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Tumor-associated macrophages: potential therapeutic strategies and future prospects in cancer

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ABSTRACT

Macrophages are the most important phagocytes in vivo. However, the tumor microenvironment can affect the function and polarization of macrophages and form tumor-associated macrophages (TAMs). Usually, the abundance of TAMs in tumors is closely associated with poor prognosis. Preclinical studies have identified important pathways regulating the infiltration and polarization of TAMs during tumor progression. Furthermore, potential therapeutic strategies targeting TAMs in tumors have been studied, including inhibition of macrophage recruitment to tumors, functional repolarization of TAMs toward an antitumor phenotype, and other therapeutic strategies that elicit macrophage-mediated extracellular phagocytosis and intracellular destruction of cancer cells. Therefore, with the increasing impact of tumor immunotherapy, new antitumor strategies to target TAMs are now being discussed.

INTRODUCTION

Macrophages are the most important phagocytes in vivo and play a role in engulfing cellular debris, bacteria, intracellular parasites, aging and abnormal cells, cancer cells and apoptotic cells.[1] Macrophages exist in nearly all tissues and organs (figure 1) and serve as the first line of defense against exogenous and endogenous damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns.[2] In 1883, Elie Metchnikoff published a key paper describing phagocytic cells in frogs. His descriptions were not only about phagocytes involved in host defense, but also described how these specialized cells eliminated degenerating or dying cells of the very same host during metamorphosis.[3] In 1905, his findings suggested that macrophages from infected animals could promote the ability of killing bacteria, thereby proposing the basis of the concept of macrophage activation.[4] Thus, the mechanisms by which macrophages kill bacteria have been gradually revealed after six decades of research.[5 6] In the 1930s, Ebert and Florey found that monocytes in the blood migrated to different tissues and organs to differentiate into macrophages.[7] In 1968, researchers discovered the presence of macrophage precursor cells in bone marrow, a discovery that further developed the mononuclear phagocyte system theory, which was confirmed and put forward formally as the first systematic theory on the origin of macrophages in 1972.[8 9] North and Mackaness found that cytokines alone could cause inflammation even in the absence of pathogens.[10] Rosenstreich *et al* also found that lymphocytes are the most important cells causing the antimicrobial response of macrophages.[11] Subsequently, the role of interferon-γ (IFN-γ) secreted by lymphocytes as a bridge between lymphocytes and macrophages was discovered, as was the transformation of resting macrophages to macrophages with increased antibacterial and regulatory phagocytosis capacities and secretion of proinflammatory cytokines; macrophages with this activated phenotype were officially named 'classically activated macrophages' or M1 macrophages, and this recognition of macrophage subtypes represented a first and important step in the study of macrophage polarization.[12] Over the next 30 years, the study of macrophage polarization made rapid progress. In 1989, with the finding of Th1 and Th2 cells, it was found that interleukin-4 (IL-4) secreted by Th2 cells could polarize macrophages into a phenotype different from the M1 type.[13] When macrophages are activated by IL-4, their respiratory burst is suppressed, and the expression of Major Histocompatibility Complex (MHC) II is enhanced significantly; concomitant upregulation of the mannose receptor was proved in later studies.[14] Combined with these characteristics, the concept of 'alternatively activated macrophages' was first proposed in 1992.[15] Based on the plasticity and adaptability of macrophages in response to different environments, Mosser and Edwards proposed that M1 and M2 were the two extremes in

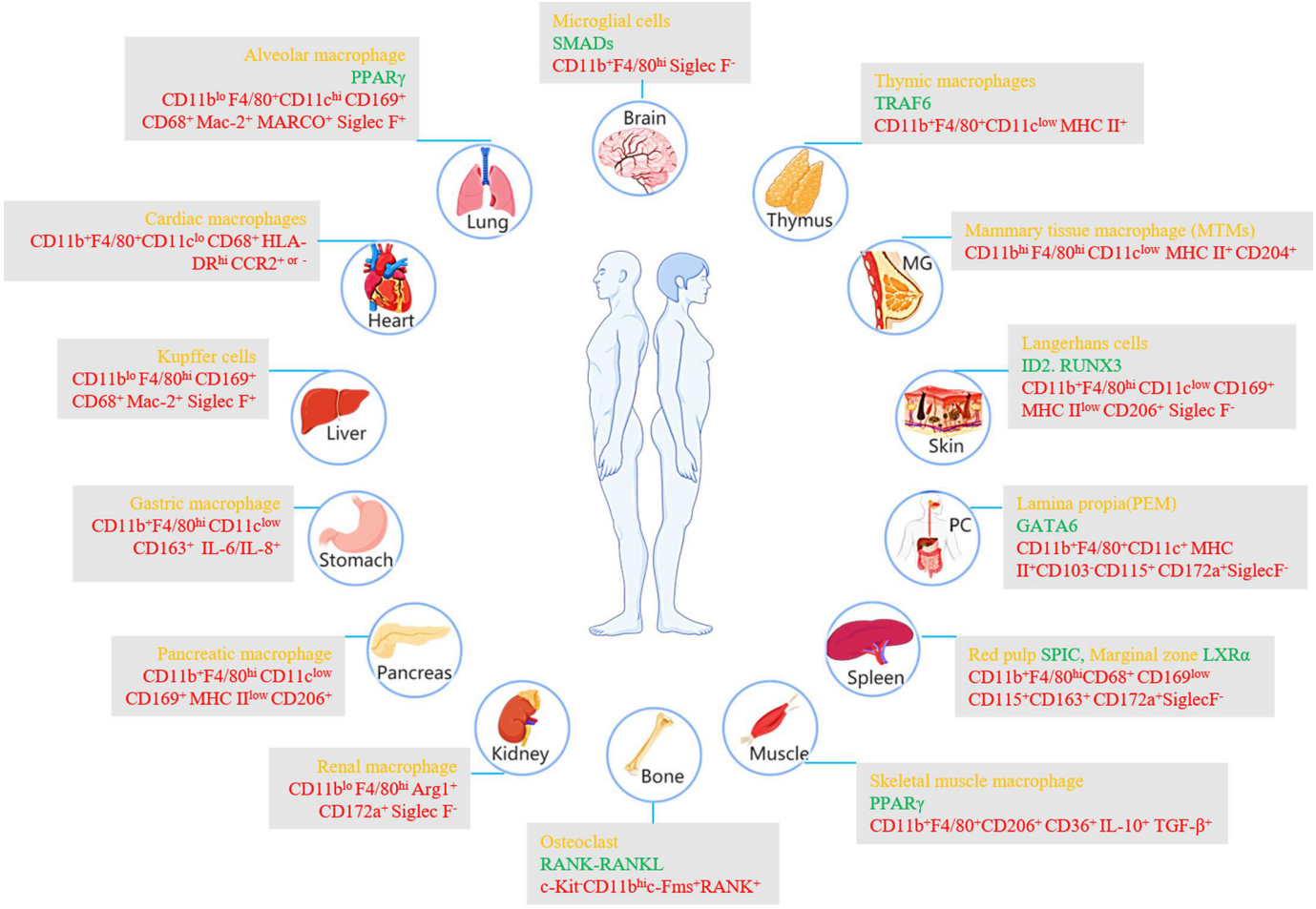

Figure 1 The distribution of macrophages in different tissues and organs. Macrophages are heterogeneous, showing different names, specific transcription factors and markers. Here, different colors correspond to different items, yellow for names, green for transcription factors and red for markers. IL-6, interleukin 6; MG, mammary gland; PC, peritoneal cavity; TGF-ß, transforming growth factor-β.

the polarization process of macrophages.[16] In 2010, the concept of macrophage polarization was modified again with the presentation of M2-like macrophages that were stimulated to transform into yet different phenotypes by immune complexes (M2b phenotype) or IL-10, transforming growth factor-β (TGF-β), and glucocorticoids (M2c phenotype), among others.[17] These special environmental factors trigger switches in the phenotype and function of macrophages, allowing them to play different roles under different stimuli and to change dynamically between the two extremes of the M1 and M2 phenotypes[18] (figure 2).

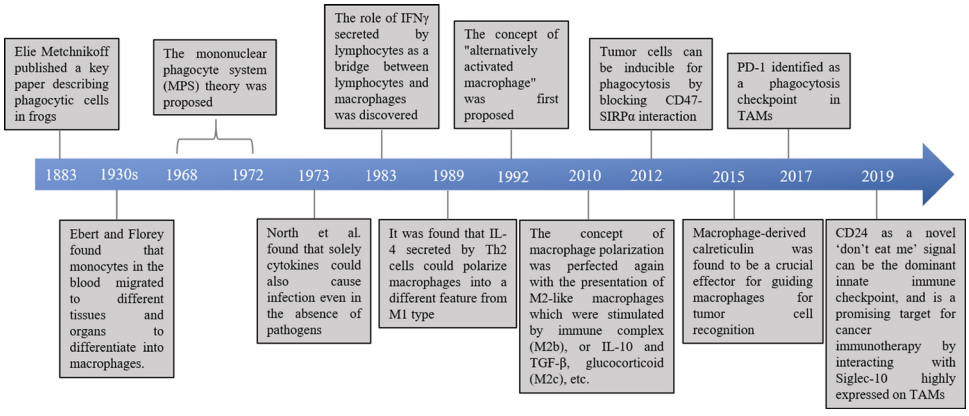

Figure 2 History of macrophages in cancer. Advances made over the past decades in the identification of macrophages including checkpoints and stimulatory signals. IFNγ, interferon-γ; IL-10, interleukin 10; PD-1, programmed cell death protein 1; SIRPα, signal regulatory protein α; TAMs, tumor-associated macrophage; TGF-β, transforming growth factor-β.

