



Review

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Roles of macrophages in tumor immunotherapy: metabolism, immune checkpoints, and combination strategies

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Abstract: Macrophages influence anti-tumor immunity through tractable metabolic and checkpoint programs. Within hypoxic, nutrient-competitive tumor beds, tumor-associated macrophages (TAMs) adopt oxidative and lipid-based metabolisms, dampening antigen presentation and T-cell support. Converging evidence shows that lactate flux, fatty acid oxidation (FAO), and glutamine handling steer TAMs toward immunosuppressive states that blunt checkpoint efficacy. Here, we synthesize the mechanism by which discrete pathways—glycolysis/pentose phosphate pathway (PPP) switching, FAO–lysosomal lipolysis, and glutamine– α -ketoglutarate signaling—mechanistically reprogram TAMs. We then map these circuits onto combination strategies involving programmed cell death (ligand) protein 1/cytotoxic T-lymphocyte antigen-4 (PD-(L)1/CTLA-4) blockade, chemotherapy/radiotherapy, and chimeric antigen receptor T-cell (CAR-T). Rather than providing a catalogue, we emphasize when metabolic rewiring dominates, which TAM subsets are affected, and how these levers can be timed or co-inhibited to restore cytotoxic T-cell function. This approach provides operational guidance for pairing macrophage-directed agents with immune checkpoint inhibitors in solid tumors.

Key words: Tumor immunotherapy; Macrophage polarization; Immune checkpoint regulation; Metabolic reprogramming; Combined therapy strategies

1 Introduction

The tumor microenvironment (TME) is a spatially and temporally dynamic network comprising malignant cells, stromal elements, vascular structures, extracellular matrix components, and diverse immune populations, among which tumor-associated macrophages (TAMs) are particularly abundant and influential (Anderson and Simon, 2020). Responding to a variety of local cytokines and metabolic cues, TAMs display marked functional heterogeneity, adopting phenotypes that range from classically activated, pro-inflammatory macrophages with tumor-restrictive activity to alternatively activated, immunoregulatory macrophages that

favor tumor expansion (Cassetta and Pollard, 2020). Classically activated macrophages secrete interleukin-12 (IL-12) and tumor necrosis factor- α (TNF- α), up-regulate antigen presentation, and can suppress tumor cell proliferation. In contrast, alternatively activated populations release IL-10 and transforming growth factor- β (TGF- β), stimulate angiogenesis, facilitate extracellular matrix remodeling, and enhance tumor cell motility, invasion, and metastatic spread (Petty et al., 2019; Liu Q et al., 2020; Wei F et al., 2021; Gao J et al., 2022; Shao et al., 2025). Most advanced cancers have M2-like characteristics in the predominant TAM TME phenotype, which is closely associated with immuno-evasion and disease progression.

TAMs inhibit T-cell activation and secrete growth factors, such as vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF), that promote tumor cell proliferation. They also secrete matrix-degrading enzymes, e.g., matrix metalloproteinase-9 (MMP-9), and recruit regulatory T cells (Tregs), which hinder anti-tumor immunity while enhancing metastatic spread (Chen et al., 2017; Deng et al., 2017; Stöth et al.,

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2019; Zhang et al., 2020; Bourhis et al., 2021; Petty et al., 2021; Song et al., 2024). According to Shao et al. (2025), the link between TAMs' immune functions and metabolism causes metabolic activities in the TME to drive an M2-like phenotype that promotes tumorigenesis. In addition, TAM-regulated immune checkpoints such as programmed cell death protein 1/programmed cell death-ligand 1 (PD-1/PD-L1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) contribute to immune escape (Wei F et al., 2021). Therefore, tumor immunity may be enhanced by effectively manipulating TAMs using metabolic reprogramming, immune checkpoint blockade, or a combination of both.

Recent research suggests that the metabolic environment within the TME can also be reprogrammed. This could influence the functions of immune cells, especially TAMs. M2-like TAMs, for example, frequently depend on fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) to maintain their immunosuppressive phenotype (Jia et al., 2024). It has

also been reported that the metabolic crosstalk between tumors and macrophages—such as the tumor's lactate or fatty acid transfer—promotes immune evasion and tumor progression in a variety of cancers (Chen et al., 2022). These findings suggest that targeting TAM metabolism may be an exciting new therapeutic approach to cancer treatment. Fig. 1 summarizes the polarization, roles, and therapeutic relevance of TAMs in the TME.

2 Macrophage function and metabolic reprogramming

Abnormal metabolic pathways in the TME significantly impact TAM function and polarization, particularly in terms of glucose, amino acid, and lipid metabolism. This metabolic reprogramming plays a crucial role in promoting an immunosuppressive

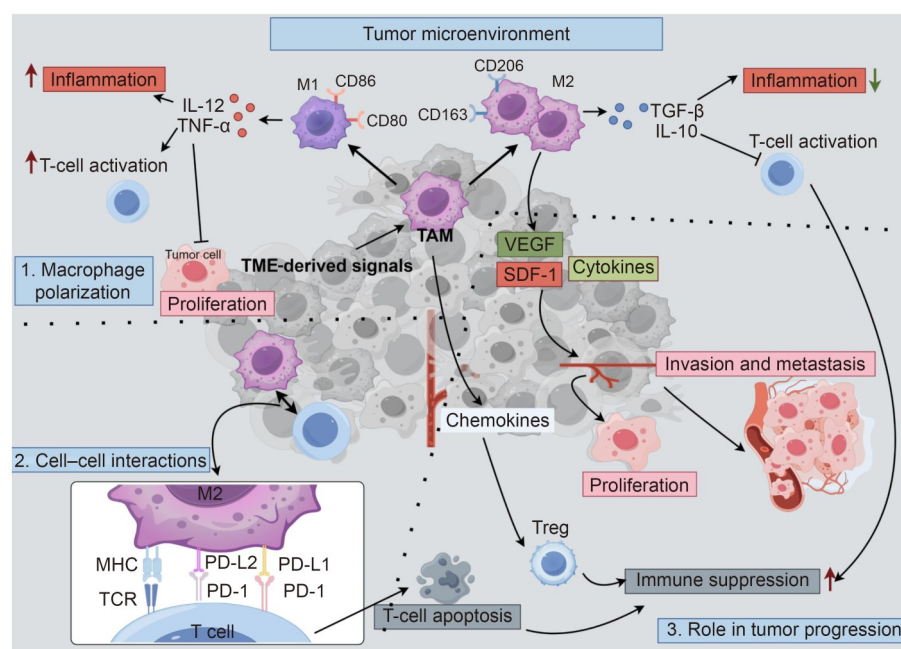


Fig. 1 Roles of tumor-associated macrophages (TAMs) in the tumor microenvironment (TME). TAMs polarize into M1 or M2 phenotype in response to TME signals. M1 macrophages express cluster of differentiation 86 (CD86) and CD80, secrete pro-inflammatory cytokines such as interleukin-12 (IL-12) and tumor necrosis factor- α (TNF- α), and inhibit tumor growth by enhancing T-cell activation and inflammation. In contrast, M2 macrophages, which dominate in the TME, express CD206 and CD163, secrete anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF- β), and promote tumor growth through immunosuppression, angiogenesis, and immune evasion. TAMs also express immune checkpoint molecules, e.g., programmed cell death-ligand 1 (PD-L1) and PD-L2, and secrete angiogenic factors, e.g., vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1 (SDF-1), recruiting regulatory T cells (Tregs) and facilitating tumor progression through multiple pathways, including immune regulation and metastasis promotion. TCR: T cell receptor; MHC: major histocompatibility complex; PD-1: programmed cell death protein-1. Created with Figdraw (<https://www.figdraw.com>).

phenotype in TAMs, ultimately contributing to tumor growth, inhibiting anti-tumor immunity, and facilitating tumor angiogenesis, metastasis, and recurrence (Yang et al., 2024; Wang et al., 2025).

