Targeting tumor-associated macrophages to synergize tumor immunotherapy

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The current treatment strategies in advanced malignancies remain limited. Notably, immunotherapies have raised hope for a successful control of these advanced diseases, but their therapeutic responses are suboptimal and vary considerably among individuals. Tumor-associated macrophages (TAMs) are a major component of the tumor microenvironment (TME) and are often correlated with poor prognosis and therapy resistance, including immunotherapies. Thus, a deeper understanding of the complex roles of TAMs in immunotherapy regulation could provide new insight into the TME. Furthermore, targeting of TAMs is an emerging field of interest due to the hope that these strategies will synergize with current immunotherapies. In this review, we summarize recent studies investigating the involvement of TAMs in immune checkpoint inhibition, tumor vaccines and adoptive cell transfer therapies, and discuss the therapeutic potential of targeting TAMs as an adjuvant therapy in tumor immunotherapies.

INTRODUCTION

Given the association of malignancies with subverted immune surveillance,1 immunotherapies provide options for advanced cancer patients, and multiple clinical trials are underway.2 Despite the impressive results achieved in several clinical trials,3–6 obstacles have been encountered with the current immune checkpoint inhibitor (ICI)-based immunotherapies.7–9 Suboptimal efficacy is among the major concerns because previous trials suggest that the response rate to ICI monotherapy is limited, and the responses vary significantly across multiple tumors and among individuals.10–13

Accumulating evidence has suggested that immune suppression in the tumor microenvironment (TME) represents a major barrier to maximizing the clinical potential of immunotherapies.14 The TME is complex with diverse populations of nontumor stromal cells that impact tumor immune evasion, response to immunotherapy, and patient survival.15 In addition to cytotoxic lymphocytes (CTLs) and natural killer cells (NKs) that are generally considered effective antitumor immune cells, the TME contains a range of other cell types that are involved in the crosstalk with anti-tumor immune cells, including cancer-associated fibroblasts (CAFs),16 endothelial cells (ECs),17 and tumor-associated macrophages (TAMs).18 CAFs can induce a robust stromal reaction characterized by fibrotic extracellular matrix (ECM) and make the TME convert to an immune-excluded type via the transforming growth factor-β (TGF-β) signaling pathway.19–21 The tumor-associated vasculature is another hallmark of advanced solid tumors.22,23 ECs of tumor vasculature can not only inhibit antitumor immunity by establishing a selective immune barrier via the vascular endothelial growth factor (VEGF)/prostaglandin E2 (PGE2)-FASL pathway,24 but can also exacerbate the hypoxia condition with low pH and cause high interstitial fluid pressure, which is unfavorable for the infiltration and activation of CTLs and NKs.19,20,22

Macrophages are involved in various processes in both homeostasis and disease.25–27 With effector functions such as phagocytosis, antigen presentation, and the plasticity to secrete a wide variety of signaling molecules, they serve as an efficient “firewall” in regulating homeostasis.26–28 They are also dynamic populations, and the resident macrophage pool can be rapidly expanded by infiltrating monocytes under pathological states such as tissue damage, inflammation and malignancy.20,21,26–28 Macrophages in the TME can be roughly induced into two contrasting groups: classically activated “M1” macrophages and alternatively activated “M2” macrophages.22 M2 and small populations of M1 cells, also known as tumor-associated macrophages (TAMs), have been generally thought to be involved in tumor initiation, progression, angiogenesis and metastasis.29 Most relevant for patients, a high TAM infiltration is often correlated with poor clinical outcomes in a wide variety of tumors and is believed to decrease responses to standard-of-care therapeutics, including radiotherapy, chemother-apy and targeted therapy.30–41 However, the “M1-M2” macrophage dichotomy is too simple to describe their complicated roles in the TME.32 Recent data acquired using unbiased large-scale techniques might help discriminate among macrophage subpopulations and have unraveled a previously unrecognized complexity in macrophage polarization, far beyond the old dogma of the binary “M1-M2” binary system.42 Furthermore, significant dynamic changes in macrophage subpopulations were observed during tumor development and were correlated with the efficacy of immunotherapy.37–49 These findings suggest a better understanding of heterogeneous TAMs and their roles in immunother-apy will be critical for developing effective immunotherapies.50,51
Here, we attempt to illustrate the regulatory functions of TAMs in the TME and different immunotherapies. We further discuss the therapeutic potential of targeting TAMs to improve current immunotherapies (immunotherapy classifications are summarized in Fig. 1).