Macrophage origin

Macrophages exist in nearly all healthy adult tissues, deriving from either an embryonic precursor (yolk sac or fetal liver) before birth or a monocyte precursor of hematopoietic origin in adults.[19 20] In the brain, lung and liver, embryonically derived macrophages can be maintained by self-renewal of tissue-resident macrophages in adults, while in the gut, skin, heart and pancreas, most subsets are progressively maintained through the differentiation of monocyte precursors from hematopoietic stem cells (HSCs).[21] During myocardial infarction, cardiac-resident macrophages can be replenished by monocytes.[22–25] The Ly6C^{high} monocyte cells are plentifully recruited to the infarct area from the bone marrow and spleen through monocyte chemoattractant protein-1 (MCP-1)/CCR2 chemokine receptor interaction.[26–29] However, the Ly6C^{low} monocytes are recruited through CX3 chemokine receptor 1 (CX3CR1) into the infarcted area.[29]

Due to the development of labeling of single cells for in vivo cell fate mapping, research on the origin of tissue-resident macrophages (TRMs) has seen recent advances.[30] Studies have shown that TRMs exist during embryonic development and are independent of the circulating monocytes in the blood. In the first trimester, macrophages first appear in the yolk sac between embryonic day 6.5 and embryonic day 8.5 (E6.5-E8.5). Then (E8.5-E10.5), HSCs appear in the aorta-gonad-mesonephros region and determine the immune cell lineages. At E10.5, HSCs migrate to the fetal liver, which becomes the main hematopoietic organ during subsequent embryonic development.[31] Until the perinatal stage, traditional bone marrow stem cells are the predominant hematopoietic cells and complement the immune cell lineages. All adult macrophages, resident or infiltrating, are progenies of classical HSCs with the exception of microglia and some epidermal Langerhans cells, which are yolk sac-derived.[32] We consider blood monocytes as tissue-macrophage progenitors because the major fraction of macrophages originates from blood-borne monocytes. Under specific circumstances, the egress of monocytes from blood to inflamed tissue is dependent on both CCR2 and CX3CR1.[33] Defining the origins and developmental pathways of TRMs should help refine our understanding of the role of these cells in various disease settings. However, the exact differentiation pathways of the embryonic progenitors that give rise to adult TRMs are still controversial, and the mechanisms of macrophage maintenance in adult tissue are undefined. Tumor-associated macrophages (TAMs) mainly originate from bone-marrow-derived monocytes[34–36] although local proliferation has been observed in some mouse tumors.[37] Chemokines (eg, CCL2 (MCP-1), CCL3 (macrophage inflammatory protein (MIP)1α), CCL4 (MIP1β) and CXCL12 (stromal cell-derived factor 1α)) and colony-stimulating factor (CSF-1) are major determinants of monocyte infiltration in tumor microenvironment (TME), as well as IL-6 and IL-1β, and vascular epidermal growth factor A (VEGFA).[38 39] Besides, the complement cascade also have been described to have a role in recruiting macrophage.[40 41] In these cases, the major recruitment factor is the chemokine CCL2, produced mostly by tumor cells, which acts through CCR2 expressed on classical monocytes.[34 38 42–44] However, other studies suggest that in pancreatic cancer and glioma, TAMs can also originate from yolk sac and fetal liver,[45–50] both recruited monocyte-derived TAMs (MoD-TAMs) and tissue-resident interstitial TAMs (Res-TAMs) can acquire different functions depending on cancer type. In humans, the breast and endometrial TAMs have a completely different transcriptional landscape and marker profile from TRMs and from each other,[51] suggesting that different niches can activate TAMs in a tumor-specific and tissue-specific way. These observations reinforce the idea that the TAMs definition should not be used just to identify bone marrow-derived macrophages that infiltrate the tumor, but it should be extended to all macrophages that play a role within the TME, including TRMs.[52] Res-TAMs in mouse lungs contribute to the pool of TAMs together with CCR2-dependent recruited MoD-TAMs. Res-TAMs largely correlate with tumor growth, while MoD-TAMs accumulation is associated with enhanced tumor spreading. Both subsets can be depleted after chemotherapy, but MoD-TAMs rapidly recover and perform phagocytosis-mediated tumor clearance.[53] Therefore, in a particular tumor, understanding the origin, function and types of TAMs is critical to the selection of targeting TAMs strategies.

Functional classifications

In contrast with the MPS theory, the current dominant view is that macrophages can be divided into two functional categories: classically activated macrophages (M1) and alternatively activated macrophages (M2), which work on two major lymphocyte subpopulations, Th1 and Th2 cells and have diametrically contrasting functions according to the pattern of cytokines they secrete (figure 3). M1 macrophages, also known as inflammatory macrophages, are mainly activated by IFN-γ secreted by Th1 cells, Cytotoxic T Lymphocytes (CTLs) and natural killer (NK) cells; TNF-α; HMGB1[54]; lipopolysaccharide (LPS),[55] a component of the outer membrane of Gram-negative bacteria and granulocyte-macrophage CSF (GM-CSF) produced through activation of nuclear factor-kappa B (NF-κB), signal transducer and activator of transcription 1 (STAT1) NFAT5,[56 57] and others; these cells show the an enhanced capacity for antigen presentation and phagocytosis and release many proinflammatory factors, including TNF-α, IL-1β, IL-12 and IL-18, nitric oxide (NO), IL-12, the intracellular protein NOS2 and suppressor of cytokine signaling 3 (SOCS3), and thus participate in the type I immune response.[58] Phenotypically, M1 macrophages express high levels of MHC II and CD68, as well as the costimulatory molecules CD80 and CD86 (figure 3).[59] In liver macrophages, glycogen synthase kinase 3β (Gsk3β) can promote innate proinflammatory immune activation by restraining

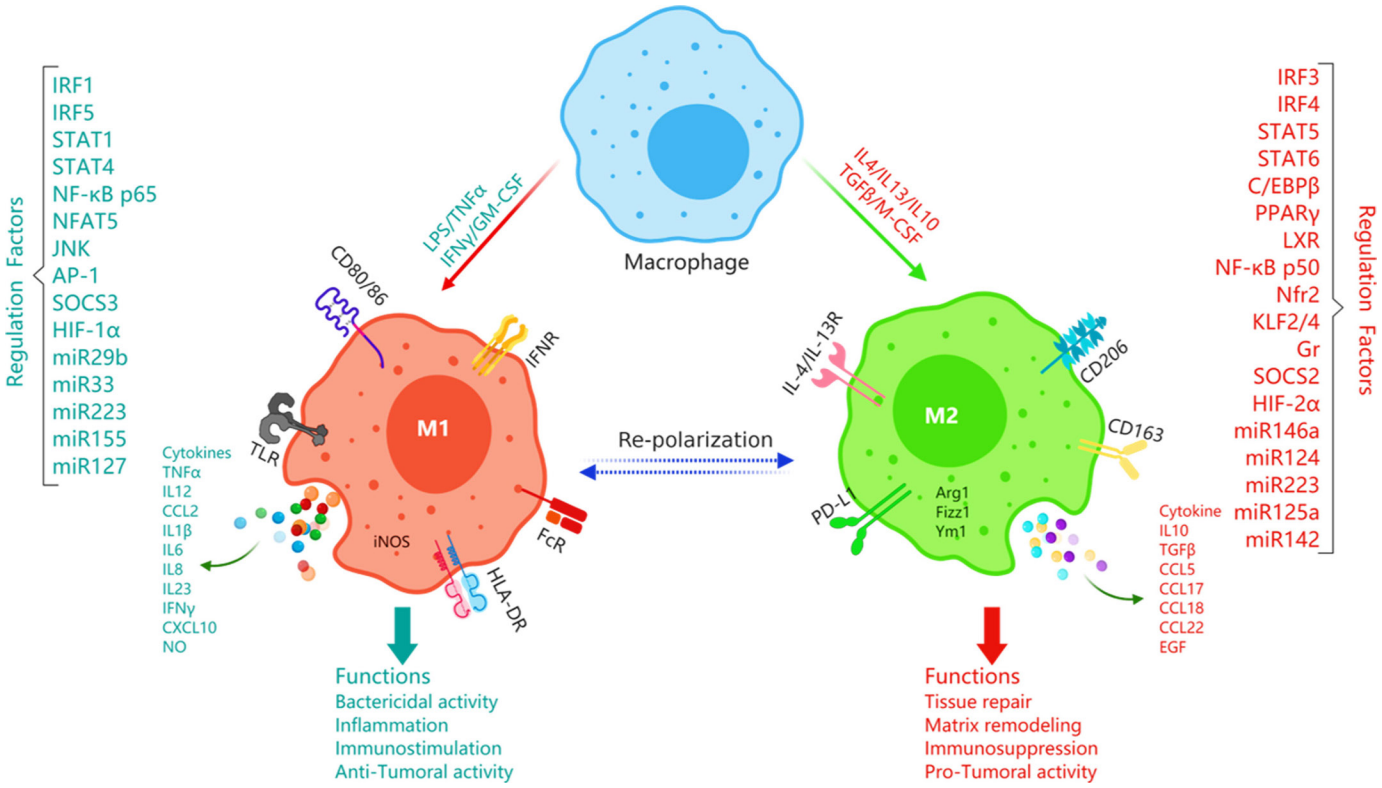

Figure 3 Macrophages can be polarized into M1 and M2 macrophages with different mechanisms. Macrophages can be polarized into two functional categories: classically activated macrophages (M1) and alternatively activated macrophages (M2) under different stimuli through different transcription factors, and show distinct specific markers on the macrophage subsets, which play important roles in pro-inflammation or anti-inflammation. FcR, Fc receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL10, interleukin 10; LPS, lipopolysaccharide; miRNA, microRNA; NF-κB, nuclear factor-kappa B; STAT1, signal transducer and activator of transcription 1; TGF-ß, transforming growth factor-β; TLR, toll-like receptor; TNFα, tumor necrosis factor-α.

AMPK activation.[60] M2 macrophages, also known as anti-inflammatory macrophages, are mainly activated by IL-4, IL-13, CSF-1, IL-10, TGF-β and helminth infections through activation of STAT6, peroxisome proliferator-activated receptor γ (PPARγ), SOCS2. (figure 3), and produce many anti-inflammatory factors, including IL-10, TGF-β and arginase 1, participating in the type II immune response, which plays a central role in the response to parasites, tissue remodeling, angiogenesis and allergic diseases.[61] Phenotypically, M2 macrophages are characterized by the expression of macrophage mannose receptor (CD206).[62–64] CD163 has also been suggested as an M2 marker, while CD163 is an M2 macrophage marker associated with the transcription factor c-Maf in human tissue; thus, CD163 cannot be recommended as an M2 marker alone.[65] c-Maf controls many M2-related genes, has direct binding sites within a conservative noncoding sequence of the csf-1r gene and promotes M2-like macrophage-mediated T cell suppression and tumor progression.[66] Macrophage galactose-type C-type lectin 1 (MGL1) and MGL2 are also expressed in M2 macrophages on stimulation.[67] Response gene to complement 32 (RGC-32) is a cell cycle regulator expressed in many cells, including macrophages but not monocytes. The absence of RGC-32 does not affect monocyte differentiation to macrophages; however, under M-CSF or IL-4 stimuli, RGC-32 has a relevant role in promoting M2 polarization, and its level of expression still increases M2 macrophages.[68] In mouse models, some characteristic profiles of M2 macrophages have been reported: MMR (Mrc1), arginase 1 (Arg1), resistin-like molecule α (FIZZ1) and chitinase-like protein Ym 1 were shown to be upregulated, especially in allergic asthma.[69] Arg1 expression, a hallmark of M2 macrophages, depends on IL-4 and IL-13 and is a direct consequence of STAT6 activation.[70] The NF-κB p50 subunit, IRF4 and PPARγ have been proposed to enhance the M2 phenotype.[71] In addition, macrophages exhibit different phenotypic characteristics in different tissues (figure 1).

