by reducing M2 macrophages in combination with adoptive cell transfer immunotherapy or other small-molecule inhibitors.\textsuperscript{186,187} FAK is indispensable for the migration and stable protrusion formation of macrophages. Small-molecule inhibitors against FAK have shown promising antitumor activity, especially when combined with chemotherapy and immunotherapy strategies.\textsuperscript{188}

PROSPECT: MACROPHAGES ARE A PROMISING TARGET IN FUTURE CANCER IMMUNOTHERAPY
To date, great endeavors to boost T cell-directed anticancer immune responses have been made. As reported, the incidence of cancerogenesis is low in invertebrates with no T or B cells, indicating that innate immune cells are of great importance for preventing the initiation and development of cancer.\textsuperscript{189–191} In addition to their supporting role in all kinds of immunotherapies, macrophages may become a promising target in future cancer immunotherapy.\textsuperscript{192,193} Many targets and pharmacological agents related to macrophages in oncotherapy have been summarized in recent reviews.\textsuperscript{126,193} We updated the typical macrophages-targeting agents that have been registered for cancer-related clinical trials (excluding projects those are in the status of terminated, withdrawn, unknown, not yet recruiting) in Table 2. The potential and promising strategies targeting macrophages have been categorized into six types based on their objectives in Fig. 4. There are several advantages to target macrophages in cancer immunotherapy. Low infiltration is a major barrier for T-cell-based anticancer therapy, and macrophages account for ~30–50% of infiltrating immune cells in the tumor microenvironment. As mentioned above, circulating monocytes are a major source of infiltrating macrophages in tumors, and the accessibility of peripheral blood mononuclear cells makes it easy to operate if a macrophage-based therapy strategy is adopted in the clinic.