2.1 Glucose metabolism

The TME generates lactate as a result of the high glucose consumption by tumor cells, leading to a hypoxic local environment characterized by low glucose and high lactate levels. These conditions prompt a metabolic shift in TAMs, transitioning their glycolytic processes to OXPHOS and lipid metabolism. The accumulation of lactate within tumors influences the metabolic programming of certain myeloid cells, causing them to lose their immune functions and adopt an anti-inflammatory or immunosuppressive M2 phenotype. This TAM subtype produces IL-10 cytokines, which suppress T-cell activity and facilitate immune evasion (Liao et al., 2021; Zeng et al., 2023; Jin et al., 2025). Recent studies have emphasized the importance of changes in TME glucose metabolism for regulating TAM function, proposing the targeting of metabolic pathways to enhance anti-tumor therapies. Furthermore, during the M2 polarization of TAMs, the long non-coding RNA small nucleolar RNA host gene 17 (*SNHG17*) enhances lactate accumulation by activating phosphoglycerate kinase 1 (PGK1) (Lin JY et al., 2023), which in turn inhibits the anti-tumor function of T cells. Additionally, TAMs compete with cluster of differentiation 8-positive ($CD8^+$) T cells for glucose. This competitive metabolic burnout offers an alternative explanation for TAM-mediated immunity suppression and supports TAM metabolic reprogramming.

The regulation of glycolysis and the pentose-phosphate pathway is crucial for various types of macrophages. M1 phenotypic macrophages predominantly utilize glycolysis, which leads to reduced nicotinamide adenine dinucleotide phosphate (NADPH) production through the oxidative pentose phosphate pathway (PPP) and promotes reactive oxygen species (ROS) production (Kennel and Greten, 2021; Erlich et al., 2022; Tsai et al., 2022). These processes are essential for the anti-tumor effects of M1 macrophages. Inhibition of glycolysis and PPP has been demonstrated to suppress the inflammatory polarization of M1 macrophages, highlighting the importance of these metabolic pathways in macrophage polarization (Tan et al., 2015; Baardman et al., 2018). Conversely, the main metabolic

feature of M2 macrophages is FAO, where glycolysis plays a crucial role in M2 polarization. By inhibiting glycolysis, 2-deoxy-D-glucose (2-DG) effectively blocks M2-like polarization (Covarrubias et al., 2016; Huang et al., 2016; Chiba et al., 2017). However, if mitochondrial OXPHOS remains functional, the impact of glycolysis inhibition on M2 polarization is less pronounced (de-Brito et al., 2020). In conclusion, despite the importance of glycolysis as a metabolic pathway in many cells, its role as an auxiliary pathway in the function of TAMs warrants further elucidation.

Glucose metabolism and glycolysis play a crucial role in the polarization of TAMs. Modifying these metabolic pathways that mediate tumor progression using immunotherapy—specifically by interfering with glucose metabolism or lactate accumulation—represents a promising approach to enhancing the effectiveness of anti-tumor therapies (Fig. 2).

2.2 Lipid metabolism

Lipid metabolism plays a crucial role in regulating TAMs. TAMs primarily rely on FAO and fatty acid synthesis (FAS) to generate energy and sustain their immunosuppressive phenotype, thereby promoting tumor growth and metastasis (Su et al., 2020). TAM polarization is influenced by excessive lipid accumulation, leading to the M2 immunosuppressive phenotype and supporting tumor-mediated immune evasion.

Inhibiting key lipid metabolic drivers, such as fatty acid synthase (FASN) or FAO, suppresses M2 polarization and helps restore innate anti-tumor immunity (Zhao et al., 2021). Endogenous and exogenous fatty acids undergo mitochondrial β -oxidation to produce reduced nicotinamide adenine dinucleotide (NADH), acetyl-coenzyme A (acetyl-CoA), and reduced flavin adenine dinucleotide ($FADH_2$). Acetyl-CoA then joins the tricarboxylic acid (TCA) cycle, producing the reducing equivalents for OXPHOS and adenosine triphosphate (ATP) production. The M2-type TAMs' immunosuppressive phenotype is strengthened by this metabolic program, which promotes tumor progression (Huang et al., 2014). According to Niu et al. (2017), caspase-1-mediated cleavage of peroxisome proliferator-activated receptor gamma ($PPAR\gamma$) can impair FAO and thereby potentiate the pro-tumor functions of TAMs; thus, modulating this axis may reverse the pro-tumor role of TAMs. Xiao et al. (2024) showed that choline-metabolism reprogramming, particularly through methylenetetrahydrofolate

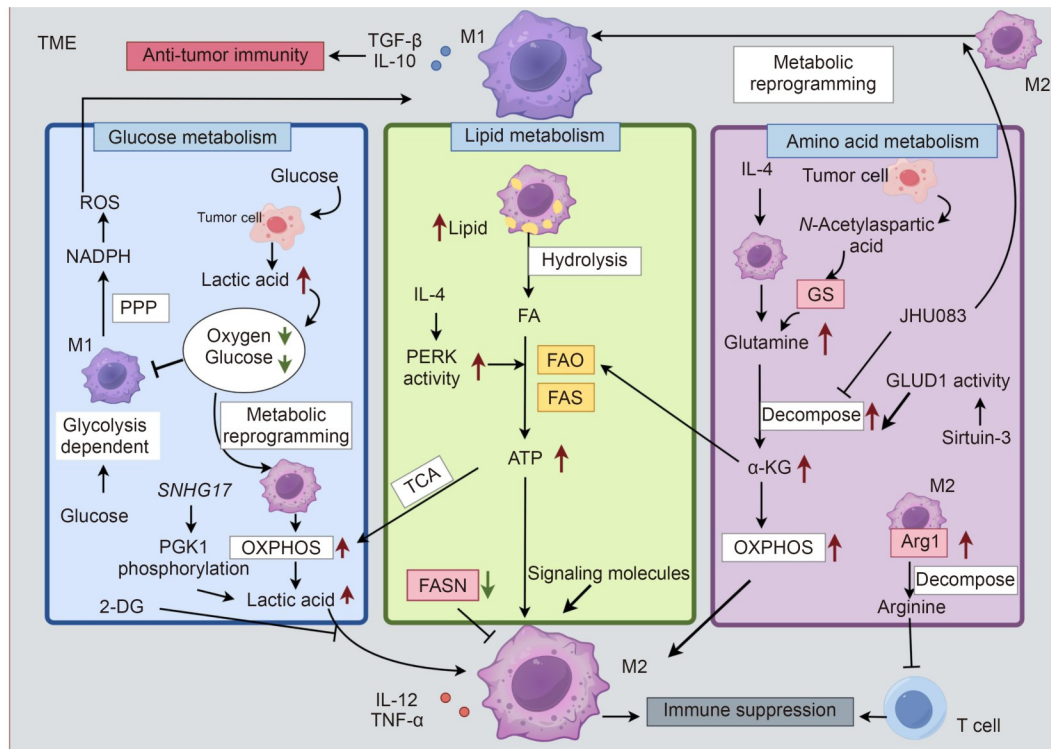


Fig. 2 Metabolic reprogramming of macrophages and immune regulation. In glucose metabolism, tumor-associated macrophages (TAMs) undergo reprogramming toward an M2 phenotype under hypoxic, low-glucose, and high-lactate conditions, favoring oxidative phosphorylation (OXPHOS) and interleukin-10 (IL-10) secretion. M1 macrophages, in contrast, rely on glycolysis, producing nicotinamide adenine dinucleotide phosphate, reduced (NADPH) via the pentose phosphate pathway (PPP) to drive reactive oxygen species (ROS) production for anti-tumor effects. Small nucleolar RNA host gene 17 (*SNHG17*) promotes lactate accumulation, while 2-deoxy-D-glucose (2-DG) inhibits M2 polarization. In lipid metabolism, elevated lipid levels in the tumor microenvironment (TME) promote M2 polarization via fatty acid oxidation (FAO) and fatty acid synthesis (FAS). Inhibiting fatty acid synthase (FASN) or FAO blocks M2 polarization and restores anti-tumor functions. Fatty acids (FAs) undergo β -oxidation to produce acetyl-coenzyme A (acetyl-CoA), which enters the tricarboxylic acid (TCA) cycle to support OXPHOS. IL-4-induced TAMs require protein kinase R-like endoplasmic reticulum kinase (PERK) for maintaining FAO and mitochondrial function, while blocking PERK reduces their immunosuppressive traits. In amino acid metabolism, M2 TAMs express high levels of arginase 1 (Arg1), degrading arginine to suppress T-cell proliferation. IL-4 increases glutamine uptake, promoting M2 polarization, while the glutamine antagonist JHU083 reprograms TAMs to the M1 phenotype. The glutamine metabolism byproduct α -ketoglutarate (α -KG) enhances FAO and OXPHOS, maintaining M2's immunosuppressive role and reducing inflammation. Sirtuin-3, activated by IL-4, enhances glutamate dehydrogenase 1 (GLUD1) activity, promoting glutamine breakdown. Tumor cells induce glutamine synthetase (GS) to enhance M2 TAM function. TGF- β : transforming growth factor-beta; PGK1: phosphoglycerate kinase 1; ATP: adenosine triphosphate; TNF- α : tumor necrosis factor-alpha. Created with Figdraw (<https://www.figdraw.com>).