Tumor-associated macrophages (TAMs), as a specialized phenotype of M2-like macrophages, are phagocytic cells with unclear origins (figure 4), while TAMs originating from circulating $CCR2^{+}$ monocytes can alter the TME through endocytic collagen turnover as they are centrally engaged in tumor-associated collagen degradation.[72–74] Although TAMs share some patterns of M1 and M2 macrophages, these cells have a unique transcriptional profile distinct from M1 or M2 macrophages. Some features of TAMs resemble M2 polarization, such as high production of IL-10 and TGF-β.[75 76] In most cases, impaired macrophage accumulation in the TME is associated with control

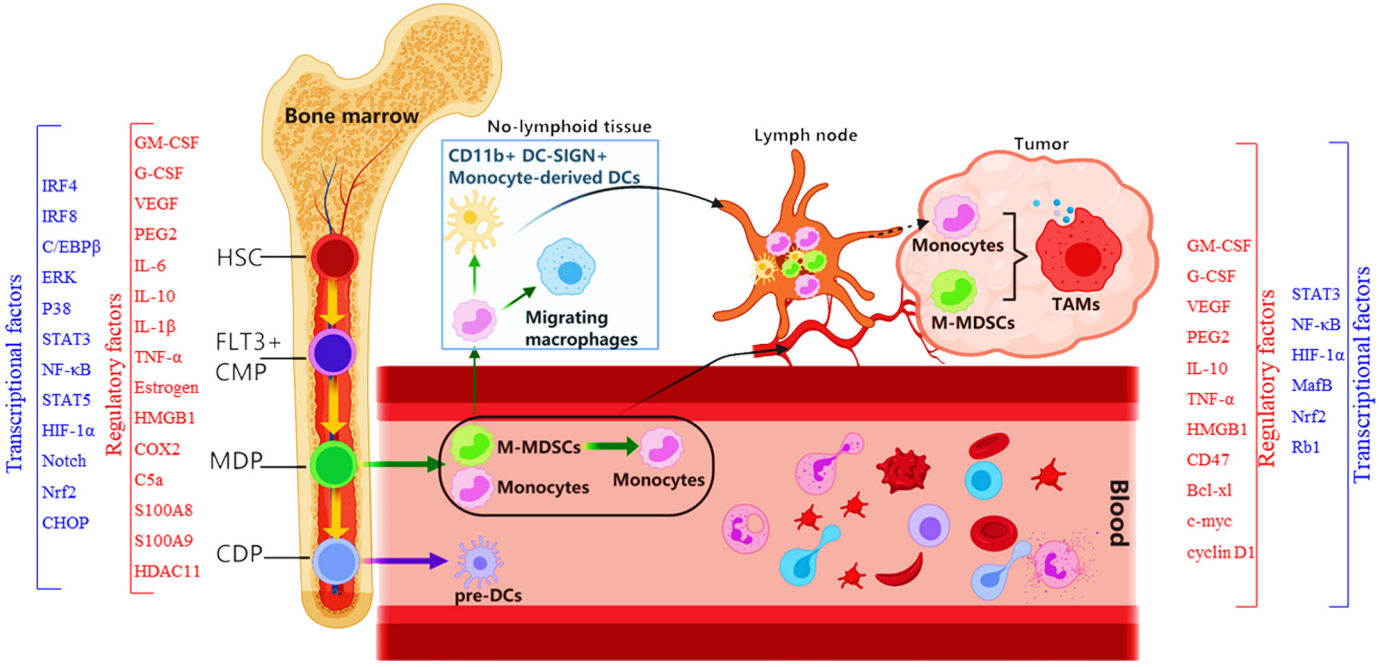

Figure 4 Overview of macrophages involvement in myeloid cell differentiation in cancer through blood circulation. Macrophages development, accumulation, suppressive activity and survival are controlled by a complex network of transcription factors, cytokines and non-cytokine immune regulatory factors. Monocytes and M-MDSCs originate from the common myeloid progenitor (CMP) cell in the bone marrow (also in the spleen of mice) during myelopoiesis (left). They can circulate in the blood and lymph node and home to sites of inflammation and to the solid tumors (right). Under different conditions such as the tumor microenvironment, a variety of factors promote cancer risk, facilitate cancer onset and progression, and polarize TAMs. DCs, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-10, interleukin; NF-κB, nuclear factor-kappa B; MDSCs, Myeloid-derived suppressor cells; TAMs, tumor-associated macrophages; TNFα, tumor necrosis factor-α; VEGF, vascular epidermal growth factor.

of the tumor and reduced metastasis, suggesting a major role of TAMs in cancer.[77] There is no exact information on how a monocyte precursor can generate TAMs sharing markers of both M1 and M2 macrophages. In regard to this, Movahedi *et al* reported that the tumor-infiltrating monocyte pool was predominantly $Ly6C^{+}CX3CR1^{low}$ and suggested that $Ly6C^{high}$ monocytes were direct precursors of TAMs.[78] Then, they subdivided TAMs into two groups according to the expression of MHC II and the suppression of T cell proliferation: (1) MHC II^{high} TAMs were found to suppress proliferation using an inducible Nitric Oxide Synthase (iNOS)-dependent pathway and (2) MHC II^{low} TAMs suppressed proliferation via an iNOS-independent pathway. However, according to the origin of the macrophage precursor cells, TAMs can be newly recruited MoD-TAMs, which are mostly generated in a CCR2-dependent manner, and TAMs derived from tissue-resident cells (ResTAMs) or embryonic origin TAMs (EmD-ResTAMs), which locally self-maintain without the contribution of adult hematopoiesis and accumulate with tumor expansion in lung tumors.[78] In mice, the growth of breast tumors induces the accumulation of TAMs, which differ in phenotype and function from mammary tissue macrophages.[34 79] TAMs express the adhesion molecule Vcam1 and proliferate when they differentiate from inflammatory monocytes but do not transform into the M2 phenotype through the Notch signaling transcription regulator RBPJ.[34 80] It is worth mentioning that Notch mediates the expression of IL-1β and CCL-2 in tumor cells, TAM recruitment and TGFβ-mediated activation of tumor cells by TAMs in basal-like breast cancer.[81] Thus far, it is not entirely clear how the cascade of events generating TAMs is orchestrated. Resting TAMs are often characterized by high expression of CCL2, CCL5[82] and IL-10 and surface markers, including MGL1, MGL2, dectin-1, CD81, MHC II and macrophage scavenger receptor 1, which can facilitate M2-like polarization by enhancing mitochondrial oxidative phosphorylation (OXPHOS) by activating the phosphoinositide 3-kinase (PI3K)/AKT/GSK3β/β-catenin pathway.[83] As previously mentioned, CCR2 depletion plays a driving role in shaping the TME as it leads to largely reduced infiltration of TAMs but strong infiltration of CTLs. In $CCR2^{-/-}$ mice engrafted with colorectal cancer, decreased infiltration of TAMs is associated with reduced tumor burden along with altered extracellular matrix (ECM) composition. It has been described that the TAM activation pathway enhances IRF3 and STAT1 and the release of CCL2, CCL3, CCL5 and IL-10, as well as other molecules, such as PGE2 and VEGF. In murine PDAC models, both inflammatory monocytes and tissue-resident macrophages were identified as sources of TAMs.[45] Moreover, TAM-released pyrimidines inhibited gemcitabine through molecular competition at the level of drug uptake and metabolism.[84] Unexpectedly, significant

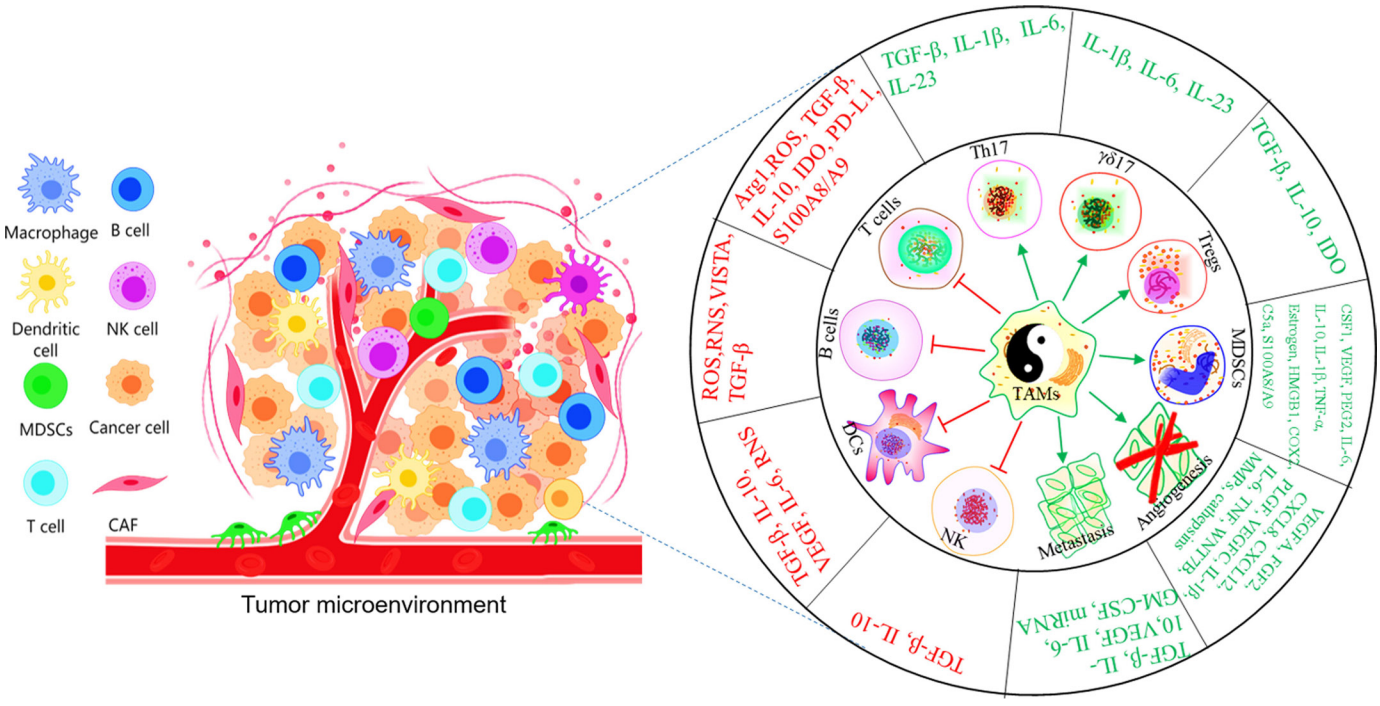

Figure 5 Immunoregulatory effects of TAMs. TAMs in TME can exert the immune regulatory roles on the different immune cells with different mechanisms by producing a variety of cytokines and effector molecules. On the one hands, TAMs inhibit T cell, B cells, NK cells and DCs. On the other hands, TAMs can promote Tregs, Th17 cells, γδT cells and MDSCs, as well as angiogenesis and metastasis of tumor. DCs, dendritic cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-10, interleukin; MDSCs, Myeloid-derived suppressor cells; NK, nuclear factor-kappa B; PD-1, programmed cell death protein 1; TAMs, tumor-associated macrophages; TGFβ, transforming growth factor-β; TME, tumor microenvironment; TNFα, tumor necrosis factor-α; VEGF, vascular epidermal growth factor.

