Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *in press*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2500179

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

Shan WANG[1,2*]✉, Yage FU[1,2*], Yamei HUANG[1]

[1]*Department of Oral Pathology, School of Stomatology, Hainan Medical University, Haikou 571199, China*
[2]*Department of Stomatology, The Second Affiliated Hospital of Hainan Medical University, Haikou 570216, China*

Abstract: Macrophages influence antitumor 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/PPP switching, FAO–lysosomal lipolysis, and glutamine–α-ketoglutarate signaling—mechanistically reprogram TAMs. We then map these circuits onto combination strategies involving PD-(L)1/CTLA-4 blockade, chemotherapy/radiotherapy, and 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 & 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 & Pollard, 2020). Classically activated macrophages secrete IL-12 and TNF-α, upregulate antigen presentation, and can suppress tumor cell proliferation. In contrast, alternatively activated populations release IL-10 and TGF-β, stimulate angiogenesis, facilitate extracellular matrix

✉ Shan WANG, birchtree20032003@126.com
* The two authors contributed equally to this work
Shan WANG, https://orcid.org/0000-0003-1921-1644
Yage FU, https://orcid.org/0009-0001-6526-4184
Yamei HUANG, https://orcid.org/0009-0001-0735-9265

Received Apr. 10, 2025; Revision accepted Aug. 15, 2025;
Crosschecked xxx. xx, 20xx; Published online xxx. xx, 20xx

remodeling, and enhance tumor cell motility, invasion, and metastatic spread (Shao et al., 2024; Wei et al., 2021; Petty et al., 2019; Gao et al., 2022; Liu et al., 2020). 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., MMP-9) and recruit regulatory T cells, which hinder anti-tumor immunity while enhancing metastatic spread (Bourhis et al., 2021; Petty et al., 2021; Deng et al., 2017; Chen et al., 2017; Stöth et al., 2019; Zhang et al., 2020; Song et al., 2024). According to Shao and colleagues (2024), 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 like PD-1/PD-L1, CTLA-4 contribute to immune escape (Wei 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 and oxidative phosphorylation to maintain their immunosuppressive phenotype (Jia et al., 2024) It has also been reported that 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. Figure 1 summarizes the polarization, roles, and therapeutic relevance of TAMs in the TME.

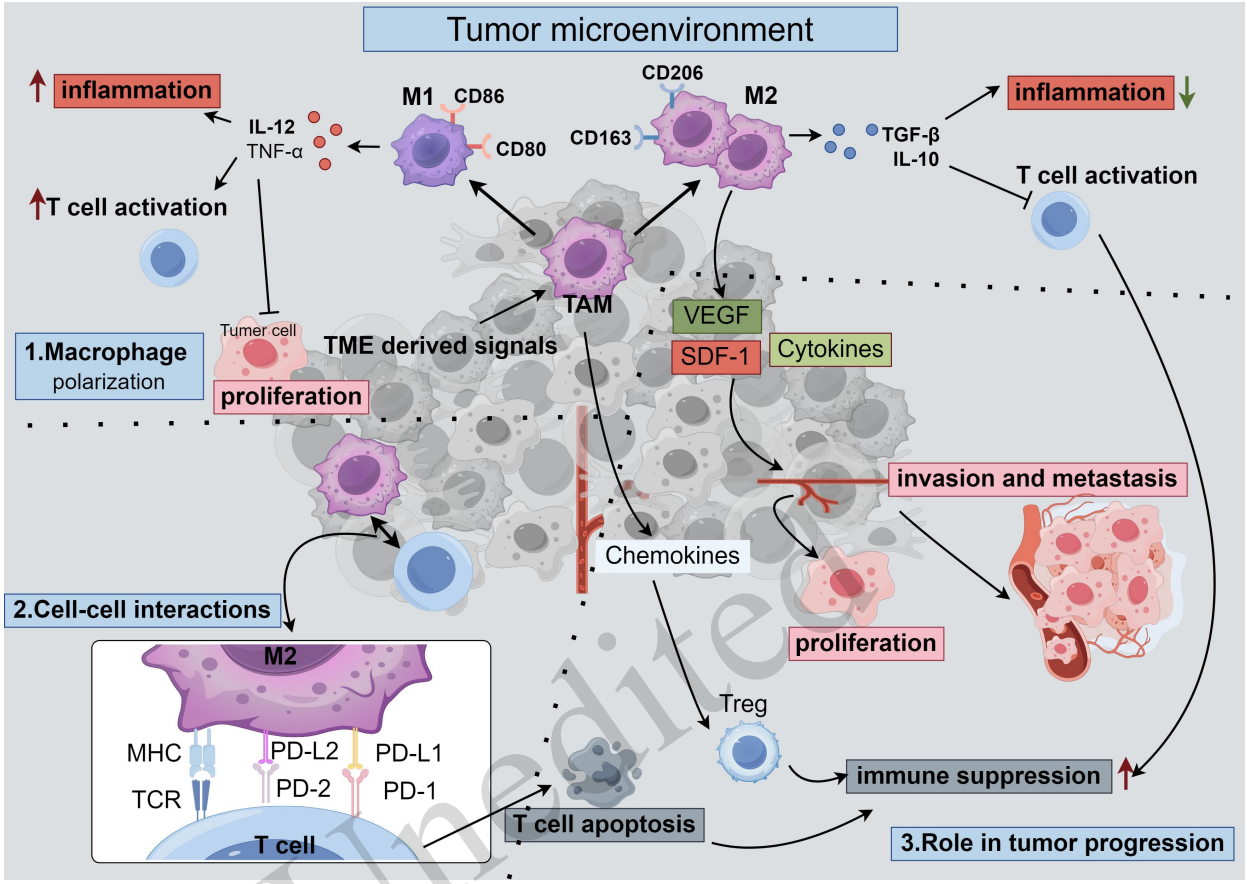

Fig. 1 Roles of tumor-associated macrophages (TAMs) in the tumor microenvironment (TME).
TAMs polarize into M1 or M2 phenotypes in response to TME signals. M1 macrophages express cluster of differentiation 86 (CD86) and cluster of differentiation 80 (CD80), secrete pro-inflammatory cytokines [interleukin-12 (IL-12), tumor necrosis factor-alpha (TNF-α)], and inhibit tumor growth by enhancing T cell activation and inflammation. In contrast, M2 macrophages, which dominate in the TME, express cluster of differentiation 206 (CD206) and cluster of differentiation 163 (CD163), secrete anti-inflammatory cytokines [interleukin-10 (IL-10), 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 death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2)] and secrete angiogenic factors [e.g., vascular endothelial growth factor (VEGF), 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. *Created with Figdraw (www.figdraw.com).*

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 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 oxidative phosphorylation 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 immune-suppressive M2 phenotype. This TAM subtype produces IL-10 cytokines, which suppress T cell activity and facilitate immune evasion (Liao et al., 2021; Jin et al., 2025; Zeng et al., 2023). 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 SNHG17 enhances lactate accumulation by activating pgk1, which in turn inhibits the anti-tumor function of T cells. Additionally, TAMs compete with CD8+ T-cells for glucose. Tharp et al. found that TAMs upregulate glucose metabolic pathways and consume large quantities of glucose, depriving CD8+ T cells in the TME of energy and impairing their tumor-killing ability (Tharp et al., 2024). 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 NADPH production through oxidative PPP and promotes ROS production (Erlich et al., 2022; Kennel and Greten, 2021; Tsai et al., 2022). These processes are essential for the anti-tumor effects of M1 macrophages. Inhibition of glycolysis and the 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 fatty acid oxidation (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; Chiba et al., 2017; Huang et al., 2016). However, if mitochondrial oxidative phosphorylation 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).