Currently, it is generally believed that cancer cells originate from endogenous cells in humans. Even if numerous tumor-specific antigens have been identified, most specific antigens still exist in a few normal cells. In contrast, not all cancer cells express just one specific antigen because of tumor heterogeneity. Clearance of specific antigen-expressing cancer cells may only result in temporary and limited antitumor efficacy. Nevertheless, as a type of innate immune cell, macrophages can exert a tumor-suppressive function without targeting one specific antigen.\textsuperscript{194,195}
| Target | Agent | Organization | ClinicalTrials.gov Identifier | Tumors | Other interventions | Phase |
|--------|-------|--------------|-------------------------------|--------|--------------------|-------|
| CSF1   | Lacnotuzumab (MCS110) | Novartis Oncology | NCT02435680 | Advanced triple-negative breast cancer | Carboplatin, gemcitabine | II |
|        |        |              | NCT01643850 | Pigmented villonodular synovitis | None | II |
|        |        |              | NCT03694977 | Gastric cancer | PDR001 | II |
| CCL2   | Carlumab (CNTO 888) | Centocor Research & Development | NCT01204996 | Solid tumors | Standard of care | I |
|        |        |              | NCT00992186 | Prostate cancer | None | II |
| SIRPα  | TTI-622 | Trillium Therapeutics | NCT03530683 | Advanced relapsed or refractory lymphoma or myeloma | Rituximab, PD-1 inhibitor, proteasome-inhibitor regimen | I |
|        |        |              | NCT01204996 | Solid tumors | BI 754091 | I |
|        |        |              | NCT00992186 | Prostate cancer | None | II |
| TIE2   | CEP-11981 (ESK981) | Karmanos Cancer Institute | NCT04159896 | Advanced cancer | Pembrolizumab | I |
|        |        |              | NCT03456804 | Prostate cancer | None | II |
|        | Regorafenib (BAY 73-4506) | Bayer | NCT04170556 | Hepatocellular carcinoma | Pembrolizumab, nivolumab | I/II |
| Arginase | INCB001158 (CB1158) | Incyte | NCT03910530 | Advanced solid tumors | Pembrolizumab | I |
| HER2   | CAR-macrophage | Carisma Therapeutics Inc. | NCT04660929 | HER2 overexpressing solid tumors | Pembrolizumab | I |
|        | EF-022 | Efranat | NCT02052492 | Solid tumors | None | I |
| CD40   | SEA-CD40 | Seattle Genetics | NCT02376699 | Solid tumors | Pembrolizumab | I |
|        | APX005M | Apexigen | NCT03389802 | Pediatric CNS | None | I |
|        |        |              | NCT04130854 | Locally advanced rectal adenocarcinoma | Doxorubicin | II |
|        |        |              | NCT02482168 | Non-small-cell lung cancer, melanoma, urethelial carcinoma, head and neck cancer | Pembrolizumab | I |
| CP-870,893 | Selicrelumab (R7009879) | VLST Corporation | NCT01103635 | Metastatic melanoma | Tremelimumab (anti-CTLA-4) | I |
|        |        |              | NCT02760797 | Advanced solid tumors | Anti-PD-L1 | I |
|        |        |              | NCT02665416 | Advanced solid tumors | Bevacizumab or vanucizumab | I |
|        |        |              | NCT02304393 | Solid tumors | Atezolizumab | I |
|        |        |              | NCT02588443 | Pancreatic ductal adenocarcinoma | Gemcitabine, nab-paclitaxel | I |
| Target | Agent | Organization | ClinicalTrials.gov Identifier | Tumors | Other interventions | Phase |
|--------|-------|--------------|-------------------------------|--------|--------------------|-------|
| CDX-1140 | Celldex Therapeutics | NCT04491084 | Non-small-cell lung cancer, lung cancer | CDX-301 | I/II |
| | | NCT04520711 | Malignant epithelial neoplasms | TCR-T, pembrolizumab | I |
| | | NCT04616248 | Unresectable or metastatic breast cancer | Poly ICLC, radiation therapy, recombinant Fc ligand | I |
| | | NCT04364230 | Melanoma | 6MPH, NeoAg-mBRAF, Poly ICLC | I/II |
| | | NCT03329950 | Advanced malignancies | CDX-301, pembrolizumab, chemotherapy | I |
| | | NCT00525447 | Multiple myeloma | Lenalidomide, dexamethasone | I |
| | | NCT0079716 | Multiple myeloma | None | I |
| | | NCT0043916 | Large B-cell diffuse lymphoma, non-Hodgkin lymphoma | None | II |
| | | NCT0103779 | Non-Hodgkin lymphoma | None | I |
| | | NCT0065837 | Large B-cell diffuse lymphoma, non-Hodgkin lymphoma | Rituximab, gemcitabine | I |
| | | NCT0056699 | None | Rituximab | I |
| | | NCT0064898 | Multiple myeloma | Bortezomib | I |
| | | NCT00283101 | Lymphocytic, chronic leukemia | None | I/II |
| | | NCT00670592 | Non-Hodgkin’s lymphoma, Hodgkin’s lymphoma | None | II |
| | | NCT01275209 | Follicular lymphoma | None | I |
| | | NCT00231166 | Multiple myeloma | None | I |
| | | NCT04547777 | Glioma | None | I |
| | | NCT04059588 | Solid tumor, skin cancer | D2C7-IT | I |
| | | NCT02829099 | Advanced solid neoplasms | None | I |
| | | NCT04635995 | Cancer | None | I |
| | | NCT15061911 | Neoplasms, lymphoma, non-Hodgkin, B cell | None | I |
| | | NCT03852511 | Metastatic cancer, epithelial tumor | None | I |
| | | NCT02599324 | Renal cell, urothelial, gastric, colon, pancreatic adenocarcinoma | None | Ib/II |
| | | NCT01478581 | Multiple myeloma | Dexamethasone | I |
| | | NCT01752426 | Leukemia | heavy water (H2O) | I, II |
| | | NCT01236391 | Mantle cell lymphoma | None | II |
| | | NCT01105247 | B-cell chronic lymphocytic leukemia, small lymphocytic lymphoma | None | I, II |
| | | NCT01614821 | Waldenstrom’s macroglobulinemia | None | II |
| | | NCT01292135 | B-cell chronic lymphocytic leukemia, small lymphocytic lymphoma | None | I |
| | | NCT01520519 | Leukemia | Rituximab | II |
| | | NCT01109069 | B-cell lymphoma, chronic lymphocytic leukemia | None | II |
| | | NCT01217749 | Chronic lymphocytic leukemia | Ofatumumab | I, II |
| | | NCT02403271 | Chronic lymphocytic leukemia | Durvalumab | I, II |
| Target | Agent | Organization | ClinicalTrials.gov Identifier | Tumors | Other interventions | Phase |
|--------|-------|--------------|--------------------------------|--------|--------------------|-------|
|        |       |              |                                | Non-small-cell lung cancer, breast cancer, pancreatic cancer | Temsirolimus | III |
|        |       |              | NCT01646021 | Mantle cell lymphoma | | |
|        |       |              | NCT01855750 | Lymphoma | Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone | II |
|        |       |              | NCT01980628 | Marginal zone lymphoma, B-cell lymphoma | None | II |
|        |       |              | NCT01589302 | Prolymphocytic leukemia, small lymphocytic lymphoma, chronic lymphocytic leukemia | None | II |
|        |       |              | NCT01325701 | Diffuse large B lymphoma | None | II |
|        |       |              | NCT01578707 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Ofatumumab | III |
|        |       |              | NCT01722487 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Chlorambucil | III |
|        |       |              | NCT02436668 | Metastatic pancreatic adenocarcinoma | Gemcitabine, nab-paclitaxel | III |
|        |       |              | NCT01980654 | Follicular lymphoma, B-cell lymphoma, non-Hodgkin's lymphoma | Rituximab | II |
|        |       |              | NCT01973387 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Rituximab | III |
|        |       |              | NCT01611090 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Bendamustine, hydrochloride, rituximab | III |
|        |       |              | NCT02401048 | Diffuse large B-cell lymphoma, follicular lymphoma | MEDI4736 | I, II |
|        |       |              | NCT02639910 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Tafasitamab, idelalisib, venetoclax | II |
|        |       |              | NCT02902965 | Multiple myeloma | Bortezomib dexamethasone | II |
|        |       |              | NCT01744691 | Chronic lymphocytic leukemia with 17p deletion, small lymphocytic lymphoma with 17p deletion | None | II |
|        |       |              | NCT02264574 | Chronic lymphocytic leukemia, small-cell lymphoma | Obinutuzumab, chlorambucil | III |
|        |       |              | NCT02514083 | Chronic lymphocytic leukemia, small lymphocytic lymphoma | Fludarabine | II |