portions of pancreas-resident macrophages were found to originate from embryonic development and to expand through in situ proliferation during tumor progression. Whereas MoD-TAMs played more potent roles in antigen presentation, EmD-ResTAMs exhibited a profibrotic transcriptional profile, indicative of their role in producing and remodeling molecules in the ECM.[45] Whether this assumption can be generalized to other models deserves further study, and the generalizability in human tumors is even more hypothetical due to the lack of knowledge of macrophage ontogeny.[53] TAMs contribute to tumor progression at different levels: by promoting genetic instability, nurturing cancer stem cells, supporting metastasis and taming protective adaptive immunity.[85] TAMs are critical players in the crosstalk between cancer cells and their microenvironment, contribute to all aspects of tumor progression and are often associated with poor prognosis in cancer patients.[86 87] These cells have been poorly categorized. However, there is experimental evidence that TAMs appear to share M1 and M2 polarization signatures. In general, TAMs affects tumor progression in the following ways[88–90]: (1) TAMs can promote the proliferation of tumor cells by producing growth factors, cytokines and chemokines including basic fibroblast growth factor-2, TGF-β, platelet derived growth factor (PDGF), IL-10, CXCL and VEGF, which not only promotes cell division directly but also indirectly accelerate this process by promoting angiogenesis. (2) TAMs can promote tumor angiogenesis by secreting cytokines, including VEGF, COX-2, and PDGF. In addition, under hypoxia, TAMs upregulate hypoxia-inducible transcription factors and activate expression programs that appear to be proangiogenenic, protumor growth, prometastatic and immunosuppressive.[91] (3) TAMs is involved in tumor invasion and metastasis by producing several enzymes which can degrade the ECM. Such enzymes include several metalloproteinases (eg, MMP-2, MMP-7, MMP-9 and MMP-12) as well as urokinase-type plasminogen activator that degrade the ECM. Dissolution of the ECM leads to cleavages through which tumor cells can evade and metastasize.[79 92 93] (4) TAM mediates immunosuppression, shape and remodel tumor immune microenvironment (TIME), and is involved in tumor immune escape[94 95] (figure 5). In TIME, TAMs inhibits the immune microenvironment and plays an immunosuppressive role by secreting chemokines and cytokines, such as IL-10, TGF-β and IDO1, and recruit Tregs to tumor sites, which promote the progression of cancer.[76 96–98] TAMs can also inhibit T cells by L-arginine depletion through arginase-1 activity, which decreases the expression of the T-cell receptor CD3ζ chain and impairs T-cell responses.[99 100] Additionally, TAMs is involved in tumor immune escape, for example, CD24 on the surface of tumor cells interacts with Siglec-10 on the surface of TAMs to promote the immune escape of tumor cells.[101–103] It is worth noting that chemotherapy will increase the infiltration of TAMs

into tumor tissues, and the combination of TAM-targeted drugs with chemotherapy can improve the therapeutic effect of chemotherapeutic drugs.[104]

THERAPEUTIC STRATEGIES TARGETING TAMS

As an important component in the TME, TAMs show high plasticity.[105] To date, some therapeutic strategies targeting macrophages in animal models and clinical trials have been proposed (all therapeutic strategies targeting TAMs in clinical trials are included in table 1), including reducing or depleting TAMs, repolarizing TAMs toward M1-like macrophages, blocking the inhibitory receptors (immune checkpoints) on TAMs, blocking 'don't eat me' signals, and other potential strategies targeting TAMs (figure 6).

Reducing or depleting TAMs

CSF1/CSF1R signaling pathway

Targeting the CSF1/CSF1R signaling pathway is another important effective strategy for treating malignant cancer. Currently, CSF1 is recognized as a classic tumor-stimulating factor that recruits macrophages to the tumor site and promotes the polarization of TAMs.[106] Clinically, blocking CSF1R by AMG 820 can significantly reduce the accumulation of immunosuppressive TAMs in solid tumors.[107] The CSF1R c.1085A>G genetic variant causes a change of histidine to arginine in the receptor dimerization domain, which confers sensitivity to CSF-1R inhibitors.[108] Experimentally, BLZ945, a highly selective small molecule CSF-1R inhibitor, can inhibit TAM recruitment in murine breast cancer. In addition, BLZ945 can markedly augment the infiltration of $CD8^+$ CTLs in cervical cancer and breast cancer and inhibit the growth of neuroblastoma.[109] RG7155, a CSF1R monoclonal antibody, can inhibit the activation of CSF1R and cause the death of CSF1-dependent macrophages, which can also significantly decrease the intratumoral number of $CSF1R^+$ and $CD68^+CD163^+$ macrophages, as well as inhibit the growth of several types of cancer.[110] However, studies in mice and clinical trials in humans have shown that it is insufficient to treat tumors using CSF1/CSF1R blockers alone, and the antitumor efficacy was significantly elevated by treatment with a combination of CSF1/CSF1R blockers and chemotherapy or checkpoint inhibitors.[111] In murine PDAC, CSF1/CSF1R blockers can enhance the antigen presentation of macrophages and antitumor T cell responses via inhibition of CSF1R signal transduction; however, programmed cell death protein 1 (PD-1) expressed on these T cells was obviously upregulated, which weakened the antitumor effect of the CSF1R inhibitor. However, CSF1/CSF1R blockers combined with ICB can strengthen antitumor efficiency.[111] In tumors, CSF1 expression correlates with the abundance of $CD8^+$ T cells and $CD163^+$ TAMs. Human melanoma cell lines consistently produce CSF1 after exposure to melanoma-specific $CD8^+$ T cells or T cell-derived cytokines in vitro, reflecting a broadly conserved mechanism of CSF1 induction by activated $CD8^+$ T cells.[112] Mining of publicly available transcriptomic datasets suggests co-enrichment of $CD8^+$ T cells and CSF1 or various TAM-specific markers in human melanoma, which was associated with nonresponsiveness to PD-1 checkpoint blockade in a small patient cohort. The combination of anti-PD1 and anti-CSF1R antibodies induced the regression of transplanted melanoma in mice, a result that was dependent on the effective elimination of TAMs.[112] In addition, the use of CSF1R inhibitors to target TAMs is therapeutically appealing but has shown very limited antitumor effects. One limitation to the effect of CSF1R-targeted therapy is that carcinoma-associated fibroblasts (CAFs) are major sources of chemokines that recruit granulocytes to tumors. CSF1 produced by tumor cells caused HDAC2-mediated downregulation of granulocyte-specific chemokine expression by CAFs, which limited the migration of these cells to tumors.[113] Treatment with CSF1R inhibitors disrupted this crosstalk and triggered a profound increase in granulocyte recruitment to tumors. Combining a CSF1R inhibitor with a CXCR2 antagonist blocked granulocyte infiltration of tumors and showed strong antitumor effects.[113 114]

Targeting chemokine

Targeting chemokines to reduce the infiltration of TAMs into the TME is the main approach used. CCL2 can recruit monocytes expressing CCR2 from peripheral blood to the tumor site, where they further mature into TAMs.[115] The inactivation of serine-threonine kinase 11 or liver kinase B1 (LKB1) can lead to abnormal production of CCL2, while the loss of LKB1 can increase the expression of CCL2 and thereby elevate the density of macrophages in tumors. Thus, the recruitment and infiltration of macrophages into the TME can be blocked by inhibiting the release of CCL2 from tumor and stromal cells or by using small molecule inhibitors of CCR2. Blockade of the CCL2/CCR2 axis as a therapeutic strategy affecting the recruitment of monocytes/macrophages in HCC suppresses murine liver tumor growth by activating the T cell antitumor immune response.[43] Zoledronic acid, a kind of diphosphate compound, can suppress CCL2/MCP-1 production in tumor cells to reduce the infiltration of TAMs and promote the proliferation of CTLs. However, interruption of CCL2 inhibition exacerbates metastasis and accelerates death because of monocyte release from the bone marrow and enhancement of cancer cell mobilization from the primary tumor, as well as blood vessel formation and increased proliferation of metastatic cells in the lungs in an IL-6- and VEGF-A-dependent manner.[39] In addition to CCL2, it is worth mentioning that CCL5, another C-C motif chemokine ligand, can also recruit TAMs and promote the metastasis and recurrence of tumors, which can be limited by the CCL5 receptor antagonist maraviroc and Raf kinase inhibitor protein.[116] Macrophage-derived CCL5 facilitates the immune escape of colorectal cancer cells via the NF-κB p65/STAT3-CSN5-PD-L1 pathway, which is significantly

Table 1 The combination molecules on TAMs of targeted drugs in clinical trials

Targets	Drugs	Cancer type	NCT
CSF1	PD-0360324	▶ Recurrent fallopian tube carcinoma ▶ Recurrent ovarian carcinoma ▶ Recurrent primary peritoneal carcinoma	NCT02948101
	PD-0360324	▶ Advanced cancer	NCT02554812
CSF1R	Edicotinib	▶ Recurrent adult acute myeloid leukemia ▶ Refractory ▶ Acute myeloid leukemia	NCT03557970
		▶ Recurrent adult acute myeloid leukemia ▶ Refractory acute myeloid leukemia	NCT03557970
	TPX-0022	▶ Advanced solid tumor ▶ Metastatic solid tumors	NCT03993873
	Cabiralizumab	▶ Peripheral T cell lymphoma	NCT03927105
		▶ Tenosynovial giant cell tumor	NCT02471716
		▶ Lung cancer ▶ Head and neck cancer ▶ Pancreatic cancer ▶ Ovarian cancer ▶ Renal cell carcinoma ▶ Malignant glioma	NCT02526017
		▶ Advanced melanoma ▶ Non-small cell lung cancer ▶ Renal cell carcinoma	NCT03502330
		▶ Peripheral T cell lymphoma	NCT03927105
	IMC-CS4	▶ Neoplasms	NCT01346358
		▶ Pancreatic cancer	NCT03153410
		▶ Neoplasms	NCT01346358
	SNDX-6352	▶ Solid tumor ▶ Metastatic tumor ▶ Locally advanced malignant neoplasm ▶ Unresectable malignant neoplasm	NCT03238027
		▶ Unresectable intrahepatic cholangio carcinoma	NCT04301778
	BLZ945	▶ Advanced solid tumors	NCT02829723
	ARRY-382	▶ Advanced solid tumors	NCT02880371
		▶ Metastatic cancer	NCT01316822
	Sunitinib	▶ Lymphoma, Non-hodgkin ▶ Multiple myeloma ▶ Advanced solid tumors	NCT02693535
		▶ Metastatic renal cell carcinoma	NCT01265901
	Nilotinib	▶ Malignant solid neoplasms	NCT02029001
	DCC-3014	▶ Sarcoma ▶ Advanced sarcoma ▶ High grade sarcoma ▶ Leiomyosarcoma ▶ Undifferentiated pleomorphic sarcoma ▶ Myxofibrosarcoma ▶ Dedifferentiated liposarcoma	NCT04242238
		▶ Advanced malignant neoplasm ▶ Tenosynovial giant cell tumor, Diffuse	NCT03069469
		▶ Advanced malignant neoplasm ▶ Tenosynovial giant cell tumor, Diffuse	NCT03069469
	PLX73086	▶ Solid tumors ▶ Tenosynovial giant cell tumor	NCT02673736
	RG7155	▶ Solid cancers	NCT02323191
		▶ Neoplasms	NCT02760797
		▶ Fallopian tube adenocarcinoma ▶ Fallopian tube clear cell adenocarcinoma ▶ Fallopian tube endometrioid adenocarcinoma	NCT02923739
		▶ Advanced solid tumors	NCT01494688
		▶ Lymphoma, Non-Hodgkin	NCT03369964