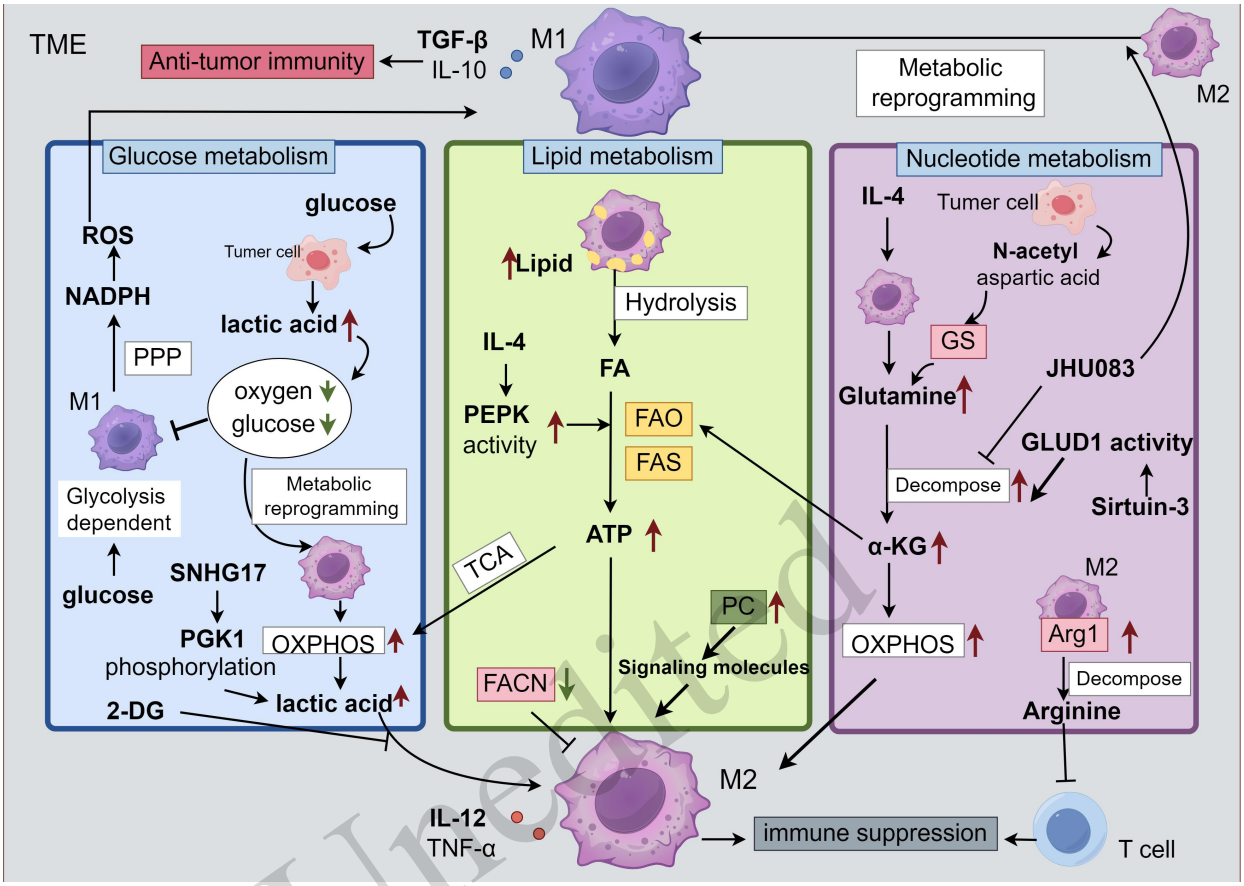

Fig. 2 Metabolic reprogramming of macrophages and immune regulation.
In glucose metabolism, TAMs undergo reprogramming towards an M2 phenotype under hypoxic, low-glucose, and high-lactate conditions, favoring oxidative phosphorylation (OXPHOS) and IL-10 secretion. M1 macrophages, in contrast, rely on glycolysis, producing nicotinamide adenine dinucleotide phosphate (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 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 undergo β-oxidation to produce acetyl-coenzyme A (acetyl-CoA), which enters the tricarboxylic acid (TCA) cycle to support OXPHOS. Interleukin-4 (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. Glutamine metabolism byproduct alpha-ketoglutarate (α-KG) enhances FAO and OXPHOS, maintaining M2's immunosuppressive role and reducing inflammation. Sirtuin-3 (SIRT3), activated by IL-4, enhances glutamate dehydrogenase 1 (GLUD1) activity, promoting glutamine breakdown. Tumor cells induce glutamine synthetase (GS) to enhance M2 TAM function *Created with Figdraw (www.figdraw.com).*

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 FASN or FAO suppresses M2-like polarization and restores innate anti-tumor immunity (de Brito et al., 2020).

Inhibiting key lipid metabolic drivers such as 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 NADH, acetyl-CoA, and FADH2. Acetyl-CoA then joins the TCA cycle, producing the reducing equivalents for OXPHOS and 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 and colleagues, inhibiting TAMs' FAO function may change their role within tumors from pro-tumor to anti-tumor. Xiao and co-workers elucidate that reprogramming choline metabolism promotes PC synthesis, which can promote M2 polarization by inducing cytokine secretion, thereby inhibiting T cell function. IL-4-activated TAMs exhibit elevated PERK activity, which is vital for sustaining FAO and mitochondrial functionality, as explained by Raines et al. in 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).

TLR9 agonists activate the FAO and lipid synthesis pathways to boost TAM metabolic reprogramming, which may contribute to their anti-tumor effects. The commitment of M1 macrophages to lipid accumulation relies on the joint coordination of carnitine palmitoyl transferase 1 (CPT-1) and ATP citrate lyase (ACL), which regulate the metabolic flux between FAO and lipid synthesis (Liu et al., 2019). 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 immune-suppressive 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 & Mantovani, 2012). 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 in conditions of glutamine deprivation (Tavakoli et al, 2017; Jha et al, 2015).

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 (Praharaj et al., 2024; Oh et al., 2020). 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 NF-κB 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 glutamine synthetase in macrophages in mouse models reprograms TAMs to an anti-tumor M1-like state (Menga et al. 2020; Palmieri et al. 2017), 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 & Morrell, 2020; Plotkin et al., 2021). High BCAA

levels can increase the risk of heart disease and cause overly agitated platelets (Hilt & Morrell, 2020). In some lung cancer models, TAMs further support a TME populated by regulatory T cells, 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 MRI, allow for noninvasive, longitudinal monitoring of TAMs in gliomas, providing insights into their spatial and temporal dynamics during disease progression and treatment (Croci 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 towards 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 EMT-related cytokines, including IL-1β, 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 A2A receptors, blocking their activation and promoting TAM differentiation into an immunosuppressive M2-like cell phenotype. Together, these activities inhibit antitumor responses and facilitate malignant growth. Research suggests that the glutamine antagonist JHU083 reduces purine metabolites within TAMs, resulting in a decrease in adenosine production. Our findings show that decreased IL-10 expression reprograms the TAMs to an anti-tumor M1 phenotype. This leads to enhanced antigen presentation and T cell activation. 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 IDH3a regulates xanthine oxidoreductase, which modifies the 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 IDH3α, and macrophage polarization occurs through M2 state macrophages (activated macrophages). The 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-IDH3α interferes with TAMs, thus serving as an immunodynamic target, offering a new immunotherapeutic technique for treating HCC (Lu et al., 2023).