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| Target | Agent | Organization | ClinicalTrials.gov Identifier | Tumors | Other interventions | Phase |
|--------|-------|--------------|--------------------------------|--------|---------------------|-------|
|        |       |              | NCT02777710 | Pancreatic or colorectal cancers | Durvalumab | I |
|        |       |              | NCT02734433 | Advanced solid tumors | None | I |
|        |       |              | NCT03158103 | Gastrointestinal stromal tumor | MEK162 | I |
|        | BLZ945| Novartis     | NCT02829723 | Advanced solid tumors | PDR001 | I |
|        | ARRY-382| Array Biopharma | NCT01316822 | Metastatic cancer | None | I |
|        |        |              | NCT02880371 | Advanced solid tumors | Pembrolizumab | II |
|        | Edicotinib (JNJ-40346527) | Johnson & Johnson | NCT03177460 | Prostate cancer | None | I |
|        | IMC-CS4(LY3022855) | Eli Lilly | NCT01346358 | Advanced solid tumors | None | I |
|        |        |              | NCT02265536 | Advanced breast, prostate cancer | None | I |
|        |        |              | NCT02718911 | Solid tumor | Durvalumab, tremelimumab | I |
|        |        |              | NCT03101254 | Melanoma | Vemurafenib cobimetinib | I & II |
|        |        |              | NCT03153410 | Pancreatic ductal adenocarcinoma | Cyclophosphamide, pembrolizumab, GVAX | I |
|        |        |              | NCT02471716 | Tenosynovial giant cell tumor | None | II |
|        |        | Five Prime Therapeutics | NCT03927105 | Peripheral T-cell lymphoma | Nivolumab | II |
|        |        |              | NCT03502330 | Melanoma, non-small-cell lung cancer, renal cell carcinoma | APX005M nivolumab | I |
|        |        |              | NCT04331067 | Triple-negative breast cancer | Nivolumab | Ib/II |
|        |        |              | NCT03158272 | Advanced malignancy | Nivolumab | I |
|        |        |              | NCT02526017 | Advanced solid tumors | Nivolumab | I |
|        |        | Hoffman La Roche | NCT02323191 | Advanced solid tumors | Atezolizumab | I |
|        | Emactuzumab (RO5509554) | Hoffman La Roche | NCT02760797 | Advanced solid tumors | RO7009789 | I |
|        |        |              | NCT01494688 | Advanced solid tumors | Paclitaxel | I |
|        |        |              | NCT03708224 | Advanced head and neck squamous cell carcinoma | Atezolizumab | II |
|        |        |              | NCT03193190 | Pancreatic ductal adenocarcinoma | Additional therapies | I/II |
|        |        | Turning Point Therapeutics, Inc. | NCT03993873 | Advanced solid tumor | None | I |
|        |        | Deciphera Pharmaceuticals LLC | NCT04242238 | Sarcoma | Avelumab | I |
|        |        |              | NCT03696469 | Advanced malignant neoplasm | None | I & II |
|        | Q702 | Qurient Co., Ltd. | NCT04648254 | Solid tumor | None | I |
|        | SNXD-6532 | Syndax | NCT03238027 | Solid tumor | Durvalumab | I |
|        |        |              | NCT04301778 | Unresectable intrahepatic cholangiocarcinoma | Durvalumab | II |
|        | CD47 | Magrolimab (HuSF9-G4) | NCT02211609 | Solid tumor | None | I |
|        |        | Gilead Sciences | NCT03248479 | Hematological Malignancies | Azacitidine | I |
|        |        |              | NCT02678338 | Acute myeloid leukemia, myelodysplastic syndrome | None | I |
|        |        |              | NCT03527147 | Non-Hodgkin's lymphoma | AZD9150 acalabrutinib AZD6738 rituximab AZD5153 | I |
|        |        |              | NCT04599634 | B-cell malignancies | Obinutuzumab venetoclax | I |
| Target  | Agent               | Organization                         | ClinicalTrials.gov Identifier | Tumors                                                                 | Other interventions                                                                 | Phase |
|---------|---------------------|--------------------------------------|-------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------|
|         |                     |                                      |                               |                                                                       |                                                                                      |       |
|         |                     |                                      | NCT02953782                   | Advanced solid malignancies and colorectal carcinoma                    | Cetuximab                                                                            | I     |
|         |                     |                                      | NCT03558139                   | Ovarian cancer                                                          | Avelumab                                                                             | I     |
|         |                     |                                      | NCT03248479                   | Hematological malignancies                                              | Azacitidine                                                                          | I     |
|         |                     |                                      | NCT04541017                   | T-cell lymphoma                                                         | Mogamulizumab                                                                        | I/I   |
|         |                     |                                      | NCT03922477                   | Acute myeloid leukemia                                                  | Atezolizumab                                                                          | I     |
|         |                     |                                      | NCT04435691                   | Acute myeloid leukemia                                                  | Azacitidine, venetoclax                                                               | I/I   |
|         |                     |                                      | NCT03869190                   | Urothelial carcinoma                                                    | Atezolizumab, enfortumab, vedotin, niraparib                                         | I/I   |
|         |                     |                                      | NCT02953509                   | Non-Hodgkin lymphoma                                                    | Rituximab, gemicitabine, oxaliplatin                                                 | I/I   |
|         |                     |                                      | NCT04313881                   | Myelodysplastic syndromes                                               | Azacitidine                                                                          | III   |
|         |                     |                                      | NCT02890368                   | Solid tumors and mycosis fungoides                                     | PD-1/PD-L1 inhibitor, pegylated interferon-α2a, radiation, talimogene laherparepvec | I     |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      | NCT04328831                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT04338659                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT04257617                   | Locally advanced solid tumor                                            | None                                                                                 | I     |