Continued

Table 1 Continued

Targets	Drugs	Cancer type	NCT
CSF-1R TKI	Pexidartinib	▶ Colorectal cancer ▶ Pancreatic cancer ▶ Metastatic cancer ▶ Advanced cancer	NCT02777710
	PLX3397	▶ Giant cell tumors of the tendon sheath ▶ Tenosynovial giant cell tumor	NCT02371369
		▶ Sarcoma ▶ Malignant peripheral nerve sheath tumors	NCT02584647
	NMS-03592088	▶ Acute myeloid leukemia ▶ Chronic myelomonocytic leukemia	NCT03922100
CCR2/CCR5	BMS-813160	▶ Non-small cell lung cancer ▶ Hepatocellular carcinoma	NCT04123379
		▶ Pancreatic ductal adenocarcinoma	NCT03767582
		▶ Pancreatic ductal adenocarcinoma	NCT03496662
		▶ Advanced cancer	NCT02996110
CCR2	MLN1202	▶ Metastatic cancer ▶ Unspecified adult solid tumor,	NCT01015560
	PF-04136309	▶ Metastatic pancreatic ductal adenocarcinoma	NCT02732938
	CCX872-B	▶ Pancreatic cancer	NCT02345408
CCL2	Carlumab	▶ Prostate cancer	NCT00992186
CCL5	Maraviroc	▶ Colorectal cancer ▶ Neoplasm metastasis ▶ Liver metastases	NCT01736813
		▶ Acute leukemia ▶ Chronic myelogenous leukemia ▶ Myelodysplasia	NCT02208037
Clodronate	Clodronate	▶ Breast cancer	NCT00009945
			NCT00127205
			NCT00873808
		▶ Prostatic neoplasms ▶ Multiple myeloma	NCT01198457
		▶ Bone neoplasms	NCT00909142
PI3Kγ	PI3K inhibitor	▶ Lymphoma, small lymphocytic ▶ Lymphoma ▶ Lymphoma, non-hodgkin	NCT04342117
	BYL719	▶ Estrogen receptor-positive breast cancer ▶ HER2-negative breast cancer ▶ Invasive ductal breast carcinoma	NCT01791478
		▶ Stomach neoplasms esophageal neoplasms ▶ Metastatic gastric cancer mutated PI3KCA protein overexpressed HER2 protein	NCT01613950
	BKM120	▶ Metastatic squamous neck cancer with occult ▶ Primary squamous cell carcinoma ▶ Recurrent metastatic squamous neck cancer with occult primary ▶ Recurrent salivary gland cancer	NCT01816984
		▶ Unspecified adult solid tumor	NCT01540253
		▶ Recurrent non-small cell lung cancer ▶ Stage IV non-small cell lung cancer	NCT01723800
		▶ Breast cancer	NCT01629615
	RP6530	▶ Lymphoma, B-Cell ▶ T-cell lymphoma	NCT02017613
PI3Kδ/γ	TGR-1202	▶ Recurrent diffuse large B-Cell lymphoma ▶ Refractory diffuse large B-Cell lymphoma	NCT02874404
	Tenalisib	▶ NHL	NCT03711578
	Duvelisib	▶ Lymphoma	NCT02598570
		▶ T-cell lymphoma ▶ Indolent B-cell lymphoma	NCT04331119
		▶ Hematological malignancy	NCT02711852

Continued

Table 1 Continued

Targets	Drugs	Cancer type	NCT
		▶ Indolent NHL	NCT04038359
		▶ Recurrent chronic lymphocytic leukemia (CLL) ▶ Recurrent small lymphocytic lymphoma (SLL) ▶ Refractory CLL ▶ Refractory SLL	NCT03961672
		▶ CLL	NCT03534323
		▶ Head and neck squamous cell carcinoma	NCT04193293
		▶ Lymphoma ▶ Relapsed/refractory T-cell lymphomas	NCT02783625
		▶ CLL ▶ Recurrent diffuse large B-Cell lymphoma ▶ Refractory diffuse large B-cell lymphoma	NCT03892044
		▶ Peripheral T-cell lymphoma	NCT03372057
		▶ Lymphoma, small lymphocytic ▶ Lymphoma ▶ Lymphoma, non-hodgkin	NCT04342117
		▶ SLL ▶ CLL	NCT04209621
TLR9	Imiquimod	▶ Cervical intraepithelial neoplasia	NCT02130323
			NCT02329171
			NCT00941252
			NCT02669459
			NCT02917746
		▶ Breast cancer ▶ Breast neoplasms	NCT00899574
		▶ Melanoma	NCT01264731
		▶ Superficial basal cell carcinoma	NCT00189306
		▶ Basal cell carcinoma	NCT00129519
			NCT03534947
			NCT00189241
			NCT00463359
			NCT00581425
			NCT01212562
		▶ Metastatic melanoma ▶ Stage IIIB cutaneous melanoma AJCC v7 ▶ Stage IIIC cutaneous melanoma AJCC v7 ▶ Stage IV cutaneous melanoma AJCC v6 and v7	NCT03276832
		▶ Cervical cancer ▶ Precancerous condition	NCT00031759
		▶ Carcinoma, basal cell	NCT00204555
TLR7/8	Resiquimod	▶ Cutaneous T cell lymphoma	NCT01676831
		▶ Melanoma	NCT00470379
		▶ Tumors	NCT00821652
		▶ Recurrent melanoma	NCT01748747
		▶ Advanced malignancies	NCT00948961
		▶ Melanoma ▶ Metastatic melanoma ▶ mucosal melanoma	NCT02126579
CD40	Chi Lob 7/4	▶ Cancer ▶ Neoplasms ▶ Lymphoma	NCT01561911
	NG-350A	▶ metastatic cancer ▶ epithelial tumor	NCT03852511
	SGN-40	▶ multiple myeloma	NCT00664898
		▶ NHL	NCT00556699
	ADC-1013	▶ Neoplasms ▶ Solid tumors	NCT02379741

Continued

Table 1 Continued

Targets	Drugs	Cancer type	NCT
	2141 V-11	▶ Cancer ▶ Solid tumor ▶ Cancer of skin	NCT04059588
	Selicrelumab	▶ Recurrent B-cell NHL ▶ Refractory B-cell NHL	NCT03892525
	HCD122	▶ Multiple myeloma	NCT00231166
EGFR TKI	Gefitinib	▶ Non-small cell lung cancer	NCT03157310
Chloroquine	Chloroquine	▶ Breast cancer ▶ Invasive breast cancer	NCT02333890
		▶ Pancreatic cancer	NCT01777477
		▶ Glioblastoma ▶ Astrocytoma, grade IV	NCT02432417
		▶ Glioblastoma multiforme	NCT00224978
		▶ Glioblastoma WHO grade IV ▶ Diffuse midline glioma histone 3 K27M WHO grade IV ▶ Anaplastic astrocytoma WHO grade III	NCT03243461
CD24	CD24Fc	▶ Metastatic melanoma	NCT04060407
CD47	ZL1201	▶ Locally advanced solid tumor	NCT04257617
	Hu5F9-G4	▶ Acute myeloid leukemia	NCT02678338
		▶ Solid tumor	NCT02216409
		▶ Acute myeloid leukemia	NCT03248479
		▶ Colorectal neoplasms ▶ Solid tumors	NCT02953782
		▶ NHL ▶ DLBCL ▶ NHL ▶ Diffuse large B cell lymphoma	NCT03527147
		▶ Lymphoma, non-hodgkin ▶ Lymphoma, large B-cell, diffuse ▶ Indolent lymphoma	NCT02953509
	IBI188	▶ Advanced malignancies	NCT03717103
			NCT03763149
	IBI322	▶ Advanced malignancies	NCT04338659
			NCT04328831
	HX009	▶ Advanced solid tumor	NCT04097769
	AO-176	▶ Solid tumor	NCT03834948
	CC-90002	▶ Hematological neoplasms	NCT02367196
	AK117	▶ Neoplasms malignant	NCT04349969
	TTI-621	▶ Hematological malignancies ▶ Solid tumor	NCT02663518
		▶ Solid tumors ▶ Melanoma	NCT02890368
		▶ Lymphoma ▶ Myeloma	NCT03530683
	SRF231	▶ Advanced solid cancers ▶ Hematological cancers	NCT03512340
	ALX148	▶ Metastatic cancer ▶ Solid tumor ▶ Advanced cancer ▶ NHL	NCT03013218
SIRPα	Anti-SIRPα	▶ Hepatocellular carcinoma	NCT02868255
CD47-SIRPα	SRF231	▶ Advanced solid cancers ▶ Hematological cancers	NCT03512340

CSF1, colony-stimulating factor 1; DLBCL, diffuse large B cell lymphoma; PI3K, phosphoinositide 3-kinase; TAMs, tumor-associated macrophages; TKI, tyrosine kinase inhibitor; TLR, toll-like receptor.