3 Macrophages in Immune Checkpoint Regulation

3.1 The 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 immune checkpoint inhibitors (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 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 PDL1) 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 & Mellman, 2013). 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 et al. found that TAMs release IL-1b, causing the expression of PDL1 in tumor cells and diminishing the efficacy of immune checkpoint blockade treatment. Other research has produced similar results. Therapies aiming to bolster the IL-1β pathway may be instrumental in augmenting the impact of checkpoint blockades (Xu et al., 2023a). Tsuruta et al. (2022) confirmed that TAMs modulate anti-tumor activity through the circadian rhythm of PD-1 and Dec2. In addition, PD-L1 expression is regulated in TAMs through the MyD88/IL-1R axis via the NF-kB pathway, and, in combination with 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 TAM regulation. Tang et al. 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 (Tang et al., 2024). Liu et al. identified a link between 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 (Liu et al., 2024).

3.2 The 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 also demonstrated a strong correlation between macrophage PD-L1 expression and lack of response to checkpoint blockade therapy (Homicsko et al., 2023). Peckert-Maier et al. note 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 (Peckert-Maier et al., 2023). Therapeutic strategies such as blocking the PD-L1 expression on TAMs and other immune checkpoint molecules may therefore be a promising approach to cancer treatment (Park et al., 2021; Jelinic et al., 2018; Jing et al., 2020).

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 research studies have investigated a new combination of PD-1 inhibitors with CSF1R inhibitors—colony-stimulating factor 1 receptor (CSF1R) has been 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 (Fujiwara et al., 2021; Cannarile et al., 2017). A B301 study (NCT 02804825) was conducted on multiple solid cancers. Findings from a phase I/IIa

clinical trial (Wainberg et al., 2016) demonstrated that the combination of the CSF1R inhibitor Pexidartinib and the PD-1 inhibitor Pembrolizumab was safe and showed preliminary efficacy in patients with advanced solid tumors, including melanoma. Specifically, they showed that targeting the CSF1R pathway decreased immunosuppressive TAM infiltration and enhanced the anti-tumor immune response mediated by the PD-1 inhibitor. According to Wainberg et al., it led to disease shrinkage as well as progression-free survival in several patients, but also caused immune-related side effects in some.

A preclinical investigation assessed the use of a CSF1R inhibitor in combination with an anti-PD-L1 agent in a malignant mesothelioma animal model using the BLZ945 strain. 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 activities of a combination of a CSF1R inhibitor and a PDL1 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 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. demonstrated that preconditioning cancer-associated fibroblasts (CAFs) and macrophages with synthetic retinoids enhanced cancer responses to checkpoint blockade (Owaki et al., 2024). Shimizu et al. demonstrated that concomitant targeting of CSF1R with the inhibitor Pexidartinib, in combination with checkpoint inhibitors, resulted in the deletion of CAF-induced M2 macrophages, leading to tumor immunity in a colorectal cancer model (Shimizu et al., 2024). Additionally, previous studies have demonstrated that the depletion of TAMs, through 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 (Magkouta et al., 2021; Peranzoni et al., 2018; 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 PD-1 antibody increases M1 macrophage proportion, thus improving anti-tumor immunity (Sharma et al., 2022; Wang et al., 2021).

Recent research has indicated that combining treatments aimed at TAMs with PD-1, PD-L1, and CTLA-4 inhibiting ICIs may have a synergistic impact on tumors. TAMs that express the immune checkpoint molecule PDL1 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 towards M1 macrophages (Wei et al., 2021). Research by Lin and colleagues indicated that ADAR1 downregulation and interferon-γ treatment of macrophages alter the TME and increase the efficacy of immune checkpoint blockade (Lin et al., 2023). Furthermore, Zuo et al. showed that DPP4 inhibitors may reduce TAM numbers and improve the anti-tumor efficacy of anti-PD-L1 therapy in non-small cell lung cancer (Zuo et al., 2023).

In a nutshell, the response being encouraged in this area of cancer immunology is an anti-tumor response. The potential benefits of cancer immunotherapies may be realized by combining TAM-targeting strategies with ICIs.

3.4 TAMs and 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.'s 2023 study suggests that TAMs promote an immunosuppressive environment via IGFBP2-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 (Galluzzi et al., 2017; Mantovani et al., 2013).

Research has shown that chemotherapy agents, such as paclitaxel, induce PD-L1 expression on macrophages by activating the NF-κB 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, something that 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 deliver drugs directly into the tumor site, increasing T cell anti-tumor activity by reducing TAMs that suppress immunity. Additionally, Choi et al. 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 (Choi et al., 2024). In a model of head and neck cancer, researchers found that an increase in 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 CCR2 inhibitor improved radiotherapy outcomes by limiting TAMs and increasing tumor control rate and survival (Mondini et al. 2017). 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 Cell Therapy

While 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. suggest that re-educating TAMs and microglia enhances CAR-T cell glioblastoma treatment (Zhu et al., 2024). According to Anami et al., loss of sinusoidal macrophage function in the tumor host promotes resistance to immunotherapy; therefore, adjusting macrophages may overcome resistance to CAR-T in solid tumors (Singh et al., 2022).

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 techniques and technologies 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 understanding of the human TME by transplanting human immune cells into immunodeficient mice. This approach has also been used to measure combinations and evaluate 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 heterogenization post-treatment, high-dimensional imaging techniques were deployed, revealing that breast cancer subtypes activate TAM 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., 2017; 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 (3D) co-culture systems are commonly used in vitro to facilitate improved mimicry of in vivo niches and more accurate studies on the interaction between TAMs and 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. Ravi et al. used a 3D co-culture

system to determine the effect of paclitaxel on TAM polarization in order to define its mechanism of action in the enhancement of PD-1 blockade therapy (Ravi et al., 2015).

4.2 Emerging Technologies for Tumor-Associated Macrophage (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 TA (tumor-associated) macrophage puzzle. Single-cell RNA sequences 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 the 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 single-cell RNA sequences (scRNAseq) or spatial transcriptomics, enable novel investigations into the functional states of different TAM subgroups and their roles in tumor immunity.

4.2.2 The Principles of Single-Cell RNA Sequencing (scRNA-seq)

Single-cell RNA sequencing, or 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 (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 reverse 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 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 XYZeq platform, which merges single-cell RNA sequencing data with spatial metadata to accurately pinpoint the TME localization of cells alongside their gene expression features, correlating it with the infiltration of specific TAM subgroups (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 gram-scale data to improve personalized therapies using immune checkpoint

blockade. As Saqib et al. suggest, integrating these multi-dimensional data can lead to a more precise characterization of the spatial and functional heterogeneity of TAMs, proposing explicit directions for therapeutic strategies targeting specific TAM subpopulations (Saqib et al., 2023).