**TAMS IN TUMOR INITIATION AND PROGRESSION**

The strong relationship between inflammation and tumorigenesis has long been recognized. Approximately 90–95% of all types of tumors are connected to environmental exposures including tobacco, obesity, smoke, radiation, chemicals, and chronic infections, all of which could induce a smoldering inflammatory state. TAMs help establish a pro-inflammatory microenvironment and the link between TAMs and tumor initiation has been extensively studied in various clinical samples and preclinical models. For instance, liver macrophages were found to be the key source of steatosis-induced Wnt expression and the active Wnt/β-catenin signaling in macrophages can promote the growth of tumor progenitor cells, underlying the increased risk of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) in obese individuals.

TAMs can promote tumor progression by producing mediators that remodel the tumor-supportive TME. Such mediators include growth factors and cytokines that support tumor cell proliferation; NF-κB-mediated factors that protect against apoptosis (for example, interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, C-C motif chemokine (CCL)2, C-X-C motif chemokine (CXCL)8, and CXCL10); pro-angiogenic growth factors, such as VEGF, platelet derived growth factor (PDGF), TGF-β and fibroblast growth factor (FGF); and other factors that modulate tissue architecture and favor tumor cell migration, invasion and metastasis.

TAMs also subvert local immune surveillance because they can directly reduce the activities of T cells and NKs by expressing cell surface proteins or by releasing soluble factors that display immunosuppressive functions (for example, arginase 1 (ARG1), indoleamine 2,3-dioxygenase (IDO), IL-10, programmed death ligand 1 (PD-L1), and TGF-β) or indirectly suppress T cell activities through recruitment of other immune suppressive cells such as regulatory T cells (Tregs). Overall, TAMs play a dual role as “tumor promoters” and “immune suppressors” because they can promote tumor initiation and act as central drivers of the immunosuppressive TME through...
their expression of cell surface receptors, secreted cytokines, chemokines, and enzymes that regulate the recruitment and function of multiple immune cell subtypes.

TAMs in checkpoint inhibitor therapy
Inhibition of immune checkpoints, such as PD-1/L1 and CTLA-4, removes inhibitory signals of T cell activation, which enabling tumor-reactive T cells to overcome regulatory mechanisms and mount an effective antitumor response. However, the underlying cellular mechanisms remain unclear, namely, the expression patterns of checkpoint molecules and the interplay among ICIs and different components within the TME. In the setting of ICI therapy, the impact of TAMs should be carefully considered (Fig. 2, left).

**Upregulation of checkpoint molecules.** Early in the 2000s, the overexpression of checkpoint ligand B7-H1 (PD-L1) on tumor cells was considered a key mechanism of immune evasion. However, it was not until 2009 that two independent studies first demonstrated that macrophages are the predominant immune cells that express PD-L1 in HCC. These PD-L1+ TAMs, activated by tumor-derived IL-10, could mediate CD8+ T cell dysfunction via the PD-1/PD-L1 interaction. Similar results were also observed in other tumor types including head and neck squamous cell carcinoma (HNSC), ovarian cancer, soft tissue sarcoma, bladder cancer and CCA. In the last decade, other B7 family checkpoints ligands were found to be expressed on TAMs, including B7-DC (PD-L2) and B7-H4 (B7-S1), as well as alternative checkpoints ligands such as galectin-9 and V-domain Ig-containing suppressor of T cell activation (VISTA). Thus, TAMs have been regarded as carriers of checkpoint ligands that are upregulated in response to TME-derived factors, resulting in
immune exhaustion via the checkpoint ligand/receptor interaction in a cell-to-cell contact manner. However, these findings do not provide a comprehensive picture. The expression of checkpoint molecules on macrophages might reflect the immune status within the TME. PD-L1 expression on macrophages, rather than on tumor cells, was positively correlated with patients’ overall survival (OS) and might be used as an independent prognostic factor based on a cohort study of 453 HCC patients.87 Surprisingly, PD-L1+ TAM-enriched tumors exhibited an activated immune status, with high levels of CD8+ T cell infiltration and immune-related gene expression, which indicates that a substantial proportion of tumor might be a amenable to ICI therapy.87–89