dehydrogenase 1 (MTHFD1) in TAMs, promotes M2-like polarization and an immunosuppressive micro-environment in non-small cell lung cancer. IL-4-activated TAMs exhibit elevated protein kinase R-like endoplasmic reticulum kinase (PERK) activity, which is vital for sustaining FAO and mitochondrial functionality, as explained by Raines et al. (2022). Blocking PERK results in a decrease in FAO, FAS, and the immunosuppressive activity of TAMs, reprogramming them into an anti-tumor phenotype (Reinfeld et al., 2021; Raines et al., 2022).

Toll-like receptor 9 (TLR9) agonists activate the FAO and lipid synthesis pathways to boost TAM metabolic reprogramming, which may contribute to their anti-tumor effects. Liu et al. (2019) showed that CpG-activated macrophages coordinate carnitine palmitoyltransferase 1A (CPT1A)-dependent FAO with ATP citrate lyase (ACLY)-dependent de novo lipid biosynthesis rather than adopting a classical M1 or M2 program, and that this hybrid metabolic state supports antitumor phagocytic activity. While cholesterol has been implicated in boosting the anti-tumor effects of

TAMs, the role of other lipid species in regulating macrophage function remains largely unknown (Zhao et al., 2024).

2.3 Amino acid metabolism

The immunosuppressive function of TAMs depends on the metabolism of amino acids, specifically arginine and glutamine. M2-type TAMs are marked by high levels of arginase 1 (Arg1), an enzyme that depletes arginine, ultimately inhibiting T-cell proliferation and leading to immune suppression (Biswas and Mantovani, 2012). Tharp et al. (2024) further showed that TAMs initiate a collagen-biosynthesis program, consume environmental arginine, and secrete ornithine, thereby restricting CD8⁺ T-cell function in the TME. Glutamine, the blood's most abundant amino acid, supports the metabolic needs of TAMs by delivering TCA cycle intermediates and participating in the production of the antioxidant glutathione (Yang et al., 2017). Under IL-4 stimulation, glutamine influx increases within macrophages due to the increased expression of glutamine transporters, which favors M2 polarization. M1 macrophages show no effect on polarization under conditions of glutamine deprivation (Jha et al., 2015; Tavakoli et al., 2017).

Recent research has revealed that the glutamine antagonist JHU083 is capable of inhibiting glutamine metabolism, driving TAMs from the immunosuppressive M2 type to the anti-tumor M1 type and enhancing antigen presentation for T-cell activation (Oh et al., 2020; Praharaj et al., 2024). The breakdown product of glutamine metabolism, alpha-ketoglutarate (α -KG), is a key factor in M2 polarization, sustaining the M2 immunosuppressive phenotype by enhancing FAO and OXPHOS (Jha et al., 2015; Liu et al., 2017). Furthermore, a high α -KG/succinate ratio dampens the activity of the nuclear factor-kappa B (NF- κ B) signaling pathway, thereby inhibiting the expression of inflammatory genes (Liu et al., 2017).

Research demonstrates that actions targeting glutamine metabolism alter the metabolic state of TAMs and enhance anti-tumor immune activity within the TME (Zhou et al., 2022). The mitochondrial protein Sirtuin-3, which is dependent on IL-4, stimulates the function of glutamate dehydrogenase 1 (GLUD1) to accelerate glutamine degradation, thereby promoting M2 macrophage activation (Zhou et al., 2022). *N*-Acetylaspartate, secreted by tumor cells, can enhance

glutamine synthetase (GS), which improves the M2 functionality of TAMs (Menga et al., 2021). Deleting GS in macrophages in mouse models reprograms TAMs to an anti-tumor M1-like state (Palmieri et al., 2017; Menga et al., 2020), further supporting the competition between TAMs and tumor cells for glutamine (Lyu et al., 2025).

Many amino acids, beyond arginine or glutamine, affect the phenotype of TAM and its function. These include tryptophan and branched-chain amino acids (BCAAs). BCAAs are involved in protein synthesis, energy production, and the regulation of cell growth (Hilt and Morrell, 2020; Plotkin et al., 2021). High BCAA levels can increase the risk of heart disease and cause overly agitated platelets (Hilt and Morrell, 2020). In some lung cancer models, TAMs further support a TME populated by Tregs, which may limit the efficacy of immunotherapies. Combining TAM targeting with anti-angiogenic therapies and immune checkpoint blockade shows the potential to improve outcomes (Martinez-Usatorre et al., 2021). Recent advancements in imaging techniques, such as fluorine-19 magnetic resonance imaging (¹⁹F MRI), allow for noninvasive, longitudinal monitoring of TAMs in gliomas, providing insights into their spatial and temporal dynamics during disease progression and treatment (Crocì et al., 2022).

2.4 Purine metabolism

TAMs undergo metabolic reprogramming in the TME, switching from lipid metabolism to purine metabolism. TAMs exhibit enhanced purine metabolism, which advances the tumor phenotype and makes them less responsive to immune checkpoint inhibitors (ICIs). TAMs exhibit activation of purine metabolism, reduced expression of genes involved in phagocytosis and antigen presentation, and increased expression of immunosuppressive and angiogenic genes (Zhao et al., 2024). Macrophages induced by granulocyte-macrophage colony-stimulating factor (GM-CSF) exhibit M1-like activity, whereas those differentiated in the presence of macrophage colony-stimulating factor (M-CSF) are skewed toward the M2 phenotype. The purinergic pathway can drive TAMs toward the M2 phenotype, as ectonucleotidases such as CD39 and CD73 convert extracellular ATP into immunosuppressive adenosine. This can cause macrophages to adopt the immunosuppressive M2 phenotype. As a result, the

secretion of epithelial-mesenchymal transition (EMT)-related cytokines, including IL-1 β , IL-6, IL-10, and VEGF, further enhances immune suppression in the TME (Ohradanova-Repic et al., 2018).

The generation of adenosine via purine metabolism is an important mechanism by which macrophages suppress immunity. As adenosine builds up in the TME, it binds to A_{2A} receptors, blocking their activation and promoting TAM differentiation into an immunosuppressive M2-like cell phenotype. Together, these activities inhibit anti-tumor responses and facilitate malignant growth. Research suggests that the glutamine antagonist JHU083 reduces purine metabolites within TAMs, resulting in a decrease in adenosine production. These findings show that decreased IL-10 expression contributes to reprogramming TAMs toward an anti-tumor M1 phenotype, with enhanced antigen presentation and T-cell activation (Praharaj et al., 2024; Lyu et al., 2025). Blocking the purine metabolism pathway (primarily adenosine production) could be a potential strategy for reprogramming TAMs to inhibit their immunosuppressive properties or to counteract tumor immunity (Praharaj et al., 2024; Lyu et al., 2025).