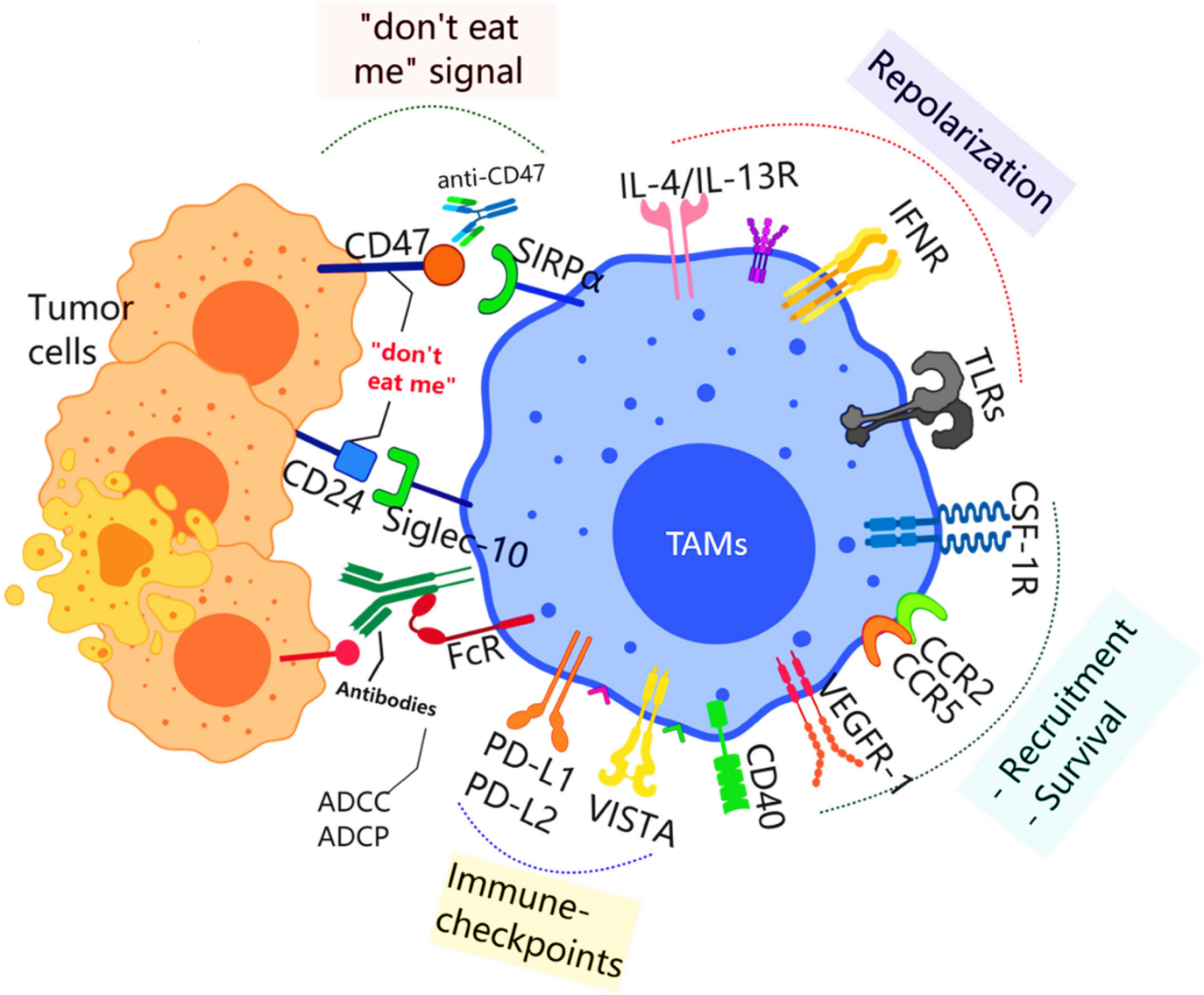

Figure 6 Main therapeutic strategies targeting TAMs. These therapeutic ways are aimed at either activating the anti-tumoral activity, or inhibiting the recruitment, survival and protumoral functions of macrophages. The process of macrophage-mediated antibody-dependent cellular cytotoxicity (ADCC) involves recognition of the therapeutic antibodies by Fc receptors (FcRs) on TAMs. The 'don't eat me' signal including SIRPα-CD47 pathway and CD24-Siglec 10 pathway. The antibodies against SIRPα-CD47 pathway and CD24-Siglec 10 pathway can activate macrophage-mediated antibody-dependent cellular phagocytosis (ADCP). Here, the main therapeutic strategies targeting TAMs are generally summarized including the 'don't eat me' signal pathways, repolarization, reducing and decreasing the recruitment and survival, and immune-checkpoints blockades with antibodies. IFNR, interferon receptor; TAMs, tumor-associated macrophages; VEGFR, vascular epidermal growth factor R.

activated by LPS- or HCD-driven macrophage infiltration in an animal model of CRC.[117]

Clodronate

Clodronate, a chemical agent that induces depletion of macrophages, can significantly deplete TAMs in the TME.[118] In proof of function experiments, clodronate depleted macrophages in a genetic mouse model of chronic hepatitis and HCC, leading to a significant reduction in F4/80$^+$ cells in the livers and spleens of treated mice.[119] In B16/F10 subcutaneous melanoma, clodronate significantly reduced the size of primary tumors. In tumors, the expression of F4/80 and α-SMA was significantly lowered.[119] In the B16/F10 lung metastatic melanoma model, treatment with clodronate significantly reduced the number of pulmonary nodules. F4/80$^+$ cells and microvessel density were also statistically decreased.[119] Tumor hypoxia and aerobic glycolysis are well-known resistance factors for anticancer therapies. TAMs secrete TNFα to promote tumor cell glycolysis, whereas increased AMPK and PPARγ coactivator 1-α in TAMs facilitate tumor hypoxia. Depletion of TAMs by clodronate was sufficient to abrogate aerobic glycolysis and tumor hypoxia, thereby improving the tumor response to anticancer therapies. TAMs depletion led to a significant increase in PD-L1

expression in aerobic cancer cells as well as T cell infiltration in tumors, resulting in antitumor efficacy from anti-PD-L1 antibodies, which were otherwise completely ineffective.[120]

REPOLARIZING TAMS TOWARD M1-LIKE MACROPHAGES

PI3Kγ signaling pathway

Myeloid cell PI3Kγ plays a role in regulating tumor immune suppression by promoting integrin α4-dependent Myeloid-derived suppressor cell (MDSC) recruitment to tumors and by stimulating the immunosuppressive polarization of MDSCs and TAMs, thereby inhibiting antitumor immunity. On the one hand, PI3Kγ stimulates the activation of integrin α4 in a manner dependent on BTK, PLCγ, RAPGEF, Rap1a, RIAM, and paxillin. On the other hand, PI3Kγ can also activate BTK to promote immunosuppressive myeloid cell polarization by inducing the expression of IL-10, TGF-β, and arginase, which are dependent on mTOR, S6Kα, and C/EBPβ, and inhibiting the expression of IL-12, IFN-γ, and Nos2.[121] Duvelisib (IPI-145), an oral inhibitor of the PI3Kδ and PI3Kδγ isoforms, can induce the transformation of TAMs from the immunosuppressive M2-like phenotype to the inflammatory M1-like phenotype.[122] In PDAC, PI3Kγ selectively drives immunosuppressive transcriptional programming in macrophages that inhibits adaptive immune responses and promotes tumor cell invasion and desmoplasia. Blockade of PI3Kγ in PDAC-bearing mice reprogrammes TAMs to stimulate $CD8^+$ T cell-mediated tumor suppression and to inhibit tumor cell invasion, metastasis, and desmoplasia.[123] Additionally, tumor cell-derived C3a modulated TAMs via C3a-C3aR-PI3Kγ signaling, thereby repressing antitumor immunity.[41] PI3Kγ-deficient macrophages and monocytes produce elevated inflammatory IL-12 and IL-23 in a GSK3α/β-dependent manner on toll-like receptor (TLR) stimulation.[124] Poly(l-glutamic acid)-combretastatin A4 conjugate (PLG-CA4), a novel class of vascular disrupting agents that has notable antitumor activity, induces the polarization of TAMs toward the M2-like phenotype in 4T1 metastatic breast cancer cells. Inhibition of PI3Kγ attenuates the immunosuppressive effect of PLG-CA4 treatment by decreasing the number of M2-like TAMs. Importantly, PI3Kγ inhibition synergizes with PLG-CA4 to significantly extend mean survival time.[125]

TLR signaling pathway

TLRs are important pathogen-recognition receptors expressed by cells of the immune system. Treatment with agonist of TLRs, such as TLR3, TLR4, TLR7/8 and TLR9, is a commonly used procedure that results in rapid activation of innate and adaptive immunity.[126] The most commonly used TLR agonists are cytosine-phosphorothioate guanine oligonucleotides for TLR-9, imiquimod for TLR-7 and poly (I:C) for TLR-3. Stimulation of TLR-3 polarizes macrophages to an M1 phenotype, as evidenced by upregulation of the expression of the costimulatory molecules CD80, CD86, CD40 on macrophages and their enhanced production of cytokines such as IL-6, IL-12 and TNF-α; these changes in the macrophages occur via inhibition of the co-inhibitory receptor Tim-3, enhancement of antigen uptake, enhancement of the ability to prime T cells, and inhibition of polarization toward the M2a and M2c subtypes, thus leading to significant increases in M1 macrophages and regression in tumor growth.[127] Engineered FlaB-secreting bacteria effectively suppressed tumor growth and metastasis in mouse models and prolonged survival, which was associated with TLR5-mediated host reactions in the TME, and these effects were completely abrogated in mice with TLR4 and MyD88 knockout and partly suppressed in TLR5 knockout mice. These results indicate that TLR4 signaling is required for tumor suppression mediated by FlaB-secreting bacteria, whereas TLR5 signaling augments tumor-suppressive host reactions via induction of the infiltration of abundant immune cells such as monocytes/macrophages and neutrophils via TLR4 signaling.[128] Tumor-secreted cathepsin K, a vital mediator in the relationship between the intestinal microbiota and CRC metastasis, can bind to TLR4 to stimulate M2 polarization of TAMs via an mTOR-dependent pathway.[129] Protein S (Pros1), a Mer/Tyro3 ligand produced by tumor cells, can decrease macrophage M1 cytokine expression in vitro and in vivo. Treatment with resiquimod, a TLR7/8 agonist, did not improve survival in mice bearing Pros1-secreting tumors but doubled survival for Pros1-deleted tumors, indicating that the combination of Pros1 depletion and TLR7/8 agonists could lead to antitumor responses by way of M1 polarization.[130]

CD40 and its ligands

The cell surface molecule CD40, a highly conserved costimulatory protein found on antigen-presenting cells, is a member of the tumor necrosis factor receptor superfamily and is broadly expressed by immune cells, in particular B cells, dendritic cells (DCs), and monocytes, as well as other normal cells and some malignant cells.[131] Anti-CD40 treatment significantly increased the proportion of activated macrophages within the liver, and blockade of macrophage activation using anti-CSF1/1R mAbs abrogated the lethality of anti-CD40/Gem treatment without reducing the antitumor efficacy of the combination treatment in PDAC. Concurrent CSF1R blockade and CD40 agonism led to profound changes in the composition of immune infiltrates, causing an overall decrease in immunosuppressive cells and a shift toward a more inflammatory milieu. Anti-CD40/anti-CSF1R antibody-treated tumors contain fewer TAMs and $Foxp3^+$ Treg cells, which increases the maturation and differentiation of pro-inflammatory macrophages and DCs and drives potent priming of effector T cells in draining lymph nodes.[132] In murine CT26 and MC38 colon adenocarcinoma, the most dramatic changes in the immune infiltrate after anti-CD40/anti-CSF1R antibody treatment were observed in macrophage and monocyte populations, which can also

suppress the growth of melanoma by reducing MMP9 or CCL17/22, which are characteristic of an M2 state, and by simultaneously inducing a polyfunctional inflammatory TAMs subset secreting TNF-α, IL-6 and IL-12[133]; these results were also seen in mesothelioma and colorectal adenocarcinoma.[134] Consistent with the high CSF1R expression on Ly6C^{low} TAMs, combining anti-CSF1R inhibition and CD40 agonism resulted in significantly reduced frequencies of MHC II^{high} and MHC II^{low} TAMs in tumors. A concomitant increase in MHC II^{high} Ly6C^{int} macrophages suggested that combination therapy reduced the suppressive, tumor-educated TAMs while leaving newly differentiated, pro-inflammatory macrophages to repopulate the TME. The remaining macrophages in the tumors had high expression of the costimulatory molecules CD80 and CD86 and inflammatory cytokines and low levels of MHC II and IL-10R.[132] In tyrosine kinase inhibition (TKI) of gastrointestinal stromal tumors (GIST), CD40 ligation did not have a direct inhibitory effect on human GIST cells, while the combination of anti-CD40 antibodies and imatinib (a TKI) effectively enhanced therapy directed at TAMs expressing high levels of CD40.[135]