Moreover, the blocking effect of ICIs on the checkpoint molecules expressed on TAMs is increasingly attracting attention. Gordon et al. found that the phagocytic potency of PD-1+ macrophages is rescued by PD-1/PD-L1 inhibition, which lengthens the survival of colon cancer preclinical models in a macrophage-dependent manner.90 Another recent study based on a myeloid cell-specific PD-1 silencing model revealed the vital role of elevated intracellular cholesterol during anti-PD-1 treatment, which is required for differentiation of inflammatory macrophages and the promotion of antigen-presenting function.91 These findings show the complexity behind the checkpoint molecules expressed on TAMs. A further understanding of their intracellular regulatory mechanisms will be helpful for precise classification of TAMs and providing guidance for ICI treatment.

**Crosstalk with regulatory T cells.** Tregs are critical components of the TME and contribute to different aspects of tumor progression.92 Recent studies have revealed the compensation between TAMs and Tregs that derived immune evasion and ICI resistance.93 TAMs favor chemokine/cytokine-mediated recruitment of Tregs to the TME.94–98 TAM-derived CCL20 was found to promote the infiltration of CCR6+ Tregs in colorectal cancer (CRC) and HCC, which may be an essential mechanism of anti-PD-L1 therapy resistance.99,100 Specifically, TREM-1+ Tregs elevate the expression of the chemokine CCL20 via the extracellular signal-regulated kinase (ERK)/NF-κB pathway in response to hypoxia and tumor metabolites promoting infiltration of CCR6+ FOXP3+ Tregs.67 Thus, blocking the TAM-specific TREM-1 pathway could significantly reduce immunosuppressive Tregs recruitment, as well as restore the efficacy of anti-PD-L1 therapy.67 TAM-derived factors also play a central role in the induction of induced Tregs (iTregs) in the TME. It was shown that iTregs could be induced from CD4+ CD25+ T cells co-cultured with M2-TAMs.100 A recent study by Zhou et al. highlighted the critical roles of TAM-derived exosomes in the induction of iTregs. They identified miRNAs enriched in exosomes, including miR-29a-3p and miR-21–5p which directly suppressed T cell-intrinsic STAT3 and regulated Treg/Th17 in ovarian cancer.101

Moreover, Tregs can further enhance the immunosuppressive properties of TAMs. In laryngeal squamous cell carcinoma (LSCC), malignant pleural effusion (MPE), and CRC, Tregs were found to promote the differentiation of monocytes into immunosuppressive TAMs directly.100,105 Tregs can also modulate lipid metabolism in M2-like TAMs. Liu et al. found that Tregs could suppress CD8+ T cell secretion of IFN-γ, which would otherwise block the activation of sterol regulatory element binding protein-1 (SREBP1)-mediated fatty acid synthesis in M2 TAMs. Thus, Tregs indirectly but selectively sustained M2-like TAM metabolic fitness, mitochondrial integrity and survival.104

Therefore, a positive feedback loop exists between TAMs and Tregs that further enhances their immunosuppressive effects in the TME.

**Hijacking of anti-PD-1 antibodies.** The constitutive expression of Fcγ receptor (FcγR) on monocytes/macrophages plays a crucial role in the antibody-dependent phagocytosis (ADCP) of tumor cells.105 However, it is worth noting that TAMs may have a significant impact on the pharmacokinetics and efficacy of ICI via Fc-FcγR binding. In mouse models and primary human immune cells, anti-PD-1 antibodies were observed to be seized by macrophages depending on the Fc domain of the antibody and the FcγR expressed by macrophages, which led to ICI therapy resistance.106 Moreover, a recent study has shown that Fc-FcγR binding-mediated TAM reprogramming can even induce hyperprogression in an non-small cell lung cancer (NSCLC) cohort and NSCLC patient-derived xenograft (PDX) models, although the mechanism remains unclear.107 Thus, how to interfere with the constitutive expression of FcγR on macrophages should be explored out in the tumor immunotherapy field. The effects mediated by the Fc domain of checkpoint molecular antibodies should be carefully evaluated and mechanistically understood.108

**Macrophages in adoptive cell transfection**

Adoptive cell transfer therapies such as chimeric antigen receptor T cell (CAR-T) or TCR-engineered T cell therapies enhanced the anti-tumor response in different advanced malignancies.109–111 The transferred cells must be trafficked and infiltrate into tumor sites to exert their cytolytic effects.112 However, this approach is not feasible for solid tumor treatment because of the relatively limited blood distribution and abnormal structure of tumor neovessels.12 In addition, tumors that develop from cirrhosis are highly fibrotic and difficult to penetrate physically.13 These features complicate the infiltration of adoptively transferred cells into tumor sites. In addition, TAMs contribute to the angiogenic and fibrotic TME (Fig. 2, lower right).