Purine metabolism plays a role in TAM polarization. Research has shown that isocitrate dehydrogenase 3 α (IDH3 α) regulates xanthine oxidoreductase (XOR), which modifies polarization to a suppressive TAM phenotype. XOR is responsible for purine catabolism, which produces ROS and uric acids that accumulate in the TME. Increased ROS production activates IDH3 α , and macrophage polarization occurs through M2 state macrophages (activated macrophages). M2 TAMs produce a microenvironment favorable to tumors, thereby inhibiting anti-tumor immunity and promoting hepatocellular carcinoma (HCC). As a result, the purine metabolism pathway of XOR–IDH3 α interferes with TAMs, thus serving as an immunodynamic target, offering a new immunotherapeutic technique for treating HCC (Lu et al., 2023).

3 TAMs in immune checkpoint regulation and checkpoint blockade therapy

3.1 Interaction between macrophages and immune checkpoints

Immune checkpoints help to maintain self-tolerance and avoid excessive immune activation,

which can damage normal tissues. The immune system includes checkpoint receptors, which inhibit immune activity. Cancer immunotherapy has been revolutionized by ICIs such as PD-1, PD-L1, and CTLA-4 in recent years. However, it is not only tumor cells that are involved in the regulation of immune checkpoints; macrophages, particularly TAMs within the TME, are also involved in this process (Xu SM et al., 2023).

TAMs are one of the most critical types of immunosuppressive cells in the TME. They have the ability to generate immune checkpoint molecules (such as PD-L1) that bind to T cells and suppress their activity. PD-L1 binds to the PD-1 receptor on the surface of T cells, sending an inhibitory signal that hinders T-cell activation. This interaction between PD-L1 and PD-1 diminishes the anti-tumor functions of T cells, facilitating immune evasion (Chen and Mellman, 2013). Liu YT et al. (2020) showed that, among immune cells, PD-L1 is predominantly expressed on macrophages and macrophage PD-L1 is associated with outcome after PD-1 pathway blockade. TAMs also release immunosuppressive cytokines, such as IL-10 or TGF- β , which hamper T-cell function and promote the expansion of Tregs. This immunosuppressive microenvironment not only suppresses the anti-tumor response of T cells but also impedes the effectiveness of ICIs (Binnewies et al., 2018).

Other studies have produced more data on numerous methods for controlling TAMs. Xu C et al. (2023) found that TAMs release IL-1 β , causing the expression of PD-L1 in tumor cells and diminishing the efficacy of immune checkpoint blockade treatment. Other research has produced similar results. Therapies aiming to bolster the IL-1 β pathway may be instrumental in augmenting the impact of checkpoint blockades. Tsuruta et al. (2022) confirmed that TAMs modulate anti-tumor activity through the circadian rhythm of PD-1 and differentiated embryo-chondrocyte expressed gene 2 (DEC2). In addition, PD-L1 expression is regulated in TAMs through the myeloid differentiation primary response gene 88 (MyD88)/interleukin-1 receptor (IL-1R) axis via the NF- κ B pathway, and targeting this pathway in combination with anti-PD-1 monoclonal antibodies significantly reduces tumor burden (Tartey et al., 2021).

Beyond the PD-1/PD-L1 pathway, recent research has identified other immune checkpoints involved in the TAM regulation. Tang et al. (2024) found that different TAM subtypes play diverse roles in various

immune responses, confirming the importance of TAM heterogeneity for the efficacy of immune therapy for colorectal cancer patients. Liu et al. (2024) identified a link between apolipoprotein E-positive (ApoE⁺) macrophages and clinical resistance to immune checkpoint blockade therapy, a finding also supported by single-cell analyses, with implications for targeting specific macrophage subgroups in future therapies.

3.2 Combined use of macrophages and immune checkpoint blockade therapy

TAMs are critical for controlling immune checkpoints. They can also regulate the effectiveness of immune checkpoint blockade therapy based on both their presence and functional status. However, high levels of TAMs in the TME correlate with checkpoint inhibitor ineffectiveness due to an immunosuppressive environment upheld by TAMs (Binnewies et al., 2018). Studies on breast cancer have reported that depleting TAMs markedly enhances the effect of PD-1/PD-L1 blockade, promoting T-cell infiltration into the tumor and decreasing metastasis (Lin et al., 2018). Recent studies have also identified specific immunomolecules within TAMs that can resist immune checkpoint blockade therapy. Xu et al. (2022) suggested that blocking CD89 on TAMs may be an effective method for reversing resistance to immunotherapy, providing a theoretical basis for developing anti-tumor strategies. Homicsko et al. (2023) also demonstrated a strong correlation between macrophage PD-L1 expression and lack of response to checkpoint blockade therapy. Peckert-Maier et al. (2023) noted that CD83 is a critical immune checkpoint molecule that regulates the resolution of inflammation. Targeting CD83 on macrophages may represent a novel approach to utilizing immunotherapy against cancer. Therapeutic strategies such as blocking PD-L1 on TAMs and other immune checkpoint molecules may therefore be promising approaches to cancer treatment (Jelinic et al., 2018; Jing et al., 2020; Park et al., 2021).

In recent years, various clinical trials have evaluated the effects of combining ICIs (e.g., PD-1/PD-L1 blockers) and TAM-targeting therapies, revealing both the potential and the limitations of these approaches for treating different cancers. Relevant clinical studies have investigated a new combination of PD-1 inhibitors with colony-stimulating factor 1 receptor (CSF1R) inhibitors—CSF1R is principally implicated in macrophage survival and polarization. TAMs exhibit high

expression of CSF1R, which plays a central role in promoting immune suppression within the TME. Inhibition of CSF1R reduces the accumulation of immunosuppressive TAMs, enhancing anti-tumor immune responses (Cannarile et al., 2017; Fujiwara et al., 2021). Wainberg et al. (2016) reported a phase I/IIa dose-finding protocol of pexidartinib plus pembrolizumab in advanced solid tumors (NCT02452424); safety and recommended-phase-2-dose exploration were the stated aims, and efficacy endpoints were not presented in that citation.

A preclinical investigation assessed the use of a CSF1R inhibitor (BLZ945) in combination with an anti-PD-L1 agent in an animal model of malignant mesothelioma. The authors demonstrated that the blockade of CSF1R M2 polarization of TAMs enhanced the anti-tumor effect of the PD-L1 inhibitor. This combined therapy effectively stimulated the activation of tumor-infiltrating CD8⁺ T cells and boosted the anti-tumor immune response (Magkouta et al., 2021). A study involving hepatocellular cancer mouse models revealed significant anti-tumor activity of a combination of a CSF1R inhibitor and a PD-L1 inhibitor. The formulation inhibited TAM polarization and migration, promoted T-cell invasion into the tumor, and substantially increased animal survival. Together, both experimental CSF1R and PD-L1 blockade appear to be promising approaches to targeting immunosuppressive tumors (Zhu et al., 2019). However, CSF1R-targeted therapies have limitations. CSF1R inhibition, particularly with pexidartinib, has been shown to impact dendritic cell (DC) differentiation through FMS-like tyrosine kinase 3 (FLT3) signaling blockade. Since DCs play an essential role in T-cell activation and the optimal anti-tumoral response, this unintentional modulation may compromise the therapeutic impact of ICIs, such as durvalumab, in advanced malignancies. Therefore, although CSF1R inhibitors are potent in modulating TAMs, it is important to consider their potential effects on other immune cell subtypes, including DCs, when using them in combination approaches to avoid unintended immune suppression (Voissière et al., 2024).