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 De León-Rodríguez et al. (2024) used multiplex immunofluorescence and spectral cytometry to compare the dynamic changes in 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 TCF1+ or TCF1- subgroups. These subgroups correlate with protective immune responses to melanoma. TCF1+ TRM cells expressed more Ki67 and IFN-g, whereas TCF1 - TRM cells expressed cytotoxic molecules. The relationships among TRM cells, cDC1 distribution and function, and immunotherapy response were demonstrated in De León−Rodríguez et al.'s study, which illustrated the complex interactions between the local TME and resident immune cells, highlighting the important roles these cells play in CBI (De León-Rodríguez et al., 2024). To evaluate tissue, multiplex immunofluorescence is used to stain tumor tissue sections, facilitating the analysis of the expressions of immune checkpoint molecules, 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 by 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, 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.

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	$SPP1^+$ TAMs	SPP1, CD44	Promote angiogenesis and immune evasion	Hypoxic, perivascular regions	SPP1–CD44 axis	Fan et al.,2024
HCC	$APOE^+$ TAMs	$APOE^{high}$	Lipid metabolism, immunosuppression	Fibrotic tumor regions	APOE signaling	Huang et al.,2025

Breast Cancer	Sca-1$^+$ PD-L1$^+$ TAMs	Sca-1, CD206, PD-L1	Immunosuppressive, self-renewing	Tumor stroma	Notch4, PD-L1	Wu et al,2021;Cha et al.,2024

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 LC–MS/MS and GC–MS, can be employed to detect metabolites generated by TAMs and elucidate the metabolic effects on infiltrating macrophages caused by various treatment conditions. Additionally, the potential molecular mechanisms of metabolic adaptation in TAMs have been elucidated by a metabolomics analysis in the immune microenvironment of pancreatic cancer, where TAM metabolism is key to treatment (Shimazaki et al., 2021).

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 multidimensional 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 activities. For example, hybrid nanovesicles of exosomes from M1 macrophages with liposomes (M1E/AALs) enhanced anti-tumor effects in mouse models by relieving tumor hypoxia and facilitating TAM polarization (Zhen et al., 2024). Solid lipid nanoparticles (SLNs) containing VCP/p97 inhibitors, PD-L1 inhibitors, and immunoadjuvants inhibited Tregs and TAMs in PC, thereby improving T cell infiltration (Lo et al., 2024). SLNs (SLNP@CpG) encapsulating the immunostimulatory CpG ODN reprogrammed TAMs and significantly extended the survival of mice, even eliminating tumors in some models (Kim et al., 2024). Furthermore, D@MLL nanoparticles combined with circulating monocytes enhanced TAM M1 polarization with low-dose radiotherapy, improving treatment for glioblastoma (Kuang et al., 2023). Bio-inspired liposomal bacterial platforms (DOX@Bio-Bac) re-educated TAMs and significantly inhibited tumor growth and metastasis (Meng et al., 2023), while sialic acid-derivatized liposomes (SA-CH) reduced immunosuppressive cells and improved anti-tumor immune responses (Sui et al., 2022). PEG-modified liposomes regulated the immunosuppressive TME through metabolic control, significantly enhancing anti-tumor effects in combination with radiotherapy (Shen et al., 2022). Injectable hydrogels (LPR@CHG) delivered RNA to reprogram the immune microenvironment in pancreatic cancer, promoting M1 macrophage formation (Gao 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 et al., 2022). The combination of spatial transcriptomics data and scRNA-seq offers an opportunity to reevaluate 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.

Table.2 Overview of Clinical Strategies Targeting Tumor-Associated Macrophages (TAMs) Across Cancer Types

Cancer Type	Therapeutic Approach	Mechanism of Action	Clinical Phase	Outcomes/Status
Advanced Melanoma and Other Solid Tumors	CSF1R Inhibitor (Pexidartinib) + PD-1 Inhibitor (Pembrolizumab)	Decreases immunosuppressive TAM infiltration, enhances PD-1 inhibitor-mediated anti-tumor immune response	Phase I/IIa (NCT02452424)	Disease shrinkage and extended progression-free survival in some patients; immune-related adverse effects observed in some cases
Malignant Mesothelioma	CSF1R Inhibitor (BLZ945) + Anti-PD-L1 Agent	Suppresses M2 polarization of TAMs, enhances anti-tumor effect of PD-L1 inhibitor, activates tumor-infiltrating CD8+ T cells	Preclinical	Increased anti-tumor immune response, enhanced efficacy of PD-L1 inhibitor
Hepatocellular Carcinoma	CSF1R Inhibitor + PD-L1 Inhibitor	Inhibits TAM migration and polarization, promotes T cell infiltration into the tumor	Preclinical	Significant anti-tumor activity, extended survival in mouse models
Colorectal Cancer	CSF1R Inhibitor (Pexidartinib) + Checkpoint Inhibitors	Depletes M2 macrophages differentiated by cancer-associated fibroblasts, enhances tumor immunity	Preclinical	Enhanced tumor immunity, reduced M2 macrophage population
Triple-Negative Breast Cancer	Low-Dose Chemotherapy (Cyclophosphamide) + CSF1R Inhibitor	Mitigates immunosuppressive effects of TAMs, promotes T and B cell infiltration into the tumor	Preclinical	Durable tumor regression, increased immune cell infiltration
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
Breast Cancer	Paclitaxel + PD-1 Inhibitor	Modulates TAM polarization to enhance PD-1 inhibitor efficacy	Preclinical	Improved efficacy of PD-1 blockade, enhanced anti-tumor immune response
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, extended survival
Pancreatic Cancer	Solid Lipid Nanoparticles (SLNP@CpG) + Immunostimulatory CpG ODN	Reprograms TAMs, enhances anti-tumor immunity	Preclinical	Extended survival, tumor elimination in some mouse models
Glioblastoma	D@MLL Nanoparticles + Low-Dose Radiotherapy	Enhances TAM M1 polarization, improves anti-tumor immune response	Preclinical	Improved treatment outcomes in glioblastoma models
Melanoma	IL-13 Functionalized Liposomes + Chemotherapeutics	Targets TAMs and tumor cells to inhibit tumor growth	Preclinical	Effective inhibition of melanoma growth and angiogenesis

Breast Cancer	Tumor Microcalcification-Based Nanoparticles + Photodynamic Therapy	Enhances TAM polarization, improves anti-tumor immune response	Preclinical	Enhanced breast cancer treatment outcomes through photodynamic immunotherapy

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.