TAMs support tumor angiogenesis mainly by the production of factors such as VEGFA, PDGF, TGF-β and FGF.59–61 The subpopulation of TAMs characterized by the expression of antigiopeitin 1 (TIE2+) in the blood or TME were considered to be close associated with intratumor neovessel formation.13,114 The molecular events of TAM-mediated angiogenesis were identified in a study based on a chronic HBV infection cohort, which showed that the individuals who finally developed HCC had higher serum levels of IL-23.115 IL-23, which is produced by inflammatory macrophages, enhanced macrophage-mediated angiogenesis by upregulating IL-23 receptor expression in macrophages. This “chronic inflammation-macrophage-IL-23” positive feedback loop might partially explain the significant role of macrophages in formation of the TME. Furthermore, high TAM infiltration correlated with a small number of IFN-γ-expressing active NKS in HCC,116 which might have a negative impact on the activation or survival of adoptive transferred NKS.

**Macrophages in tumor vaccines therapy**

The identification of tumor antigens led to the development of tumor vaccination strategies in the 1980s. The host anti-tumor immune response is induced by tumor-antigen-pulsed dendritic cells (DC-based vaccines) or tumor-derived antigens released from lysed tumor cells (oncolytic virus).117–119 However, the results from clinical trials, were not as striking as expected.120–122 The limited efficacy of tumor vaccines in solid tumors may be ascribed to different possible causes, one of these being the strong immunosuppressive TME. As in other immunotherapies, studies have shown the accumulation of immune suppressive CD11b+ myeloid cells in response to the tumor vaccine treatment, which may result in therapy resistance125–127 (Fig. 2, upper right).

Currently, TAM-targeting strategies combined with tumor vaccination are under evaluation. Anti-CD11b antibody-mediated depletion of myeloid cells showed a synergistic effect along with the vaccine by further prolonging the survival of tumor-bearing mice, although no significant reduction in tumor burden was observed.128 Injection of tumor lysate-pulsed DC also prolonged the survival of mouse models, and this therapeutic effect was
further enhanced by injection of PLX3397, a CSF1R inhibitor that reprograms macrophages.126

THERAPEUTIC TARGETING OF TAMS IN TUMOR

Given that TAMs have a profound impact on tumor immunotherapies, there is considerable interest in the therapeutic targeting of TAMs to synergize current ICI-based immunotherapy. The different approaches that have been explored for targeting TAMs can be roughly sorted into three major categories: (1) eliminating TAMs already present in the TME; (2) inhibition of monocyte recruitment; and (3) reprogramming of TAMs (Fig. 3).31,35,129 These strategies have been investigated in preclinical models, and some have been translated into the clinical setting as adjuvant to immunotherapy.130 Here, we summarize current preclinical and clinical studies and discuss the potential strengths and weaknesses of these approaches in different solid tumors (Table 1).

Macrophage elimination

The clearance of TAMs is an option to counter their negative impact during immunotherapy. Bisphosphonates, which are traditionally been used to prevent the bone metastases or excessive bone resorption, can be taken up by phagocytes and have cytotoxic effects on myeloid cells.131,132 Based on their structure, bisphosphonates can be divided into two categories: non-nitrogen-containing and nitrogen-containing bisphosphonates.131 Clodronate belongs to the family of non-nitrogen bisphosphonates. In early studies, clodronate-loaded liposomes (clodrolip) were often used to deplete liver macrophages.133,134 Liposomes are artificially prepared vesicles that undergo phagocytosis by macrophages after injection, and then, the intracellular release and accumulation of clodronate can induce apoptosis of macrophages.135 Administration of clodrolip depleted TAMs resulted in reduced tumor growth in preclinical models.134,138–140