3.3 Targeting TAMs to enhance the efficacy of immune checkpoint blockade

Reprogramming and depleting TAMs can increase the effectiveness of immune checkpoint blockade therapies. For example, Owaki et al. (2024) demonstrated

that preconditioning cancer-associated fibroblasts (CAFs) and macrophages with synthetic retinoids enhanced cancer responses to checkpoint blockade. Shimizu et al. (2024) demonstrated that concomitant targeting of CSF1R with the inhibitor pexidartinib, in combination with checkpoint inhibitors, resulted in the depletion of CAF-induced M2 macrophages, leading to tumor immunity in a colorectal cancer model. Additionally, previous studies have demonstrated that the depletion of TAMs, through the treatment with CSF1R inhibitors such as PLX3397 or BLZ945, either reduces tumoral infiltration of TAMs or improves the effects of anti-PD-1 monoclonal antibodies in anti-tumor therapy (Peranzoni et al., 2018; Magkouta et al., 2021; Sato et al., 2025). In previous studies on animals, PLX3397 has been shown to reduce TAMs and enhance the power of PD-1/PD-L1 blockade. BLZ945 not only decreased CD163⁺ macrophages but also promoted the polarization of M1 macrophages, thereby enhancing the immune response (Cui et al., 2020; Chen et al., 2025). Further studies have shown that the combination of hydroxychloroquine with anti-PD-1 antibody increases the M1 macrophage proportion in certain models, thus improving anti-tumor immunity (Sharma et al., 2020; Wang et al., 2021). Metformin-loaded mannose-modified macrophage-derived microparticles similarly reprogram M2-like TAMs toward an M1-like state and enhance the efficacy of anti-PD-1 therapy (Wei ZH et al., 2021).

Recent research has indicated that combining treatments aimed at TAMs with anti-PD-1, anti-PD-L1, and anti-CTLA-4 ICIs may have a synergistic impact on tumors. TAMs that express the immune checkpoint molecule PD-L1 directly hinder T-cell activities. In addition, TAMs secrete immunosuppressive cytokines, thus facilitating immune evasion in the TME. Consequently, an increasing trend in research is the development of an integrated therapeutic strategy that targets TAMs and ICIs. Modulation of the TME can further augment the efficacy of immune checkpoint inhibition, driving a synergistic anti-tumor effect (Ostuni et al., 2015).

Co-administration of DB071 and BLZ945 results in a lower proportion of type 2 TAMs present in the TME and improved immune therapy efficiency. This combination treatment can block the growth of a tumor and restore CD8⁺ T-cell activity (Cui et al., 2020). Additionally, preclinical studies have yielded

promising results for antimetastatic strategies that aim to prevent TAM recruitment or reprogram TAM polarization toward M1 macrophages (Wei F et al., 2021). Research by Lin WW et al. (2023) indicated that *ADAR1* (one major adenosine deaminase acting on RNA) downregulation and interferon-gamma (IFN- γ) treatment of macrophages alter the TME and increase the efficacy of immune checkpoint blockade. Furthermore, Zuo et al. (2023) showed that dipeptidyl peptidase 4 (DPP4) inhibitors may reduce TAM numbers and improve the anti-tumor efficacy of anti-PD-L1 therapy in non-small cell lung cancer.

3.4 TAMs, tumor burden, and immune evasion

The number and diversity of TAMs within the TME are closely associated with tumor immune evasion and therapy response. Wen et al. (2023) suggest that TAMs promote an immunosuppressive environment via the insulin-like growth factor-binding protein 2 (IGFBP2)–signal transducer and activator of transcription 3 (STAT3)–PD-L1 pathways, which dampens ICI efficacy. Targeting this signaling pathway may offer new ways to overcome immunotherapy resistance.

3.4.1 TAMs in combination with chemotherapy and radiotherapy

Chemotherapy and radiotherapy are traditional cancer treatment strategies, but their efficacy is often influenced by immunosuppressive cells within the TME, particularly TAMs. Studies have shown that radiotherapy and chemotherapy not only kill tumor cells directly, but also induce “immunogenic death,” enhancing the immune system. These treatments can create a microenvironment that is immunosuppressive and polarizes TAMs to the immunosuppressive M2 type, thereby weakening anti-tumor immunity. At the same time, strategies targeting TAMs to enhance responses to chemotherapy and radiotherapy, including the inhibition of their recruitment or reprogramming of their function, are emerging. These strategies can mitigate the suppressive role of TAMs, thereby potentiating the effects of chemotherapy and radiotherapy (Mantovani et al., 2013; Galluzzi et al., 2017).

Research has shown that chemotherapy agents, such as paclitaxel, induce PD-L1 expression on macrophages by activating the NF- κ B signaling pathway, thereby promoting immune evasion via the suppression

of naive T cells. This phenomenon elucidates the mechanism of TAM-mediated chemo-immunosuppression (Roux et al., 2019). Radiotherapy has a similar impact on the immunosuppressive activity of TAMs, causing the immunogenic death of tumor cells and initiating the expression of PD-L1 on macrophages (Wan et al., 2020). As a result, employing therapeutic strategies to reprogramme their activity or inhibit their activation may diminish these pro-tumorigenic effects, which strengthens the rationale for integrating chemotherapy, radiotherapy, and immunotherapy.

Recent studies have shown that a newly developed liposomal topotecan (FF-10850) optimizes the properties of TAMs for drug delivery within the TME. According to Shimoyama et al. (2023), this drug is safe for use in humans and can be delivered directly into the tumor site, increasing T-cell anti-tumor activity by reducing TAMs that suppress immunity. Additionally, Choi et al. (2024) discovered that paclitaxel enhances PD-1 inhibitor efficacy by regulating the polarization of TAMs in breast cancer. This suggests the potential for improving the synergy between chemotherapy and immunotherapy by modulating TAMs. In a model of head and neck cancer, researchers found that an increase in C-C motif chemokine ligand 2 (CCL2) following radiotherapy was associated with the recruitment of monocytes, which subsequently differentiated into TAMs. These TAMs then secreted factors that conferred resistance to radiotherapy. Blocking monocyte recruitment using a C-C motif chemokine receptor 2 (CCR2) inhibitor improved radiotherapy outcomes by limiting TAMs and increasing the tumor control rate and survival (Mondini et al., 2019). The regulation of TAMs' function and quantity can significantly enhance the anti-tumor effects of chemotherapy and radiotherapy when combined with immune checkpoint blockade therapy. This opens up new avenues and possibilities for treating cancer in the future.

3.4.2 TAMs and CAR-T therapy

While chimeric antigen receptor T-cell (CAR-T) therapy is highly successful in treating hematologic cancers, it is less effective in solid tumors due to the immunosuppressive TME. TAMs in the TME can inhibit CAR-T cell activity by secreting cytokines or co-opting the checkpoint receptors. Attacking and inhibiting TAMs, or reprogramming them to M1, can enhance the efficacy of CAR-T cells in solid tumors.

Humanized mouse models have been widely used to study macrophage-T cell interactions and provide a valuable system for investigating such mechanisms (Rongvaux et al., 2014). Zhu et al. (2024) suggest that re-educating TAMs and microglia enhances CAR-T cell glioblastoma treatment. According to Anami et al. (2024), loss of sinusoidal macrophage function in the tumor host promotes resistance to immunotherapy. Adjusting macrophages (for example through inhibition or reprogramming) may help overcome resistance to CAR-T therapy in solid tumors, as supported by studies showing enhanced T-cell infiltration and anti-tumor responses upon macrophage modulation (Singh et al., 2022; Zhu et al., 2024).

TAMs are a key component of combination therapies. Combining TAM-targeting approaches with other existing treatments, such as chemotherapy, radiotherapy, ICIs, and CAR-T cells, may help improve therapeutic efficacy and induce anti-tumor immune responses in patients. Recent research indicates that combination therapies can be optimized through TAM metabolic pathways, immune regulatory functions, and interactions with competitor cells. Thus, future studies should investigate how to more effectively use TAM heterogeneity, set up combinatorial strategies, and leverage the potential of TAMs in the context of cancer immunotherapy.

4 Methodological analysis

Recent studies have used various approaches to explore the role of TAMs in cancer immunotherapy. With the rapid development of these research methods, researchers are using new strategies to reveal the functions of TAMs within the TME. This paper presents a review of the literature to illustrate how such techniques are studied.