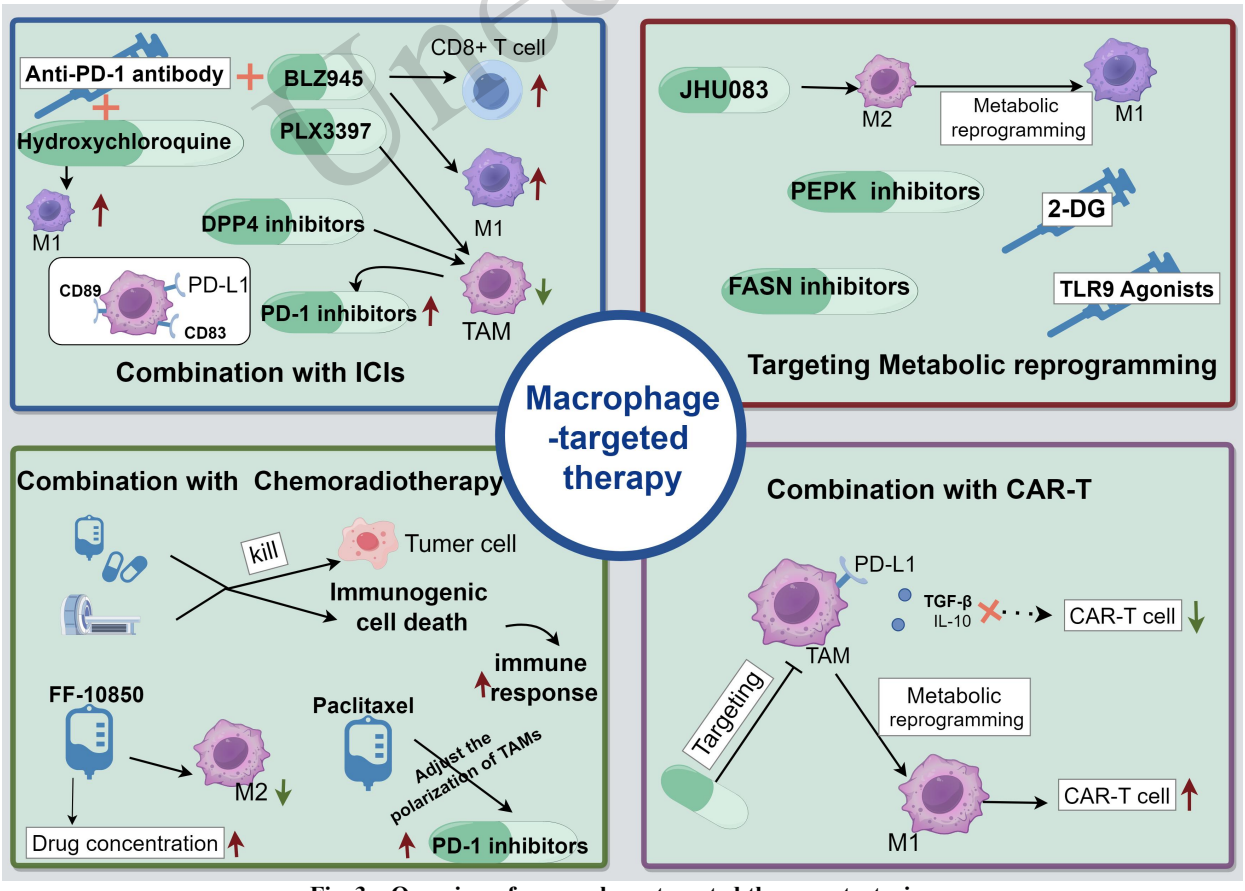

Fig. 3 Overview of macrophage-targeted therapy strategies.
TAMs' metabolic reprogramming and immunoregulatory functions can be targeted to enhance anti-tumor immune responses.

Combining immune checkpoint inhibitors (ICIs) with macrophage-targeted strategies, such as colony-stimulating factor 1 receptor (CSF1R) inhibitors [e.g., PLX3397, BLZ945], can enhance anti-tumor efficacy and promote M1 polarization. Dipeptidyl peptidase-4 (DPP4) inhibitors and hydroxychloroquine also help reverse immunosuppression, improving ICI effectiveness. Optimizing dosing times by regulating programmed death-1 (PD-1) circadian rhythm may further enhance efficacy. 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 PD-1 inhibitor efficacy. Targeting TAM metabolism, Toll-like receptor 9 (TLR9) agonists activate FAO, and 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 (CAR-T) cell therapy improves CAR-T efficacy by reprogramming TAMs to an M1 phenotype, reducing immunosuppression in the TME. *Created with Figdraw (www.figdraw.com).*

5 Conclusions

Tumor-associated macrophages (TAMs) critically shape the tumor microenvironment 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.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgment

This work was supported by the Research Foundation of Hainan Medical University (XRC2022008), the Hainan Province Science and Technology Special Fund (ZDYF2024SHFZ099), the Hainan Provincial Natural Science Foundation of China (825RC765), and in part by the National Natural Science Foundation of China (Grant No. 82560476).

Authors' contributions

Shan Wang: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration.
Yage Fu: Visualization.
Yamei Huang: Writing – editing.

Compliance with ethics guidelines

This experiment does not involve the collection of human specimens

References

1. Anderson, N., Simon, M.C., 2020. The tumor microenvironment. *Curr. Biol*, 30(16):R921-R925. http://dx.doi.org/10.1016/j.cub.2020.06.081
2. Baardman, J., Verberk, S.G.S., Prange, K.H.M., et al., 2018. A defective pentose phosphate pathway reduces inflammatory macrophage responses during hypercholesterolemia. *Cell Rep*, 25(8):2044-2052.e5. http://dx.doi.org/10.1016/j.celrep.2018.10.092
3. Binnewies, M., Roberts, E.W., Kersten, K., et al., 2018. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med*., 24(5):541-550. http://dx.doi.org/10.1038/s41591-018-0014-x
4. Biswas, S.K., Mantovani, A., 2012. Orchestration of metabolism by macrophages. *Cell Metab*, 15(4):432-437. http://dx.doi.org/10.1016/j.cmet.2011.11.013
5. Bourhis, M., Palle, J., Galy-Fauroux, I., et al., 2021. Direct and indirect modulation of T cells by VEGF-A counteracted by anti-angiogenic treatment. *Front. Immunol*, 12:616837. http://dx.doi.org/10.3389/fimmu.2021.616837