The benefits of macrophage elimination have not only been seen with clodrolip, but also with other bisphosphonates, such as zoledronate.132 Zoledronate belongs to the third-generation nitrogen-containing bisphosphonate that has been shown to exhibit selective cytotoxicity towards matrix metalloproteinase-9 (MMP9)-expressing TAMs and to impair differentiation of monocytes into TAMs.137 Zoledronate acid (ZA) reduced the infiltration of TAMs, decreased tumor angiogenesis and inhibited tumor progression in different preclinical tumor models.134,138–140

A possible major barrier to this therapeutic approach might be the fact that general depletion of monocytes/macrophages is not TAM-specific and coincides with loss of tissue-resident...
| Mechanism of action | TAM-target | Compound | Clinical phase | Tumor type | Combinational immunotherapy | Results | ClinicalTrial |
|---------------------|------------|----------|---------------|------------|-----------------------------|---------|--------------|
| Elimination         | Zoledronate acid | Zoledronate acid | Phase I/II (Completed) | Kidney Cancer and Lung Metastases | therapeutic autologous lymphocytes and IL-2 | NA | NCT00588913 |
| Recruitment block   | CCR2/5 inhibitor | BMS-813160 | Phase II (Ongoing) | Metastatic Kidney Cancer | IL-2 | NA | NCT00582790 |
| Recruitment inhibition | CCR2/5 inhibitor | BMS-813160 | Phase II (Ongoing) | NSCLC and HCC | Nivolumab (anti-PD-1 mAb) | NA | NCT04123379 |
| Recruitment inhibition | CCR2/5 inhibitor | BMS-813160 | Phase II (Ongoing) | Locally advanced PDAC | Nivolumab (anti-PD-1 mAb) and GVAX (Tumor vaccine) | NA | NCT03767582 |
| Recruitment inhibition | CCR2/5 inhibitor | BMS-813160 | Phase II (Ongoing) | Locally advanced PDAC | Nivolumab (anti-PD-1 mAb) | NA | NCT03496662 |
| Recruitment inhibition | CCR2/5 inhibitor | BMS-813160 | Phase II (Ongoing) | Advanced RCC | Nivolumab (anti-PD-1 mAb) | NA | NCT02996610 |
| Recruitment inhibition | CXCR4 antagonist | BL-8040 | Phase II (Ongoing) | Metastatic pancreatic adenocarcinoma | Pembrolizumab AND Chemotherapy | Tolerable and efficient | NCT02826486 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase I (Completed) | HNSCC | Pembrolizumab (anti-PD-1 mAb) | NA | NCT04058145 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Ongoing) | Advanced pancreatic cancer or CRC | Pembrolizumab (anti-PD-1 mAb) | NA | NCT02337710 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Completed) | Solid tumors, melanoma, NSCLC | Pembrolizumab (anti-PD1 mAb) | NA | NCT02880371 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Ongoing) | Solid tumors | Pembrolizumab (anti-PD1 mAb) | NA | NCT02829723 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase I (Ongoing) | Pancreatic cancer | Pembrolizumab (anti-PD1 mAb) and GVAX (Tumor vaccine) | NA | NCT03153410 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase I (Ongoing) | Solid tumors | Atezolizumab (anti-PD-L1 mAb) | NA | NCT02323191 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase I (Completed) | Advanced solid tumors | RO7009789 (CD40 agonist) | NA | NCT02760797 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Ongoing) | Resectable biopsiable BTC | Nivolumab (anti-PD1 mAb) | NA | NCT03768531 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Completed) | Advanced HCC | Nivolumab (anti-PD1 mAb) | NA | NCT04050462 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase I (Ongoing) | Solid tumors | Nivolumab (anti-PD1 mAb) | NA | NCT02526017 |
| Reprogramming       | CSF-1R inhibitor | Pexidartinib | Phase II (Completed) | Advanced solid tumors | Pembrolizumab (anti-PD-1 mAb) | Tolerable toxicity and moderate efficiency | NCT02713529 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Early Phase I (Ongoing) | Pancreatic cancer | Pembrolizumab (anti-PD1 mAb) | NA | NCT02807844 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Ongoing) | Solid tumors | Pembrolizumab (anti-PD1 mAb) | NA | NCT02554812 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | Solid tumors | Avelumab (anti-PD-L1 mAb) | NA | NCT03558139 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | Solid tumors | Avelumab (anti-PD-L1 mAb) | NA | NCT02890368 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Ongoing) | Urothelial Carcinoma | Atezolizumab (anti-PD-L1 mAb) | NA | NCT02663518 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | Solid tumors | Different kinds of anti-PD-1/L1 mAb | NA | NCT03869190 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Ongoing) | Hematologic Malignancies and solid tumors | Nivolumab (anti-PD-1 mAb) | NA | NCT02890368 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | Recurrent or stage IV melanoma | Tremelimumab (anti-CTLA-4 mAb) | NA | NCT01036353 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | NSCLC and metastatic melanoma | Nivolumab (anti-PD-1 mAb) | NA | NCT03123783 |
| Reprogramming       | Anti-CSF-1 mAb | IMC-CS4 | Phase I (Completed) | Solid tumors | Atezolizumab (anti-PD-L1 mAb) | NA | NCT02304393 |
Macrophage recruitment inhibition