4.1 In vivo and in vitro experimental models

Historically, humanized immune mouse models have been critical for studying the role of TAMs within the TME. Some studies have attempted to improve the understanding of the human TME by transplanting human immune cells into immunodeficient mice. This approach has also been used to evaluate combinations and T cell receptor (TCR) responses to immune checkpoint blockade therapy. One example is the research

by Zhu et al. (2024), who built humanized immune mouse models to study the efficacy of a combination of CAR-T and TAM reprogramming therapy in treating glioblastoma. Other preclinical models have shown promising results for combinations of TAM-targeting therapies with conventional therapies such as chemotherapy and radiotherapy. In a mouse model of triple-negative cancer, cyclophosphamide inhibitors and CSF1R, together with low-dose chemotherapy, significantly increased T-cell and B-cell infiltration into tumors and reduced the immunosuppressive effect in TAMs. To study TAM heterogeneity post-treatment, high-dimensional imaging techniques were deployed, revealing that breast cancer subtypes activate TAMs via different mechanisms. TAM regulation has the potential to enhance the effectiveness of chemotherapy, as demonstrated by in vivo proof-of-concept data (Singh et al., 2022).

In head and neck cancer models, TAM recruitment was systematically inhibited following radiotherapy. A CCR2 inhibitor was used to reduce TAMs and improve the therapeutic efficacy of radiotherapy. The experimental results showed improvements in both the tumor control rate and survival with the decrease in TAMs (Mondini et al., 2019). The in vivo experimental data confirm the essential role of TAMs and provide critical support for testing therapeutic strategies targeting TAMs in future clinical trials.

Three-dimensional co-culture systems are commonly used in vitro to facilitate the mimicry of in vivo niches and to more accurately study the interactions between TAMs, tumor cells, and T cells. These systems usually include tumor cells, immune cells, and stromal cell types, which are then grouped into multiple cell types to investigate TAM polarization and metabolic changes. Three-dimensional co-culture systems are widely used in vitro to more accurately recapitulate the TME and to investigate interactions between TAMs, tumor cells, and T cells (Ravi et al., 2015).

4.2 Emerging technologies for TAM typing and their principles

Extensive research has been conducted on the heterogeneity of TAMs within the TME. Modern techniques, such as spatial transcriptomics and single-cell RNA sequencing (scRNA-seq), have been used extensively to unravel the TAM puzzle. scRNA-seq can be used to identify immune checkpoint-related molecule

expression and further distinguish TAM subtypes. This method is also helpful in determining the contribution of TAM subtypes to the effectiveness of immune-checkpoint blockade therapy. Spatial transcriptomics helps identify where TAMs are found in the TME and where they interact, revealing the complex interaction networks between TAMs, tumor cells, and T cells (Wang et al., 2025).

4.2.1 How do emerging technologies precisely type TAMs?

Recognizing inflammation within the TME is crucial to the accurate classification of TAMs. Recent technologies, including scRNA-seq or spatial transcriptomics, enable novel investigations into the functional states of different TAM subgroups and their roles in tumor immunity.

4.2.2 Principles of scRNA-seq

scRNA-seq reveals the transcriptomes of single cells, thus revealing each cell's gene expression profile. With this technology, we can detect TAM heterogeneity inside tumor tissues and create a gene expression map at multiple time points for each cell. scRNA-seq may reveal the functional differences of TAM subgroups and determine their immune suppression or activation potential by analyzing immune checkpoint-related molecules. Using this strategy, macrophages can be grouped into functional categories such as the anti-tumor-associated (M1-like) and tumor-associated (M2-like) TAMs (Yan et al., 2022). A more detailed classification of the different TAM subtypes will be beneficial for designing therapies targeted at specific subtypes. Further studies will help improve the classification.

4.2.3 Principles of spatial transcriptomics

Spatial transcriptomics aims to capture tissue section-unique information in gene expression form. By examining RNA expression levels in specific areas of tumor tissue, the spatial distribution of TAMs in the TME can also be analyzed. This technology addresses a major limitation of individual cell RNA sequencing by providing insight into the spatial interplay between neighboring cells. Spatial transcriptomics can illustrate how TAMs interact with other immune cells (for instance, T cells) and tumor cells throughout various tumor regions. TAMs, for example, may

exhibit anti-tumor activity in the tumor margin but may have immunosuppressive functions within the tumor core (Li QY et al., 2022). Thus, the utilization of this technology further elucidates TAM function within the TME and suggests innovative approaches to target them in niche sites.

4.2.4 Multi-omics data integration and computational biology tools

Images of the TME can be integrated using various techniques, including several forms of scRNA-seq with spatial transcriptomics. The rationale for such integration is to connect different sources of omics data (transcriptomics, epigenomics, etc.) through computational biology paradigms to model the dynamic behavior of TAMs. These data are also analyzed using machine learning algorithms to identify novel TAM subpopulations and therapeutic targets. An example of this is the XYSeq platform, which merges scRNA-seq data with spatial metadata to accurately pinpoint both the TME localization of cells and their gene-expression features, and to correlate these features with infiltration of specific TAM subsets (Lee et al., 2021).

Integrating the results of scRNA-seq with spatial transcriptomic analysis will generate a whole-scale distribution map of TAMs and their functional differences in the corresponding microenvironments. This model offers the potential to use computational biology alongside the prediction of TAM subgroups' dynamic behavior to achieve more granular-scale data to improve personalized therapies using immune checkpoint blockade. As Saqib et al. (2023) suggest, integrating these multi-dimensional data can lead to a more precise characterization of the spatial and functional heterogeneity of TAMs, providing explicit directions for therapeutic strategies targeting specific TAM subpopulations.

Additionally, advanced research methods such as multiplex immunofluorescence and flow cytometry are used to evaluate immune infiltration in the TME and the characteristics of different TME populations to explore the response to checkpoint blockade immunotherapy (CBI). de León-Rodríguez et al. (2024) used multiplex immunofluorescence and spectral cytometry to compare the dynamic changes in tissue-resident memory (TRM) CD8 T cells and classical dendritic cells type 1 (cDC1) between two groups of patients with melanoma: those who received immunotherapy and those who did not. They concluded that TRM T

cells were heterogeneous and could be divided into T cell factor 1-positive (TCF1⁺) or TCF1⁻ subgroup. These subgroups correlate with protective immune responses to melanoma. TCF1⁺ TRM cells expressed more Ki-67 and IFN- γ , whereas TCF1⁻ TRM cells expressed cytotoxic molecules. The relationships among TRM cells, cDC1 distribution and function, and immunotherapy response were demonstrated in the study by de León-Rodríguez et al. (2024), which illustrated the complex interactions between the local TME and resident immune cells, highlighting the important roles these cells play in CBI. To evaluate tissue, multiplex immunofluorescence is used to stain tumor tissue sections, facilitating the analysis of the expression of immune checkpoint molecules and helping to identify therapeutic anti-tumor bioactive agents. Several diseases have benefited from this technique (Stack et al., 2014).

In addition to general functional states, various TAM subpopulations with tumor specificity have been documented, each with a particular phenotype, location, and function. They are found in a range of neoplasms, for example, HCC and breast cancer. The differences between malignancies are likely due to the different TME terrains and evolutionary pressures that each cancer type faces. The hypoxic and fibrotic microenvironment in HCC may promote the expansion of proangiogenic and immunosuppressive TAMs. In contrast, certain breast cancer subtypes, such as triple-negative breast cancer, can sustain a TAM phenotype that is more inflammatory with enhanced antigen-presenting capacity. This variability underscores the importance of interpreting TAM function within a tumor-type-specific context rather than applying a uniform model across malignancies.