6. Cannarile, M., Weisser, M., Jacob, W., et al., 2017. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. *J. Immunother. Cancer*, 5(1):53. http://dx.doi.org/10.1186/s40425-017-0257-y
7. Cassetta, L., Pollard, J.W., 2020. Tumor-associated macrophages. *Curr. Biol*, 30(6):R246-R248. http://dx.doi.org/10.1016/j.cub.2020.01.031
8. Cha SM, Park JW, Lee YJ, Lee HJ, Lee H, Lee IW, Gong G, Park SH, Lee HJ, Jeong BK, 2024. SPP1^{+} macrophages in HR^{+} breast cancer are associated with tumor-infiltrating lymphocytes. *NPJ Breast Cancer,* 10(1):83. https://doi.org/10.1038/s41523-024-00695-7
9. Chen, D.S., Mellman, I., 2013. Oncology meets immunology: the cancer-immunity cycle. *Immunity*, 39(1):1-10. http://dx.doi.org/10.1016/j.immuni.2013.07.012
10. Chen, J., Zhu, H., Yin, Y., et al., 2022. Colorectal cancer: metabolic interactions reshape the tumor microenvironment. *Biochim. Biophys. Acta Rev. Cancer*, 1877(5):188797. http://dx.doi.org/10.1016/j.bbcan.2022.188797
11. Chen, K., Li, X., Dong, S., et al., 2025. Modulating tumor-associated macrophages through CSF1R inhibition: a potential therapeutic strategy for HNSCC. *J. Transl. Med*. 23(1):27. http://dx.doi.org/10.1186/s12967-024-06036-3
12. Chen, X.W., Yu, T.J., Zhang, J., et al., 2017. CYP4A in tumor-associated macrophages promotes pre-metastatic niche formation and metastasis. *Oncogene*, 36(35):5045-5057. http://dx.doi.org/10.1038/onc.2017.118
13. Chiba, S., Hisamatsu, T., Suzuki, H., et al., 2017. Glycolysis regulates LPS-induced cytokine production in M2 polarized human macrophages. *Immunol. Lett*, 183:17-23. http://dx.doi.org/10.1016/j.imlet.2017.01.007
14. Choi, Y., Kim, S.A., Jung, H., et al., 2024. Novel insights into paclitaxel's role on tumor-associated macrophages in enhancing PD-1 blockade in breast cancer treatment. *J. Immunother. Cancer*, 12(7):e008864. http://dx.doi.org/10.1136/jitc-2024-008864
15. Covarrubias, A.J., Aksoylar, H.I., Yu, J., et al., 2016. Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. *eLife*, 5:e11612. http://dx.doi.org/10.7554/eLife.11612
16. Croci, D., Santalla Méndez, R., Temme, S., et al., 2022. Multispectral fluorine-19 MRI enables longitudinal and noninvasive monitoring of tumor-associated macrophages. *Sci. Transl. Med*, 14(667):eabo2952. http://dx.doi.org/10.1126/scitranslmed.abo2952
17. Cui, X., Ma, C., Vasudevaraja, V., et al., 2020. Dissecting the immunosuppressive tumor microenvironments in glioblastoma-on-a-chip for optimized PD-1 immunotherapy. *eLife*, 9:e52253. http://dx.doi.org/10.7554/eLife.52253
18. de Brito, N.M., Duncan-Moretti, J., da Costa, H.C., et al., 2020. Aerobic glycolysis is a metabolic requirement to maintain the M2-like polarization of tumor-associated macrophages. *Biochim. Biophys. Acta Mol. Cell Res*, 1867(2):118604. http://dx.doi.org/10.1016/j.bbamcr.2019.118604
19. De León-Rodríguez, S.G., Aguilar-Flores, C., Gajón, J.A., et al., 2024. TCF1-positive and TCF1-negative TRM CD8 T cell subsets and cDC1s orchestrate melanoma protection and immunotherapy response. *J. Immunother. Cancer*, 12(7):e008739. http://dx.doi.org/10.1136/jitc-2023-008739
20. Deng, L., Stafford, J.H., Liu, S.C., et al., 2017. SDF-1 blockade enhances anti-VEGF therapy of glioblastoma and can be monitored by MRI. *Neoplasia*, 19(1):1-7. http://dx.doi.org/10.1016/j.neo.2016.11.010
21. Erlich, J.R., To, E.E., Luong, R., et al., 2022. Glycolysis and the pentose phosphate pathway promote LPS-induced NOX2 oxidase- and IFN-β-dependent inflammation in macrophages. *Antioxidants (Basel)*, 11(8):1488. http://dx.doi.org/10.3390/antiox11081488
22. Fan, G., Xie, T., Li, L., et al., 2024. Single-cell and spatial analyses revealed the co-location of cancer stem cells and SPP1+ macrophage in hypoxic region that determines the poor prognosis in hepatocellular carcinoma. *NPJ Precis. Oncol*, 8(1):75. http://dx.doi.org/10.1038/s41698-024-00564-3
23. Fujiwara, T., Yakoub, M.A., Chandler, A., et al., 2021. CSF1/CSF1R signaling inhibitor pexidartinib (PLX3397) reprograms tumor-associated macrophages and stimulates T-cell infiltration in the sarcoma microenvironment. *Mol. Cancer Ther*, 20(7):1388-1399. http://dx.doi.org/10.1158/1535-7163.MCT-20-0591
24. Galluzzi, L., Buqué, A., Kepp, O., et al., 2017. Immunogenic cell death in cancer and infectious disease. *Nat. Rev. Immunol*, 17(2):97-111. http://dx.doi.org/10.1038/nri.2016.107
25. Gao, C., Cheng, K., Li, Y., et al., 2022. Injectable immunotherapeutic hydrogel containing RNA-loaded lipid nanoparticles reshapes tumor microenvironment for pancreatic cancer therapy. *Nano Lett*, 22(22):8801-8809. http://dx.doi.org/10.1021/acs.nanolett.2c01994
26. Gao, J., Liang, Y., Wang, L., 2022. Shaping polarization of tumor-associated macrophages in cancer immunotherapy. *Front. Immunol*, 13:888713. http://dx.doi.org/10.3389/fimmu.2022.888713
27. Hilt, Z.T., Morrell, C.N., 2020. Essential amino acids—essential in arterial thrombosis. *Circulation*, 142(1):65-67. http://dx.doi.org/10.1161/CIRCULATIONAHA.120.046817
28. Homicsko, K., Zygoura, P., Norkin, M., et al., 2023. PD-1-expressing macrophages and CD8 T cells are independent predictors of clinical benefit from PD-1 inhibition in advanced mesothelioma. J. Immunother. Cancer, 11(10):e007585. http://dx.doi.org/10.1136/jitc-2023-007585
29. Hou, S., Zhao, Y., Chen, J., et al., 2024. Tumor-associated macrophages in colorectal cancer metastasis: molecular insights and translational perspectives. *J. Transl. Med*., 22(1):62. http://dx.doi.org/10.1186/s12967-024-04856-x
30. Huang, S.C., Everts, B., Ivanova, Y., et al., 2014. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat. Immunol*, 15(9):846-855. http://dx.doi.org/10.1038/ni.2956
31. Huang, S.C., Smith, A.M., Everts, B., et al., 2016. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity*, 45(4):817-830.