Another TAM-targeting strategy is to cut off their replenishment by circulating monocytes. The recruitment of circulating monocytes is highly dependent on several chemokine signals, and thus, interference with chemokine signaling using monoclonal antibodies or small molecule inhibitors might be an effective way to prevent TAM accumulation in the TME.

CCL2/CCR2 signaling plays a central regulatory role in circulatory monocytes and their infiltration into the TME, making it a promising TAM-targeted therapy. Inhibition of CCL2/CCR2 signaling has shown antitumor efficiency in different experimental animal models. Genetic silencing and administration of a CCL2 neutralizing antibody or CCR2 antagonist reduced the recruitment of circulatory monocytes, subsequently lowered the number of TAMs, and downregulated the secretion function of M2-like TAMs. More importantly, an enhancement of the function of tumor-infiltrating CD8+ T cells and NKs was observed during the treatment, which may suggest a good immunotherapy response. Thus, several phase I/II clinical trials are in progress to assess the therapeutic effect of BMS-813160, a small molecule inhibitor of CCR2/5, in combination with Nivolumab and/or the tumor vaccine GVAX in several solid tumors including HCC, NSCLC, renal cell carcinoma (RCC) and pancreatic ductal adenocarcinoma (PDAC).

Hypoxia-induced upregulation of stromal cell-derived factor 1 alpha (SDF-1α/CXCL12) also contributes to the recruitment of the suppressive M2 macrophages. Inhibition of the SDF-1α receptor (CXCR4) using the CXCR4 antagonist AMD3100 relieved regional immunosuppression and facilitated anti-PD-1 antibody treatment in a sorafenib-resistance HCC model. This study is of great translation value because hypoxia and HIF-1α activation are the most common and significant features of solid tumors and are usually aggravated during conventional treatments including chemotherapy, transcatheter arterial chemoembolization (TACE) and sorafenib treatment. Moreover, inhibition of CXCR4 might have synergistic effects with anti-angiogenesis drugs because TAMs can regulate the expression of CXCR4 via the ERK pathway, have synergistic effects with anti-angiogenesis drugs because they have synergistic effects with anti-angiogenesis drugs. Therefore, CXCR4 antagonists, such as AMD3100 and BL-8040 should be judiciously considered in the future design of clinical trials for immunotherapies.

Although the efficacy of immunotherapies could be enhanced by myeloid cell recruitment inhibition, preclinical evidence from PDAC suggests that the resistance mechanism against this therapeutic approach may lie in the rapid compensation by tumor-associated neutrophils (TANs) and a lack of effect on tissue-resident TAM populations. Moreover, withdrawal of CCL2/CCR2 inhibitors may lead to a dramatically release of monocytes previously trapped within the bone marrow, which was shown to accelerate metastasis in a preclinical model of breast cancer. Although these limitations have not been reported in completed or ongoing clinical trials, considering them in the design of future clinical trials is critical, and alternative targets that overcome these limitations may be required for optimal and stable therapeutic responses.