4.3 Exploration of combination therapy strategies

Understanding tumor-type-specific TAM heterogeneity is essential for tailoring therapeutic strategies, given that different TAM subsets exhibit distinct responsiveness to interventions. In addition to technological advances in TAM typing, accumulating studies have delineated tumor-specific TAM subpopulations that vary in phenotype, localization, function, and therapeutic responsiveness. To summarize these differences, we compared representative TAM subtypes in HCC and breast cancer, as shown in Table 1. From a therapeutic standpoint, these distinctions suggest that immunomodulatory agents—such as checkpoint inhibitors,

Table 1 Comparison of tumor-associated macrophage (TAM) subpopulations, functions, and therapeutic targets in hepatocellular carcinoma and breast cancer

Tumor type	TAM subpopulation	Marker/phenotype	Function	Spatial localization	Therapeutic target	Reference
HCC	SPP1 ⁺	SPP1, CD44	Angiogenesis promotion, immune evasion	Hypoxic, perivascular regions	SPP1–CD44 axis	Fan et al., 2024
HCC	ApoE ⁺	ApoE ^{high}	Lipid metabolism, immunosuppression	Fibrotic tumor regions	ApoE signaling	Huang et al., 2025
Breast cancer	PD-L1 ⁺	PD-L1, CD206	Immunosuppression	Tumor stroma	PD-L1	Wu et al., 2021

HCC: hepatocellular carcinoma; SPP1: secreted phosphoprotein 1; CD44: cluster of differentiation 44; ApoE: apolipoprotein E; PD-L1: programmed cell death-ligand 1.

metabolic reprogramming drugs, or macrophage-depleting therapies—may require tumor-specific adaptation to maximize efficacy. Conversely, a “one-size-fits-all” approach risks suboptimal modulation of the TAM compartment and reduced clinical benefit.

Recent studies have highlighted the role of metabolomics and multi-omics analysis in combination therapy strategies. The metabolic regulation of TAMs is well understood, and metabolomics technologies, including liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS), can be employed to detect metabolites generated by TAMs and elucidate the metabolic effects on infiltrating macrophages caused under various treatment conditions.

Integrating such multi-dimensional datasets with tumor-specific TAM profiles could enable the development of a precision immunotherapy framework, in which metabolic vulnerabilities unique to a given cancer type are exploited in combination with immune checkpoint inhibition or other macrophage-targeted interventions. This information enables more specific immune checkpoint inhibition strategies due to its multi-dimensional nature.

Nanomedicine is being used to examine drug combinations and for dose optimization in order to improve the targeting of drugs to tumor sites. Several nanoparticle platforms have successfully manipulated TAMs within the TME to enhance anti-tumor activity. For example, hybrid nanovesicles of exosomes from M1 macrophages with AS1411 aptamer-conjugated liposomes (M1E/AALs) enhanced anti-tumor effects in mouse models by relieving tumor hypoxia and facilitating TAM polarization (Zhen et al., 2024). Small lipid nanoparticles encapsulating the immunostimulatory CpG oligodeoxynucleotide (SLNP@CpG) reprogrammed TAMs and significantly extended the survival

of mice, even eliminating tumors in some models (Kim et al., 2024). Furthermore, doxorubicin hydrochloride (DOX·HCl)-loaded matrix metalloproteinase-2 (MMP-2) peptide liposomes (D@MLL), which target monocytes via surface-modified lipoteichoic acid, combined with circulating monocytes enhanced TAM M1 polarization under low-dose radiotherapy, thereby improving the treatment of glioblastoma (Kuang et al., 2023). Bio-inspired liposomal bacterial platforms (DOX-containing bionic bacterium, namely DOX@Bio-Bac) re-educated TAMs and significantly inhibited tumor growth and metastasis (Meng et al., 2023), while sialic acid (SA)-derivatized liposomes (SA-cholesterol conjugate, namely SA-CH) reduced immunosuppressive cells and improved anti-tumor immune responses (Sui et al., 2022). Polyethylene glycol (PEG)-modified liposomes regulated the immunosuppressive TMEs through metabolic control, significantly enhancing anti-tumor effects in combination with radiotherapy (Shen et al., 2022). LPR@CHG, an in situ injectable thermosensitive chitosan hydrogel loaded with lipid-*IRF5* messenger RNA (mRNA)/*CCL5* small interfering RNA (siRNA) nanoparticle complexes, reprogrammed the immune microenvironment in pancreatic cancer and promoted M1 macrophage polarization (Gao C et al., 2022). IL-13-functionalized liposomes and PEG-coated extracellular vesicles co-delivering chemotherapeutic drugs effectively inhibited melanoma growth by targeting both TAMs and tumor cells (Negrea et al., 2022). A tumor microcalcification-based nanoparticle delivery system enhanced breast cancer treatment through photodynamic therapy and TAM polarization (Jian et al., 2022).

In recent years, big data analysis and machine learning models have been applied to elucidate the roles of TAMs within the TME. Integrated multi-omics analysis, which combines single-cell transcriptomics, metabolomics, and immune cell infiltration analysis,

can reveal the functional characteristics and regulatory networks of TAMs. Researchers can use these data to construct predictive models for evaluating patient responses to combination therapy strategies (Li Z et al., 2022). The combination of spatial transcriptomics data and scRNA-seq offers an opportunity to re-evaluate the spatial heterogeneity of the TME. Using these highly innovative modalities, researchers will be able to perform quantitative and functional analyses of macrophages within the TME and provide data to advocate for personalized therapeutic strategies.

To provide a comprehensive overview of clinical and preclinical strategies targeting TAMs across cancer types, Table 2 summarizes representative approaches, their mechanisms, clinical phases, and development outcomes.

4.4 Clinical translation and challenges of TAM-targeted therapies

Given the high plasticity of TAMs and the requirement for tailored therapies, along with the attendant issues of side effects/safety, strategies targeting TAMs must overcome several hurdles for successful translation into clinical practice. TAMs can reprogram themselves within the TME and transition dynamically between M1 and M2 when responding to environmental cues, which limits the long-term efficacy of single-targeted strategies (Hou et al., 2024). Thus, personalized treatment is crucial in clinical practice, where a treatment plan should be tailored to the TME and TAM status for each patient.

TAMs are important for both tumor immunity and preserving normal immune functions. Because TAMs are a key cell group in the tumor environment, targeting them could destroy the immune balance of the body, thereby significantly impairing the function of normal macrophages. Ensuring the specificity of TAM-targeted therapies without interfering with normal immune system functions remains a key research challenge (Li et al., 2024). Additionally, while combining TAM-targeted therapies with other treatments (such as ICIs) shows potential, optimizing combination treatment strategies to balance efficacy and side effects requires further in-depth clinical research. A schematic overview of macrophage-targeted therapeutic strategies and their combination approaches is depicted in Fig. 3.

5 Conclusions

TAMs critically shape the TME through metabolic reprogramming, immune regulation, and interactions with stromal and immune cells. Their plasticity in glucose, lipid, amino acid, and purine pathways promotes immunosuppression and limits immunotherapy efficacy. Reprogramming TAMs or combining TAM-targeted therapies with checkpoint inhibitors, chemotherapy, radiotherapy, or CAR-T therapy enhances anti-tumor responses. Advances in single-cell profiling, spatial transcriptomics, and nanomedicine enable precise TAM characterization and tumor-type-specific strategies. Future efforts should focus on personalized approaches that account for TAM heterogeneity and minimize off-target effects, paving the way for more effective and durable cancer immunotherapies.

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Author contributions

Shan WANG: conceptualization, methodology, formal analysis, writing – original draft, writing – review & editing, supervision, and project administration. Yage FU: visualization. Yamei HUANG: writing – review & editing. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Shan WANG, Yage FU, and Yamei HUANG declare that they have no conflicts of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the preparation of this manuscript, the authors used Grok (developed by xAI) to assist with language polishing, improving readability and natural tone, checking and formatting references, and suggesting revisions to address editorial comments on citation clarity and logical flow. The specific prompts included requests for reference verification, rephrasing for clarity, and ensuring consistency with journal style. All AI-generated content was thoroughly reviewed, edited, and verified by the authors for accuracy, originality, and scientific integrity. The authors take full responsibility for the final content of this manuscript.