|         |                     |                                      | NCT02367196                   | Hematologic neoplasms                                                  | Rituximab                                                                            | I     |
|         |                     |                                      | NCT04097769                   | Advanced solid tumor                                                    | None                                                                                 | I     |
|         |                     |                                      | NCT03717103                   | Advanced malignancies                                                   | Rituximab                                                                            | I     |
|         |                     |                                      | NCT03763149                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT03512340                   | Advanced solid cancers, hematologic cancers                             | None                                                                                 | I     |
|         |                     |                                      | NCT04349969                   | Neoplasms malignant                                                    | None                                                                                 | I     |
|         |                     |                                      | NCT04306224                   | Solid tumor, lymphoma                                                   | None                                                                                 | I     |
| CCR2    | BMS-813160           | Bristol-Myers Squibb                 | NCT03184870                   | Colorectal/pancreatic cancer                                            | Chemotherapy or nivolumab                                                            | I/I   |
|         |                     |                                      | NCT03496662                   | Pancreatic cancer                                                       | Nivolumab abraxane, gemicitabine                                                    | I/I   |
|         |                     |                                      | NCT03767582                   | Pancreatic cancer                                                       | Radiation therapy, nivolumab, GVAX                                                   | I/I   |
|         |                     |                                      | NCT04123379                   | Non-small-cell lung cancer, hepatocellular carcinoma                    | Nivolumab, BMS-986253                                                                | II    |
|         |                     |                                      | NCT02996110                   | Advanced cancer                                                         | Nivolumab, ipilimumab, relatlimab, BMS-98620S                                      | II    |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
| AL176   | Arch Oncology        |                                      | NCT02834948                   | Solid tumor                                                             | Paditaxel                                                                            | I/I   |
|         |                     |                                      | NCT04445701                   | Multiple myeloma                                                        | Bortezomib, dexamethasone                                                            | I/I   |
|         |                     |                                      | NCT04328831                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT04338659                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT04257617                   | Locally advanced solid tumor                                            | None                                                                                 | I     |
|         |                     |                                      | NCT02367196                   | Hematologic neoplasms                                                  | Rituximab                                                                            | I     |
|         |                     |                                      | NCT04097769                   | Advanced solid tumor                                                    | None                                                                                 | I     |
|         |                     |                                      | NCT03717103                   | Advanced malignancies                                                   | Rituximab                                                                            | I     |
|         |                     |                                      | NCT03763149                   | Advanced malignancies                                                   | None                                                                                 | I     |
|         |                     |                                      | NCT03512340                   | Advanced solid cancers, hematologic cancers                             | None                                                                                 | I     |
|         |                     |                                      | NCT04349969                   | Neoplasms malignant                                                    | None                                                                                 | I     |
|         |                     |                                      | NCT04306224                   | Solid tumor, lymphoma                                                   | None                                                                                 | I     |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
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|         |                     |                                      |                                |                                                                       |                                                                                      |       |
|         |                     |                                      |                                |                                                                       |                                                                                      |       |
Macrophages are a double-edged sword in the tumor microenvironment. As a prominent component of tumor stromal cells, macrophages can gather around blood vessels, induce angiogenesis, and promote tumor invasion. On the other hand, they could also phagocytose cancer cells and remodel the tumor microenvironment.