MicroRNA

MicroRNA (miRNAs) are a large class of small non-coding RNAs that negatively regulate transcript levels through sequence-dependent recognition mechanisms.[136] Mature miRNAs are processed from hairpin-shaped precursor miRNAs by the RNAse III enzyme double-stranded RNA (dsRNA)-specific endoribonuclease (DICER).[137] After deletion of DICER in macrophages, M1-like TAM reprogramming is prompted, characterized by hyperactive IFN-γ/STAT1 signaling, which abates the immunosuppressive capacity of TAMs and fosters the recruitment of activated CTLs to tumors. CTL-derived IFN-γ exacerbates M1 polarization of Dicer1-deficient TAMs and inhibits tumor growth.[138] Genetic deficiency of miR-21 promotes the polarization of TAMs toward the M1-like phenotype in vivo and in vitro in the presence of tumor cells. By downregulating JAK2 and STAT1, miR-21 inhibits the IFN-γ-induced STAT1 signaling pathway, which is required for macrophage M1 polarization.[139] miR-148a expression can reduce the severity of inflammation, decrease NF-κB and STAT3 activation, and inhibit both spontaneous and carcinogen-induced colon cancer development in mice. miR-148a directly targets several upstream regulators of NF-κB and STAT3 signaling, including GP130, IKKα, IKKβ, IL1R1 and TNFR2, which leads to decreased NF-κB and STAT3 activation in macrophages and colon tissues.[140] Furthermore, TAMs infiltration is associated with chemoresistance as TAMs secrete IL-6 and thereby activate the IL-6R/STAT3 pathway; activated STAT3 transcriptionally inhibits the tumor suppressor miR-204–5 p.[141] Additionally, colon cancer cells harboring the GOF mutated p53 selectively shed miR-1246-enriched exosomes, which can further reprogrammes TAMs into a pro-tumoral state with increases in TGF-β.[142] M2 macrophage-derived exosomes (MDEs) show high expression levels of miR-21–5 p and miR-155–5 p, and MDE-mediated migration and invasion of colon cancer cells depend on these two miRNAs binding to the BRG1 coding sequence and thus downregulating the expression of BRG1, which has been identified as a key factor promoting colon cancer metastasis.[143] miR-155 can regulate antitumor immune responses by promoting IFN-γ production from T cells in the TME.[144 145] In breast cancer, miR-149 downregulation functionally contributes to breast tumor progression by recruiting macrophages to the tumor site and facilitates CSF1 and EGF receptor crosstalk between cancer cells and macrophages.[146] Hypoxia, the most commonly observed characteristic in cancers, is implicated in the establishment of an immunosuppressive niche. Hypoxic exosomal miR-301a-3p generated by pancreatic cancer cells in a hypoxic microenvironment can polarize M2 macrophages by activating the PTEN/PI3Kγ signaling pathway. Coculturing pancreatic cancer cells with macrophages in which miR-301a-3p is upregulated or macrophages exposed to hypoxic exosomes enhances their metastatic capacity.[147] Notably, hypoxic lung cancer-derived extracellular vesicle miR-103a can increase the activation of AKT and STAT3 and induce the immunosuppressive and pro-tumoral activity of TAMs by targeting PTEN.[148] miR-195–5 p is significantly downregulated in CRC tissues and patients with a significantly shortened overall survival. Mechanistically, miR-195–5 p can regulate NOTCH2 expression in a post-transcriptional manner by directly binding to the 3′-UTR of Notch2 mRNA. Subsequently, miR-195–5 p/NOTCH2 suppresses GATA3-mediated IL-4 production in CRC cells and ultimately prohibits M2-like TAM polarization.[149]

PROMOTING THE PHAGOCYTOSIS AND ANTIGEN PRESENTATION OF TAMS BY BLOCKING 'DON'T EAT ME' SIGNALS

CD24-Siglec-10 signaling for cancer immunotherapy

CD24, also known as heat-stable antigen or small-cell lung carcinoma cluster 4 antigen, is a novel 'don't eat me' signal and a heavily glycosylated glycosylphosphatidylinositol-anchored surface protein[150 151] that is known to interact with the inhibitory receptor sialic-acid-binding Ig-like lectin 10 (Siglec-10) on innate immune cells to inhibit inflammatory responses.[101 152 153] In ovarian cancer and breast cancer, CD24 can be the dominant innate immune checkpoint and is a promising target for cancer immunotherapy because of its interaction with Siglec-10, which is highly expressed on TAMs. Genetic ablation and therapeutic blockade of either CD24 or Siglec-10, as well as blockade of the CD24-Siglec-10 interaction using monoclonal antibodies, robustly augment the phagocytosis of macrophages in all CD24-expressing human tumors.[103 154]

The CD47-signal-regulatory protein α axis as an innate immune checkpoint in cancer

The phagocytic activity of macrophages is regulated by both activating ('eat me') and inhibitory ('don't eat me') signals.[155] CD47, a widely expressed transmembrane glycoprotein on cancer cells, serves as a critical

inhibitory signal, suppressing phagocytosis by binding to signal-regulatory protein alpha (SIRPα) on the surface of macrophages[156–159]; CD47 can be directly regulated by two distinct superenhancers through the TNF-NFKB1 signaling pathway.[160] Additionally, an exosome-based immune checkpoint blockade strategy (SIRPα-exosomes) was developed to antagonize CD47.[161] SIRPα is a myeloid-specific immune checkpoint that engages the CD47 'don't eat me' signal on tumors and normal tissues, and this interaction can be blocked by the high-affinity monoclonal antibody KWAR23. Three subsets ($CD14^{+}SIRP\alpha^{high}$, $CD14^{-}SIRP\alpha^{low}$ and $CD14^{-}SIRP\alpha^{neg}$) of monocytes/macrophages based on CD14 and SIRPα expression have been identified.[162] Following KWAR23 antibody treatment in a human SIRPA knock-in mouse model, macrophages infiltrate human Burkitt's lymphoma xenografts and inhibit tumor growth, generating complete responses in the majority of treated animals.[163] However, CD47-SIRPα inhibition could potentiate tumor cell phagocytosis, and CD40-mediated activation of a type I IFN response provided a bridge between macrophage-mediated and T cell-mediated immunity that significantly enhanced durable tumor control and rejection.[164] MHC I can control the phagocytic function of macrophages. Expression of the common MHC I component β2-microglobulin by cancer cells directly protects them from phagocytosis, which is mediated by the inhibitory receptor LILRB1, whose expression is upregulated on the surface of macrophages, including TAMs. Disruption of either MHC I or LILRB1 potentiated phagocytosis of tumor cells both in vitro and in vivo, suggesting that the MHC I-LILRB1 signaling axis is an important regulator of the effector function of innate immune cells.[165] Recently, responsive exosome nanobioconjugates were synthesized for cancer therapy. Azide-modified exosomes derived from M1 macrophages were conjugated with dibenzocyclooctyne-modified antibodies against CD47 and SIRPα through pH-sensitive linkers. In the acidic TME, the benzoic-imine bonds of the nanobioconjugates are cleaved to release aSIRPα and aCD47, which can block SIRPα on macrophages and CD47, respectively, leading to abolished 'do not eat me' signaling and improved phagocytosis by macrophages. In addition, native M1 exosomes effectively reprogramme macrophages from the pro-tumoral M2 to the antitumoral M1 phenotype.[166] Notably, the CD47-SIRPα interaction requires Fc-FcγR interactions to maximize the antitumor efficacy of macrophages in T cell lymphomas.[167] Glutaminyl-peptide cyclotransferase-like protein (QPCTL) was identified as a major component of the CD47-SIRPα checkpoint. Interference with QPCTL activity enhances antibody-dependent cellular phagocytosis and cellular cytotoxicity against tumor cells.[168] Acute myeloid leukemia (AML) is organized as a cellular hierarchy initiated and maintained by a subset of self-renewing leukemia stem cells (LSCs). CD47 is more highly expressed on AML LSCs than on their normal counterparts, and increased CD47 expression predicted worse overall survival in three independent cohorts of adult AML patients. Furthermore, blocking CD47 with the monoclonal antibody TTI-621 preferentially enabled phagocytosis of AML LSCs and inhibited their engraftment in vivo. Finally, treatment of human AML LSC-engrafted mice with an anti-CD47 antibody targeted and depleted AML LSCs.[169 170] Moreover, macrophage phagocytosis activated by anti-CD47 antibodies primed $CD8^{+}$ T cells to exhibit cytotoxic functions in vivo.[171] Additionally, targeting the IRF7-SAPK/JNK pathway to induce M1 characteristics in TAMs contributed to prolonged survival in leukaemic mice.[172] In bladder cancer, CD47 is highly expressed by bladder tumor-initiating cells compared with the rest of the tumor.[173] Blockade of CD47 by a mAb resulted in macrophage engulfment of bladder cancer cells[174] and acute lymphoblastic leukemia in vitro,[175] and the combination of the monoclonal anti-CD20 antibody rituximab with an anti-CD47 antibody eradicated human B cell non-Hodgkin's lymphoma (NHL) through a mechanism involving combined Fc receptor (FcR)-dependent and FcR-independent stimulation of phagocytosis.[176] In canine diffuse large B cell lymphoma in a murine xenograft model, augmented responses are observed when CD47-blocking therapies are combined with 1E4-cIgGB, a canine-specific antibody against CD20, resulting in synergy in vitro and in vivo and eliciting cures in 100% of subjects.[177] In pediatric malignant primary brain tumors, a humanized anti-CD47 antibody, Hu5F9-G4, has demonstrated therapeutic efficacy in vitro and in vivo in patient-derived orthotopic xenograft models.[178] Hu5F9-G4 combined with rituximab has also shown promising activity in patients with aggressive and indolent lymphoma. No clinically significant safety events were observed in the initial study.[179 180] Calreticulin is a prophagocytic signal highly expressed on the surface of several human cancers, including AML and lymphoblastic leukaemias, chronic myeloid leukemia, NHL, bladder cancer, GBM,[181] small lung cancer and ovarian cancer, but minimally expressed on most normal cells.[157] Increased CD47 expression correlated with high calreticulin levels in cancer cells and was necessary for protection from calreticulin-mediated phagocytosis. Phagocytosis induced by anti-CD47 antibodies requires the interaction of target cell calreticulin with its receptor low-density lipoprotein-receptor related protein (LRP) on phagocytic cells, as blockade of the calreticulin/LRP interaction prevents anti-CD47 antibody-mediated phagocytosis. Last, increased calreticulin expression is an adverse prognostic factor in diverse tumors, including neuroblastoma, bladder cancer and NHL.[157 182 183]

Other potential molecular targets

Some drugs and molecular targets can also affect the polarization of TAMs. M2 macrophages show higher insulin-like growth factor-1 (IGF-1) and CD163 expression than M1 macrophages and increase hepatoma growth. Sorafenib can reduce the release of CD163 and IGF-1 by M2 macrophages and slow the proliferation of HuH7 and HepG2 cells driven by M2 macrophages.