http://dx.doi.org/10.1016/j.immuni.2016.09.016
32. Huang, Y., Xie, Y., Zhang, Y., et al., 2025. Single-cell transcriptome reveals the reprogramming of immune microenvironment during the transition from MASH to HCC. *Mol. Cancer,* 24(1):177. http://dx.doi.org/10.1186/s12943-025-02370-2
33. Jia, S., Bode, A.M., Chen, X., et al., 2024. Unlocking the potential: targeting metabolic pathways in the tumor microenvironment for cancer therapy. *Biochim. Biophys. Acta Rev. Cancer*, 1879(5):189166. http://dx.doi.org/10.1016/j.bbcan.2024.189166
34. Jelinic, P., Ricca, J., Van Oudenhove, E., et al., 2018. Immune-active microenvironment in small cell carcinoma of the ovary, hypercalcemic type: rationale for immune checkpoint blockade. *J. Natl. Cancer Inst*, 110(7):787-790. http://dx.doi.org/10.1093/jnci/djx277
35. Jha, A.K., Huang, S.C., Sergushichev, A., et al., 2015. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity*, 42(3):419-430. http://dx.doi.org/10.1016/j.immuni.2015.02.005
36. Jian, H., Wang, X., Song, P., et al., 2022. Tumor microcalcification-mediated relay drug delivery for photodynamic immunotherapy of breast cancer. *Acta Biomater*, 140:518-529. http://dx.doi.org/10.1016/j.actbio.2021.12.011
37. Jing, W., Guo, X., Wang, G., et al., 2020. Breast cancer cells promote CD169+ macrophage-associated immunosuppression through JAK2-mediated PD-L1 upregulation on macrophages. *Int. Immunopharmacol*, 78:106012. http://dx.doi.org/10.1016/j.intimp.2019.106012
38. Jin, X., Zhang, N., Yan, T., et al., 2025. Lactate-mediated metabolic reprogramming of tumor-associated macrophages: implications for tumor progression and therapeutic potential. *Front. Immunol*, 16:1573039. http://dx.doi.org/10.3389/fimmu.2025.1573039
39. Kennel, K.B., Greten, F.R., 2021. Immune cell-produced ROS and their impact on tumor growth and metastasis. *Redox Biol*, 42:101891. http://dx.doi.org/10.1016/j.redox.2021.101891
40. Kim, Y., Lee, S., Jon, S., 2024. Liposomal delivery of an immunostimulatory CpG induces robust antitumor immunity and long-term immune memory by reprogramming tumor-associated macrophages. *Adv. Healthc. Mater*, 13(6):e2300549. http://dx.doi.org/10.1002/adhm.202300549
41. Kuang, J., Rao, Z.Y., Zheng, D.W., et al., 2023. Nanoparticles hitchhike on monocytes for glioblastoma treatment after low-dose radiotherapy. *ACS Nano*, 17(14):13333-13347. http://dx.doi.org/10.1021/acsnano.3c01428
42. Lee, Y., Bogdanoff, D., Wang, Y., et al., 2021. XYZeq: spatially resolved single-cell RNA sequencing reveals expression heterogeneity in the tumor microenvironment. *Sci. Adv*, 7(17):eabg4755. http://dx.doi.org/10.1126/sciadv.abg4755
43. Li, Q., Zhang, X., Ke, R., 2022. Spatial transcriptomics for tumor heterogeneity analysis. *Front. Genet*, 13:906158. http://dx.doi.org/10.3389/fgene.2022.906158
44. Li, W., Yuan, Q., Li, M., et al., 2024. Research advances on signaling pathways regulating the polarization of tumor-associated macrophages in lung cancer microenvironment. *Front. Immunol*, 15:1452078. http://dx.doi.org/10.3389/fimmu.2024.1452078
45. Li, Z., Yu, Q., Zhu, Q., et al., 2022. Applications of machine learning in tumor-associated macrophages. *Front. Immunol*, 13:985863. http://dx.doi.org/10.3389/fimmu.2022.985863
46. Liao, Z.X., Ou, D.L., Hsieh, M.J., et al., 2021. Synergistic effect of repolarization of M2 to M1 macrophages induced by iron oxide nanoparticles combined with lactate oxidase. *Int. J. Mol. Sci*, 22(24):13346. http://dx.doi.org/10.3390/ijms222413346
47. Lin, H., Wei, S., Hurt, E.M., et al., 2018. Host expression of PD-L1 determines efficacy of PD-L1 pathway blockade-mediated tumor regression. *J. Clin. Invest*, 128(4):1708. http://dx.doi.org/10.1172/JCI120803
48. Lin, J., Liu, Y., Liu, P., et al., 2023. SNHG17 alters anaerobic glycolysis by resetting phosphorylation modification of PGK1 to foster pro-tumor macrophage formation in pancreatic ductal adenocarcinoma. *J. Exp. Clin. Cancer Res*, 42(1):339. http://dx.doi.org/10.1186/s13046-023-02890-z
49. Lin, W., Luo, Y., Wu, J., et al., 2023. Loss of ADAR1 in macrophages in combination with interferon gamma suppresses tumor growth by remodeling the tumor microenvironment. *J. Immunother. Cancer*, 11(11):e007402. http://dx.doi.org/10.1136/jitc-2023-007402
50. Liu, C., Xie, J., Lin, B., et al., 2024. Pan-cancer single-cell and spatial-resolved profiling reveals the immunosuppressive role of APOE+ macrophages in immune checkpoint inhibitor therapy. *Adv. Sci. (Weinh.)*, 11(23):e2401061. http://dx.doi.org/10.1002/advs.202401061
51. Liu, M., O'Connor, R.S., Trefely, S., et al., 2019. Metabolic rewiring of macrophages by CpG potentiates clearance of cancer cells and overcomes tumor-expressed CD47-mediated "don't-eat-me" signal. *Nat. Immunol*, 20(3):265-275. http://dx.doi.org/10.1038/s41590-018-0292-y
52. Liu, P.S., Wang, H., Li, X., et al., 2017. α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat. Immunol*, 18(9):985-994. http://dx.doi.org/10.1038/ni.3796
53. Liu, Q., Yang, C., Wang, S., et al., 2020. Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression. *Cell Commun. Signal*, 18(1):51. http://dx.doi.org/10.1186/s12964-020-00557-2
54. Liu, Y., Zugazagoitia, J., Ahmed, F.S., et al., 2020. Immune cell PD-L1 colocalizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. *Clin. Cancer Res*, 26(4):970-977. http://dx.doi.org/10.1158/1078-0432.CCR-19-1040
55. Lo, Y.L., Li, C.Y., Chou, T.F., et al., 2024. Exploring in vivo combinatorial chemo-immunotherapy: addressing p97