Table 2 Overview of clinical strategies targeting tumor-associated macrophages (TAMs) across cancer types

Cancer type	Therapeutic strategy	Mechanism/effect	Stage	Key outcome	Reference
Advanced melanoma and other solid tumors	CSF1R inhibitor (pexidartinib)+ PD-1 inhibitor (pembrolizumab)	Decreases immunosuppressive TAM infiltration, enhances PD-1-mediated anti-tumor immune response	Phase I/IIa (NCT02452424)	Efficacy endpoints not reported in this citation	Wainberg et al., 2016
Melanoma	IL-13-functionalized liposomes+ chemotherapeutics	Targets TAMs and tumor cells to inhibit tumor growth	Preclinical	Effective inhibition of melanoma growth and angiogenesis	Negrea et al., 2022
Malignant mesothelioma	CSF1R inhibitor (BLZ945)+ anti-PD-L1 agent	Suppresses M2 polarization of TAMs, enhances anti-tumor effect of PD-L1 inhibitor	Preclinical	Increased anti-tumor immune response, enhanced efficacy of PD-L1 inhibitor	Magkouta et al., 2021
Hepatocellular carcinoma	CSF1R inhibitor+ PD-L1 inhibitor	Inhibits TAM migration and polarization, promotes T-cell infiltration	Preclinical	Significant anti-tumor activity, extended survival in mouse models	Zhu et al., 2019
Colorectal cancer	CSF1R inhibitor (pexidartinib)+ checkpoint inhibitors	Depletes M2 macrophages differentiated by cancer-associated fibroblasts	Preclinical	Enhanced tumor immunity, reduced M2 macrophage population	Shimizu et al., 2024
Triple-negative breast cancer	Low-dose chemotherapy (cyclophosphamide)+ CSF1R inhibitor	Mitigates immunosuppressive effects of TAMs, promotes T and B cell infiltration	Preclinical	Durable tumor regression, increased immune cell infiltration	Singh et al., 2022
Breast cancer	Paclitaxel+PD-1 inhibitor	Modulates TAM polarization to enhance PD-1 inhibitor efficacy	Preclinical	Improved efficacy of PD-1 blockade	Choi et al., 2024
Breast cancer	Tumor microcalcification-based nanoparticles+ photodynamic therapy	Enhances TAM polarization, improves anti-tumor immune response	Preclinical	Enhanced treatment outcomes through photodynamic immunotherapy	Jian et al., 2022
Head and neck cancer	CCR2 inhibitor post-radiotherapy	Blocks monocyte recruitment to reduce TAMs, improves radiotherapy outcomes	Preclinical	Reduced TAM numbers, increased tumor control rates	Mondini et al., 2019
Pancreatic cancer	Injectable immunotherapeutic hydrogel (LPR@CHG)	Reprograms TAMs, enhances anti-tumor immunity	Preclinical	Extended survival, tumor elimination in some models	Gao C et al., 2022
Glioblastoma	D@MLL nanoparticles+ low-dose radiotherapy	Enhances TAM M1 polarization, improves anti-tumor immune response	Preclinical	Improved treatment outcomes in glioblastoma models	Kuang et al., 2023
Glioblastoma	CAR-T cell therapy+ TAM reprogramming	Re-educates TAMs and microglia to enhance CAR-T cell efficacy	Preclinical	Improved anti-tumor response in glioblastoma models	Zhu et al., 2024

CSF1R: colony-stimulating factor 1 receptor; PD-1: programmed cell death protein 1; PD-L1: programmed cell death-ligand 1; IL-13: interleukin-13; CAR-T: chimeric antigen receptor T-cell; CCR2: C-C motif chemokine receptor 2; LPR@CHG: an in situ injectable thermosensitive chitosan hydrogel loaded with lipid-*IRF5* messenger RNA (mRNA)/*CCL5* small interfering RNA (siRNA) nanoparticle complexes; D@MLL: doxorubicin hydrochloride (DOX·HCl)-loaded matrix metalloproteinase-2 (MMP-2) peptide liposomes targeting monocytes via surface-modified lipoteichoic acid.

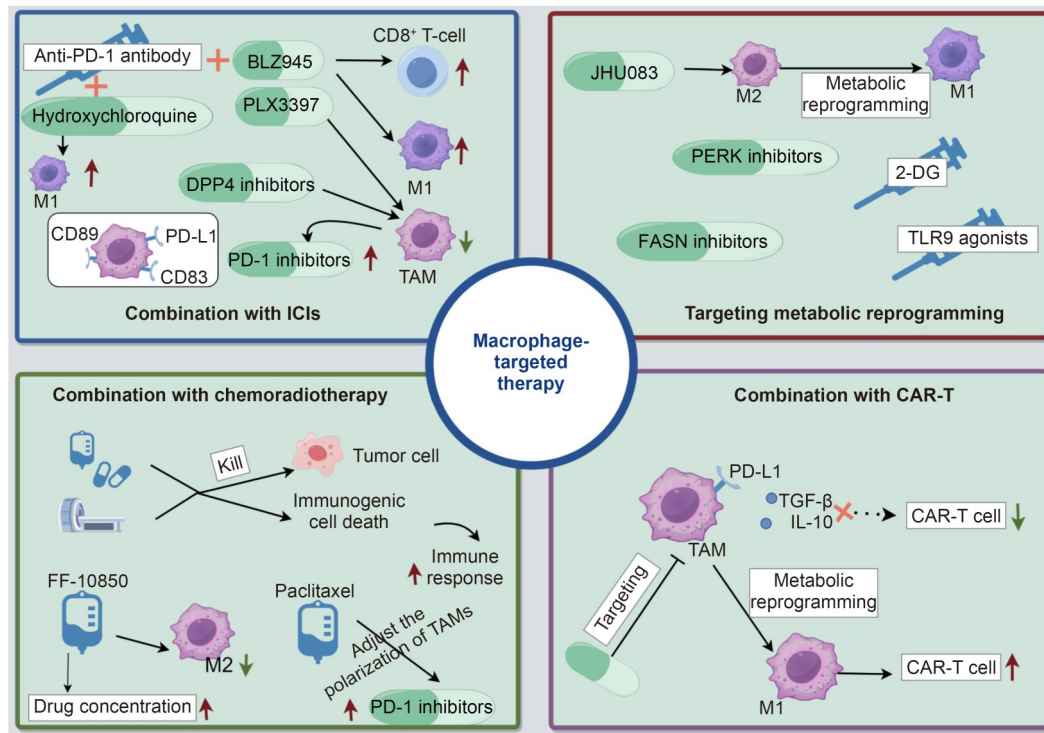


Fig. 3 Overview of macrophage-targeted therapeutic strategies. The metabolic reprogramming and immunoregulatory functions of tumor-associated macrophages (TAMs) can be targeted to enhance anti-tumor immune responses. Combining immune checkpoint inhibitors (ICIs) with macrophage-targeted strategies, such as colony-stimulating factor 1 receptor (CSF1R) inhibitors (e.g., PLX3397 and BLZ945), can enhance anti-tumor efficacy and promote M1 polarization. Dipeptidyl peptidase-4 (DPP4) inhibitors and hydroxychloroquine also help reverse immunosuppression, improving ICI effectiveness. In chemotherapy and radiotherapy, TAMs can be driven toward the M2 phenotype, compromising the anti-tumor response. Liposomal topotecan (FF-10850) enhances drug concentration and reduces TAMs, while paclitaxel modulates TAM polarization to improve programmed cell death protein 1 (PD-1) inhibitor efficacy. Targeting TAM metabolism, Toll-like receptor 9 (TLR9) agonists activate fatty acid oxidation (FAO), and 2-deoxy-D-glucose (2-DG) blocks M2 polarization, restoring anti-tumor responses. The glutamine antagonist JHU083 reprograms TAMs to an M1 phenotype, enhancing antigen presentation and T-cell activation. Combining TAM-targeted therapy with chimeric antigen receptor T-cell (CAR-T) therapy improves CAR-T efficacy by reprogramming TAMs to an M1 phenotype, reducing immunosuppression in the tumor microenvironment (TME). CD8: cluster of differentiation 8; PD-L1: programmed cell death-ligand 1; FASN: fatty acid synthase; TGF- β : transforming growth factor-beta; IL-10: interleukin-10; PERK: protein kinase R-like endoplasmic reticulum kinase. Created with Figdraw (<https://www.figdraw.com>).

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