IGF-receptor blockade with NVP-AEW541 can decelerate growth by M2 macrophage-conditioned culture media in a dose-dependent manner. A transient mCD163 (CD163 mRNA) reduction during sorafenib treatment indicated coherent M2 macrophage inhibition in patients with HCC.[184] Notably, sorafenib induces pyroptosis in macrophages and triggers NK-mediated cytotoxicity against HCC.[185] Moreover, blocking IGF in combination with paclitaxel, a chemotherapeutic agent commonly used to treat breast cancer, showed a significant reduction in tumor cell proliferation and lung metastasis in preclinical breast cancer models compared with paclitaxel monotherapy.[186] Additionally, IGF-2 can commit preprogrammed mature macrophages to OXPHOS, such that maturing macrophages can be cultured to become anti-inflammatory cells.[187] Polyinosinic-polycytidylic acid, a synthetic molecule similar to ddsRNA that potentially inhibits liver tumors, can also reprogramme TAMs toward an M1-like phenotype.[188] Gefitinib, an EGFR TKI used to treat non-small-cell lung cancer (NSCLC), can significantly inhibit IL-13-induced M2-like polarization and decrease the expression of CD206, CD163 and other specific M2 marker genes (Mrc1, Ym1, Fizz1, Arg1, IL-10 and CCL2).[189] In Lewis lung cancer, a small concentration of gefitinib significantly inhibited IL-13-induced M2-like polarization of macrophages. In RAW 264.7 cells, gefitinib inhibits IL-13-induced phosphorylation of STAT6, which was a crucial signaling pathway in macrophage M2-like polarization. In LLC mice metastasis model, oral administration of gefitinib significantly reduced the number of lung metastasis nodules, down-regulated the expression of M2 marker genes and the percentages $CD206^+$ and $CD68^+$ macrophages in tumor tissues.[190] Neferine, an antiangiogenesis reagent, is one of the most promising agents for the treatment of high-grade serous ovarian carcinoma (HGSOC) and can induce autophagy through mTOR/p70S6K pathway inhibition and suppress M2 macrophage polarization.[191] Bone morphogenetic protein (BMP)-dependent signals originate from stromal bladder tissue and mediate urothelial homeostasis. The expression of BMP4 is related to monocyte/macrophage polarization toward the M2 phenotype.[192] The inhibition of TAM infiltration can also reduce the number of TAMs. Metformin is capable of repressing prostate cancer progression by inhibiting infiltration of TAMs via inhibition of the COX2/PGE2 axis.[193] Dioscin, an herbal steroidal saponin, improves the secretion of proinflammatory cytokines (IL-6, TNF-α and IL-1β) and the phagocytic capacity of TAMs by increasing M1 phenotype polarization.[194] Notably, leucine-rich repeat-containing G protein-coupled receptor 4 (Lgr4; also known as Gpr48) promotes macrophage M2 polarization through Rspo/Lgr4/Erk/Stat3 signaling. Importantly, blockade of Rspo-Lgr4 signaling can overcome LLC resistance to anti-PD-1 therapy and improve the efficacy of PD-1-targeted immunotherapy in B16F10 melanoma.[195] CSCs contribute to the progression and androgen deprivation therapy (ADT) resistance of prostate cancer and promote the transformation of monocytes/macrophages into TAMs, and CSC-educated TAMs reciprocally promote the stem-like properties of CSCs, progression and ADT resistance through IL-6/STAT3[196]; these effects are also seen in NSCLC.[197] In human solid tumors harboring excessive STAT3 activity, hematopoietic cell kinase can suppress M2 macrophage polarization by inhibiting STAT3.[198] Additionally, inhibition of STAT3-induced gene expression can reprogramme macrophages toward an antitumor state by blocking ERK5.[199] Chloroquine, a lysosomotropic agent that is used to treat malaria, plays an important role in antitumor therapy by redirecting TAMs toward the M1 phenotype, which increases macrophage lysosomal pH, causing Ca^{2+} release via the lysosomal Ca^{2+} channel mucolipin-1 (Mcoln1), and further induces the activation of p38 and NF-κB.[200] The EMT inducer SNAIL1 regulates breast cancer metastasis, and its expression in human primary breast tumors predicts poor outcomes. The SNAIL1-dependent tumor cell secretome modulates primary TAMs polarization by regulating the production of GM-CSF, IL-1α, IL-6 and TNF-α by breast cancer cells.[201] In KRAS-mutant lung adenocarcinoma (LUAD), loss of the histone chaperone Asf1a in tumor cells sensitizes tumors to anti-PD-1 treatment, revealing that tumor cell-intrinsic Asf1a deficiency induces the polarization of M1-like macrophages by upregulating GM-CSF expression and potentiates T cell activation in combination with anti-PD-1 antibodies.[202] The p38/MAPKAP kinase 2 (MK2) axis controls the synthesis of proinflammatory cytokines that mediate both chronic inflammation and tumor progression. Blockade of this pathway can suppress inflammation and prevent colorectal tumorigenesis in a mouse model of inflammation-driven colon cancer because MK2 promotes polarization of TAMs toward protumorigenic, proangiogenic M2-like macrophages.[203] In the TME, hedgehog (Hh) signaling in myeloid cells is critical for M2 TAMs polarization and tumor growth. Furthermore, Hh-induced functional polarization of TAMs suppresses $CD8^+$ T cell recruitment to the TME through the inhibition of CXCL9 and CXCL10 production by TAMs.[204] Furthermore, TAMs exhibit antitumoral properties in sonic Hh-related medulloblastoma.[205]

Radiotherapy and TAMs

Radiotherapy (RT), besides tumor cells, also affects the TME. RT-induced inflammatory response contains five phases: innate recognition, initiation of inflammation, antigen presentation, effector response and resolution. Macrophages play an important role in all phases. RT can cause the accumulation of radioresistant M2-like TAMs.[206] Furthermore, an abscopal effect is observed. The abscopal effect is phenomenon in which local RT is associated with the regression of metastatic cancer at a distance from the irradiated site.[207–209] The abscopal effect is an immune response, which can also be mediated by macrophages, activated by inflammatory agents (cytokines, DAMPs, ROS/RNS) originating from irradiated TME. In addition, RT can also induce the transcription of

HIF-1α, which leads to increased expression of CXCL12, CCL2, CSF1 and VEGF, which recruit macrophages and promote their immunosuppressive function.[210] HIF-1α and IFN-γ signaling also induces the expression of PD-L1 in TAMs and tumor cells, which suppresses the antitumor immune response.[211 212] Moreover, RT causes cancer cell death partially via apoptosis which is known to induce immunosuppressive and anti-inflammatory response in macrophages. Apoptotic cells drive differentiation of macrophages into the M2 phenotype with enhanced secretion of anti-inflammatory cytokines such as TGF-β and IL-10 and upregulation of Arg1.[213] It's important to note that RT can recruit both M1 and M2 macrophages from bone marrow-derived myeloid cells.[213] The balance of M1 vs M2 macrophages induced by RT may depend on the radiation dose. For example, both single-dose (25 Gy) and fractionated irradiation (15×4 Gy) resulted in intratumoral macrophages with both higher expression of both M1 markers including COX2 and iNOS as well as M2 markers including Arg1 in a murine prostate cancer model.[211] In PDAC, low-dose γ irradiation led to the differentiation of iNOS +M1 macrophages, which promoted efficient recruitment of tumor-specific T-cells by helping normalize the tumor vasculature.[210] Low doses (<2 Gy) may also activate immunosuppression and angiogenesis. In mice, after a low dose of radiation, M2 macrophages suppress the antitumor response and promote metastasis through the production of Arg1 and TGF-β and IL-10. In addition, high doses of RT (>8 Gy) may promote the anti-inflammatory activation of macrophages,[214] and a dose of 20 Gy activates the M2 TAM with tolerogenic properties by inducing COX-2/PGE2 and NO.[212]

CONCLUSIONS AND FUTURE PERSPECTIVES

Although significant advances have been made in targeting TAMs to treat tumors, some risks and limitations remain. For example, in murine mammary tumors, CCR2-expressing inflammatory monocytes can be recruited to the primary tumors and metastatic sites, and CCL2 neutralization inhibits metastasis by retaining monocytes in the bone marrow. Blocking CCL2 inhibition leads to increased metastasis and accelerated death. This is due to the release of monocytes in the bone marrow and increased mobilization of cancer cells in the primary tumor, as well as the proliferation of metastatic cells and blood vessel formation in the lung. Targeting TAMs by inhibiting CSF1R has been reported to reduce tumor growth and metastasis, and such therapies are currently in clinical trials. Application of neutralizing anti-CSF1R and anti-CSF1 antibodies, or treatment with two different small molecule inhibitors of CSF1R, can actually increase spontaneous metastasis without altering primary tumor growth in mice with two independently derived breast tumors. Blocking CSF1R or CSF1 can lead to elevated serum G-CSF levels, an increased frequency of pulmonary neutrophils associated with primary tumors and metastases, and an increased number of neutrophils and Ly6C^{high} monocytes in peripheral blood. Macrophages are a key factor in the complex interaction between the immune system and tumors and play an important role in promoting tumor growth and vascular system formation and in disrupting the balance of the TME, suggesting that they are an important target for tumor prevention and treatment. TAMs in the tumor are encouraged by the tumor to undergo M2-like polarization, which promotes the growth of the tumor and seriously affects prognosis. Therefore, the development and application of drug delivery systems targeting TAMs and the TME are of great significance. Immunosuppressive agents and some natural drugs inhibit the expression of TAMs; nanoparticle drugs, phosphoric acid compounds, and some natural medicines convert TAMs from the M2 to the M1 phenotype. The replacement of TAMs with CTLs will become a new therapeutic direction for patients with advanced tumors. Targeting of CCL2 and CSF1R may have some risks, which can be eliminated with combination strategies. As mentioned above, the infiltration of TAMs in TME is associated with poor prognosis. However, instead of removing TAMs, it is better to transform TAMs into antitumor effectors, which may be the most promising strategy related to TAMs used to treat tumors in the future. Besides, at present, high dose of RT is often used in clinical. However, high dose may promote the anti-inflammatory activation of macrophages, and further suppress antitumor immunity. Therefore, combining rRT with a reprogramming strategy targeting TAMs may amplify the antitumor efficiency compared with a single treatment strategy.

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