Macrophage reprogramming

An inevitable drawback to macrophage clearance and recruitment inhibition is the loss of their potential immune-stimulatory role as the major phagocytes and professional antigen-presenting cells (APCs) within the TME. Despite generally being tumor-supportive, TAMs may be phagocytic and suppress tumor growth by activating antitumor immune responses. This suggests that macrophage plasticity can be therapeutically exploited to restore the antitumor properties to TAMs. Thus, switching TAMs toward...
an “immune-supportive” phenotype provides an opportunity to reshape the immune-suppressive or exclusive TME and therefore presents a more effective approach to optimizing current ICI-based immunotherapies. This can be achieved by using therapeutics that promote macrophage polarization and/or using nanoparticles that can selectively reprogram macrophages to a restorative phenotype.130

Restoring phagocytic capacity. In homeostasis, normal cells can avoid self-elimination by phagocytes through the expression of anti-phagocytosis molecules,154,155 which are therefore called “phagocytosis checkpoints.” However, many studies have shown that tumor cells depend even more on phagocytosis checkpoints to evade immune surveillance.156 Therefore, identification and intervention with phagocytosis checkpoints might provide a new approach for restoring the phagocytic capacity of TAMs to eliminate tumor cells.157

Signal regulatory protein alpha (SIRPα) is an ITIM-bearing inhibitory receptor expressed on myeloid cells, including macrophages.157 SIRPα recognizes CD47, which acts as a “don’t eat me” signal and is found to be overexpressed tumor cells and correlate with patients’ poor survival.158,159 Macrophage phagocytosis of tumor cells was restored after treatment with CD47 antibodies,160 and this macrophage-mediated phagocytosis was further enhanced in the presence of chemotherapeutic drugs, suggesting that patients with lower CD47 expression were more likely to benefit from adjuvant TACE treatment.158 It is worth noticing that CD47 is highly expressed in CCA.161 Interfering with the CD47-SIRPα interaction promotes phagocytosis in TAMs and consequently suppresses the progress of CCA.161 The unique overexpression of CD47 in CCA offers an exceptional opportunity for CD47-targeted therapy.

The bridging between innate and adaptive immune cells provides the rationale for combining phagocytosis checkpoint inhibitors with current ICI-based immunotherapies that boost the adaptive immune response.152 The potential for such combinations was initially observed when anti-CD47 therapy was shown to have synergistic effect with PD-L1 inhibitor in a mouse model bearing the B16F10 melanoma.162 Similarly, a bispecific antibody targeting PD-L1 on tumor cells and SIRPα on APCs showed a more significant antitumor effect against murine colon cancer compared with either anti-PD-1 or anti-SIRPα monotherapy.163 Overall, these preclinical results along with earlier observations in ICI-resistant cancer models confirm the notion that the conventional boundary between the innate immune checkpoint and adaptive immune checkpoints is becoming unclear, because more of these checkpoints have been found to function at both the innate and adaptive levels.157

Unleashing the immune-stimulatory capacity. The CSF1/CSF1R axis has been heavily investigated for its role in defining the survival, proliferation, differentiation and function of macrophages.164-166 Targeting CSF1/CSF1R signaling in protumoral TAMs represents an attractive strategy to eliminate CSF1R-dependent or reprogram M2-like TAMs.167 The altered TAM’s polarization will be key to reshaping the immunosuppressive TME and boosting a preexisting antitumor immune response.168 In preclinical models, CSF1/CSF1R blockade has been shown to improve the efficacy of different immunotherapies, including ICIs and adoptive cell transfer therapy.169-171 The positive results of these studies have led to clinical trials combining CSF1 and/or CSF1R inhibitors with ICIs or other immunotherapies.172,173 CD40, a receptor that belongs to the TNF receptor superfamily, is primarily expressed on APCs. The CD40-CD40L interaction upregulates the expression of MHC and promotes the secretion of pro-inflammatory cytokines, such as IL-12, which plays a significant role in T cell priming.129 Macrophage treatment with CD40 agonists in combination with anti-CSF1R antibodies resulted in profound TAM reprogramming before their depletion; these reprogrammed TAMs created a pro-inflammatory environment that elicited effective T cell responses, even in tumors that were nonresponsive to ICIs.174,175

Phosphatidylinositol 3-kinase γ (PI3Kγ) acts as a molecular switch that turns on an “immunosuppressive program” while shutting down “immune-stimulatory program”.176 Kaneda et al. showed that PI3Kγ determines the immunosuppressive properties of TAMs.177 It was shown that the lack of PI3Kγ activity in TAMs induced the expression of MHC-II and pro-inflammatory cytokines while reducing the immunosuppressive molecules including IL-10 and arginase.177 This dramatic shift of TAMs also enhanced adaptive immunity in the TME and significantly inhibited tumor progression.177 Another critical study by De Henau et al. also showed the potential of targeting myeloid-intrinsic PI3Kγ in overcoming ICI resistance.178 Further analysis is required to determine whether PI3Kγ inhibition could exert similar immunomodulatory function in other solid tumors.