Fortunately, the polarization of macrophages can be repolarized. The transformation from M2- to M1-phenotype macrophages is sufficient to cause a tumor-suppressive effect.\(^{194-196}\) Of note, the polarization of macrophages is independent of T cells, while M1 macrophages are able to induce Th1 immune responses, and M2 macrophages can...
trigger Th2 immune responses. This provides an opportunity to target macrophages in cancer immunotherapy. More importantly, the direction of macrophages to T or B cells does not rely on the existence of tumor-specific antigens. While IFN-γ from M1 macrophages is an incentive for Th1 responses, TGF-β and IL-10-derived M2 macrophages cause the generation of Treg cells. Trogocytosis is a process in which a tumor-derived antigen is transferred to Fcy receptor-expressing lymphocytes with the help of certain antibodies. It has been demonstrated that tumor cells decrease the expression of specific antigens by delivering them to CAR-T cells or NK cells, leading to fratricide T cells or NK cells.

Trogocytosis has also been discovered between tumor cells and macrophages and is partially responsible for tumor immune escape. However, Velmurugan et al. reported that persistent trogocytosis of macrophages eventually leads to the killing of antibody-opsonized tumor cells. They explained that these discrepancies might be caused by limited contact time between two types of cells and the lack of competing endogenous antibodies under physiological conditions. Moreover, macrophages are capable of presenting antigens. Proteins that have been passed to the plasma membrane by trogocytosis might be more likely to be processed and presented than cytotoxic proteins.

In addition, as mentioned above, macrophages from different sources may exert different functions. This offers an opportunity for more accurately targeted immunotherapy. For example, CCR2‘Ly6C’ inflammatory monocytes can be recruited to pulmonary metastasis sites by CCL2 secreted by tumor cells and then differentiate into Ly6C’ macrophages that promote metastasis. Selectively targeting this group of monocytes may reduce metastasis without damaging the homeostasis maintaining functions of residual macrophages. Macrophages also have advantages in certain types of cancer. Approximately 20% of nonparenchymal cells in the liver are macrophages. Macrophages in different locations function differently. By stimulating adaptive immune responses, they exert tumoricidal or protumoral and, in general, protumoral functions. It has been summarized in a previous review that targeting pathogenic macrophages is a promising option for patients with liver disease. Moreover, ascites is a common pathological phenomenon in liver cancer that is often accompanied by a poor prognosis. Integrated single-cell RNA sequencing revealed that lymphocytes in ascites are similar to those in peripheral blood, while myeloid cells in ascites are more likely to originate from tumor-infiltrating myeloid cells. This notion was further confirmed by RNA velocity and phylogenetic trees of macrophages from various tissues. According to this study, intratumoral macrophage-based immunotherapy for hepatocellular carcinoma can not only resolve tumor burden in situ but also relieve ascites.

Thus, macrophages provide a force to be considered in tumor immunotherapy. Research on macrophages might open a new door for oncotherapy. To address various malignancies, more strategies based on or combined with macrophages need to be explored in the future.

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AUTHOR CONTRIBUTIONS

Z.D. wrote the paper and Y.L. revised it.

ADDITIONAL INFORMATION

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