The Lmdd-MPFG (LM) vaccine activates the NF-κB pathway in TAMs through the Toll-like receptor (TLR)2-MyD88 pathways, and recruits p62 to activate the autophagy pathway.179,180 The overall effect of LM skews the TAMs from the M2-like state into the M1-like state.181 Most importantly, this approach skewed the TME cytokine profile to anti-tumor profile, and this change restored the T cell reactivity to the anti-PD-1 blockade.180

Nanoparticles in the optimization of macrophages reprogramming. Systemic targeting of TAMs using nanomedicines is an attractive approach because TAMs are ideal therapeutic targets due to their considerable propensity to phagocytose nanoparticles.182 Notably, it has been reported that myeloid cells could take up ten-fold more nanoparticles than tumor cells in a preclinical model.183 Several recent studies have used nanoparticles loaded with TLR agonists or tumor peptides to promote reprogramming of the TAMs, exploiting the capacity of nanoparticles to both target TAMs and promote antitumor immunity. For example, the TLR7/TLR8 agonist R848 loaded nanoparticles preferentially accumulated in TAMs in mouse models and promoted macrophage reeducation.184 Another study based on immunotherapy resistance tumors showed that codeelivery of a long peptide antigen, which induced antigen-presenting activity of TAMs, and TLR agonists to TAMs using a nanosized hydrogel (nanogel) can transform the resistant tumors into tumors sensitive to adaptive immune cell transfer.185 However, the development of TAM-reeducating therapies based on nanoparticles is still facing great challenges, such as how to preferentially deliver them to the protumoral M2-like TAMs or how to acquire a long-lasting and sufficient antitumor response. Fortunately, engineering of new nanomedicines provides new opportunities by (1) applying nanoparticles modified with ligands that could recognize M2 TAM’s specific markers to achieve target delivery; and (2) preparing nanoparticles to reduce the number of TAMs in the tumor via specific cytotoxicity, or reeducating TAMs in a long-lasting manner with the carriers possessing drug controlled release properties.186 Effective development of such nanomedicines could lead to a breakthrough in the field of tumor immunotherapy.

CONCLUSION AND PERSPECTIVES

TAMs are primary immune cells within the TME with high heterogeneity and complex roles as regulators of tumor immunity and immunotherapy. Thus, it is fundamental to reveal their exact regulatory mechanisms and identify macrophage-specific targets to optimize the efficacy of current immunotherapies. Recent studies have partially revealed the regulatory mechanisms and have highlighted three major TAM-targeting strategies: macrophage elimination, recruitment inhibition and reprogramming.
Early clinical trials have focused on the first two approaches. Regardless, organ homeostasis disruption induced by resident macrophages and the potential metastasis-promoting withdrawal reaction remain key barriers to practical application in clinical settings. Going forward, a better strategy for macrophage reprogramming that attenuates their immune-suppressive ability while enhancing their potential immune-stimulatory functions is favorable for current ICI-based immunotherapy. However, the actual synergistic effect of macrophage-reprogramming agents, such as PI3K inhibitors and CD40 agonists needs further evaluation. Moreover, macrophage reprogramming using nanoparticles has therapeutic potential in several preclinical models, but nanoparticle efficiency, safety and tolerability should be carefully evaluated in the human body.

Despite the recent progress in clinical and preclinical studies, some questions remain unanswered. For example, studies have highlighted the molecular events and signaling pathways of TAMs. Still, less is known about the intracellular metabolic switch during tumor progression and its potential impact on immunotherapy. Novel checkpoint receptors, such as T Cell Immunoglobulin and TIM domain (TIGIT), VISTA or Lymphocyte-activation-gene-3 (LAG-3), have attracted broad interest, but what is the significance of macrophage populations expressing these different checkpoint receptors? Finally, because macrophages in the digestive system are direct sentinel cells for changes in the gut microbiota, understanding the exact mechanisms of these interactions and their consequences could potentially aid in tailoring an antitumor microbial cocktail. This concept is based on emerging studies suggesting that the manipulation of the gut microbiome can alter cancer incidence and the responses to immunotherapy.180,187–189

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ADDITIONAL INFORMATION

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