suppression and immune reinvigoration in pancreatic cancer with tumor microenvironment-responsive nanoformulation. *Biomed. Pharmacother*, 175:116660. http://dx.doi.org/10.1016/j.biopha.2024.116660
56. Lu, Y., Sun, Q., Guan, Q., et al., 2023. The XOR-IDH3α axis controls macrophage polarization in hepatocellular carcinoma. *J. Hepatol*, 79(5):1172-1184. http://dx.doi.org/10.1016/j.jhep.2023.06.022
57. Lyu, A., Fan, Z., Clark, M., et al., 2025. Evolution of myeloid-mediated immunotherapy resistance in prostate cancer. *Nature*, 637(8048):1207-1217. http://dx.doi.org/10.1038/s41586-024-08290-3
58. Magkouta, S.F., Vaitsi, P.C., Pappas, A.G., et al., 2021. CSF1/CSF1R axis blockade limits mesothelioma and enhances efficiency of anti-PDL1 immunotherapy. *Cancers (Basel)*, 13(11):2546. http://dx.doi.org/10.3390/cancers13112546
59. Mantovani, A., Biswas, S.K., Galdiero, M.R., et al., 2013. Macrophage plasticity and polarization in tissue repair and remodelling. *J. Pathol*, 229(2):176-185. http://dx.doi.org/10.1002/path.4133
60. Martinez-Usatorre, A., Kadioglu, E., Boivin, G., et al., 2021. Overcoming microenvironmental resistance to PD-1 blockade in genetically engineered lung cancer models. Sci. *Transl. Med*, 13(606):eabd1616. http://dx.doi.org/10.1126/scitranslmed.abd1616
61. Menga, A., Favia, M., Spera, I., et al., 2021. N-acetylaspartate release by glutaminolytic ovarian cancer cells sustains protumoral macrophages. *EMBO Rep*, 22(9):e51981. http://dx.doi.org/10.15252/embr.202051981
62. Menga, A., Serra, M., Todisco, S., et al., 2020. Glufosinate constrains synchronous and metachronous metastasis by promoting anti-tumor macrophages. *EMBO Mol. Med*, 12(10):e11210. http://dx.doi.org/10.15252/emmm.201911210
63. Meng, J., Li, L., Xu, Y., et al., 2023. A simple liposome-based bionic bacterium for tumor treatment by re-education of tumor-associated microphages in combination with chemotherapy. *Colloids Surf. B Biointerfaces*, 222:113069. http://dx.doi.org/10.1016/j.colsurfb.2022.113069
64. Mondini, M., Loyher, P., Berthelot, K., et al., 2017. CCL2/CCR2-driven monocyte recruitment to the tumor following radiotherapy influences the outcome of the treatment of head and neck cancer [abstract]. *Cancer Res*, 77(13 Suppl):5668. http://dx.doi.org/10.1158/1538-7445.AM2017-5668
65. Mondini, M., Loyher, P.L., Hamon, P., et al., 2019. CCR2-dependent recruitment of Tregs and monocytes following radiotherapy is associated with TNFα-mediated resistance. *Cancer Immunol. Res*, 7(3):376-387. http://dx.doi.org/10.1158/2326-6066.CIR-18-0633
66. Negrea, G., Rauca, V.F., Meszaros, M.S., et al., 2022. Active tumor-targeting nano-formulations containing simvastatin and doxorubicin inhibit melanoma growth and angiogenesis. *Front. Pharmacol*, 13:870347. http://dx.doi.org/10.3389/fphar.2022.870347
67. Niu, Z., Shi, Q., Zhang, W., et al., 2017. Caspase-1 cleaves PPARγ for potentiating the pro-tumor action of TAMs. *Nat. Commun*, 8(1):766. http://dx.doi.org/10.1038/s41467-017-00899-4
68. Ohradanova-Repic, A., Machacek, C., Charvet, C., et al., 2018. Extracellular purine metabolism is the switchboard of immunosuppressive macrophages and a novel target to treat diseases with macrophage imbalances. *Front. Immunol*, 9:852. http://dx.doi.org/10.3389/fimmu.2018.00852
69. Oh, M.H., Sun, I.H., Zhao, L., et al., 2020. Targeting glutamine metabolism enhances tumor-specific immunity by modulating suppressive myeloid cells. *J. Clin. Invest*, 130(7):3865-3884. http://dx.doi.org/10.1172/JCI131859
70. Ostuni, R., Kratochvill, F., Murray, P.J., et al., 2015. Macrophages and cancer: from mechanisms to therapeutic implications. *Trends Immunol*, 36(4):229-239. http://dx.doi.org/10.1016/j.it.2015.02.004
71. Palmieri, E.M., Menga, A., Martín-Pérez, R., et al., 2017. Pharmacologic or genetic targeting of glutamine synthetase skews macrophages toward an M1-like phenotype and inhibits tumor metastasis. *Cell Rep*, 20(7):1654-1666. http://dx.doi.org/10.1016/j.celrep.2017.07.054
72. Park, D.J., Sung, P.S., Lee, G.W., et al., 2021. Preferential expression of programmed death ligand 1 protein in tumor-associated macrophages and its potential role in immunotherapy for hepatocellular carcinoma. *Int. J. Mol. Sci*, 22(9):4710. http://dx.doi.org/10.3390/ijms22094710
73. Peckert-Maier, K., Langguth, P., Strack, A., et al., 2023. CD83 expressed by macrophages is an important immune checkpoint molecule for the resolution of inflammation. *Front. Immunol*, 14:1085742. http://dx.doi.org/10.3389/fimmu.2023.1085742
74. Peranzoni, E., Lemoine, J., Vimeux, L., et al., 2018. Macrophages impede CD8 T cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment. *Proc. Natl. Acad. Sci. U. S. A*, 115(17):E4041-E4050. http://dx.doi.org/10.1073/pnas.1720948115
75. Petty, A.J., Dai, R., Lapalombella, R., et al., 2021. Hedgehog-induced PD-L1 on tumor-associated macrophages is critical for suppression of tumor-infiltrating CD8+ T cell function. *JCI Insight*, 6(6):e146707. http://dx.doi.org/10.1172/jci.insight.146707
76. Petty, A.J., Li, A., Wang, X., et al., 2019. Hedgehog signaling promotes tumor-associated macrophage polarization to suppress intratumoral CD8+ T cell recruitment. *J. Clin. Invest*, 129(12):5151-5162. http://dx.doi.org/10.1172/JCI128644
77. Plotkin, D.L., Delcastillo, K., Van Every, D.W., et al., 2021. Isolated leucine and branched-chain amino acid supplementation for enhancing muscular strength and hypertrophy: a narrative review. *Int. J. Sport Nutr. Exerc. Metab*, 31(3):292-301. http://dx.doi.org/10.1123/ijsnem.2020-0356
78. Praharaj, M., Shen, F., Lee, A.J., et al., 2024. Metabolic reprogramming of tumor-associated macrophages using glutamine antagonist JHU083 drives tumor immunity in myeloid-rich prostate and bladder cancers. *Cancer Immunol. Res*, 12(7):854-875. http://dx.doi.org/10.1158/2326-6066.CIR-23-1105
79. Raines, L.N., Zhao, H., Wang, Y., et al., 2022. PERK is a critical metabolic hub for immunosuppressive function in

macrophages. *Nat. Immunol*, 23(3):431-445. http://dx.doi.org/10.1038/s41590-022-01145-x
80. Ravi, M., Paramesh, V., Kaviya, S.R., et al., 2015. 3D cell culture systems: advantages and applications. *J. Cell Physiol*, 230(1):16-26. http://dx.doi.org/10.1002/jcp.24683
81. Reinfeld, B.I., Madden, M.Z., Wolf, M.M., et al., 2021. Cell-programmed nutrient partitioning in the tumour microenvironment. *Nature*, 593(7858):282-288. http://dx.doi.org/10.1038/s41586-021-03442-1
82. Rongvaux, A., Willinger, T., Martinek, J., et al., 2014. Development and function of human innate immune cells in a humanized mouse model. *Nat. Biotechnol*, 32(4):364-372. http://dx.doi.org/10.1038/nbt.2858
83. Roux, C., Jafari, S.M., Shinde, R., et al., 2019. Reactive oxygen species modulate macrophage immunosuppressive phenotype through the up-regulation of PD-L1. *Proc. Natl. Acad. Sci. U. S. A*, 116(10):4326-4335. http://dx.doi.org/10.1073/pnas.1819473116
84. Saqib, J., Park, B., Jin, Y., et al., 2023. Identification of niche-specific gene signatures between malignant tumor microenvironments by integrating single cell and spatial transcriptomics data. *Genes (Basel)*, 14(11):2033. http://dx.doi.org/10.3390/genes14112033
85. Sato, T., Sugiyama, D., Koseki, J., et al., 2025. Sustained inhibition of CSF1R signaling augments antitumor immunity through inhibiting tumor-associated macrophages. JCI Insight, 10(1):e178146. http://dx.doi.org/10.1172/jci.insight.178146
86. Shao, N., Qiu, H., Liu, J., et al., 2024. Targeting lipid metabolism of macrophages: a new strategy for tumor therapy. *J. Adv. Res*, in press. http://dx.doi.org/10.1016/j.jare.2024.02.009
87. Sharma, G., Ojha, R., Noguera-Ortega, E., et al., 2022. PPT1 inhibition enhances the antitumor activity of anti-PD-1 antibody in melanoma. *JCI Insight*, 7(20):e165688. http://dx.doi.org/10.1172/jci.insight.165688
88. Shen, W., Liu, T., Pei, P., et al., 2022. Metabolic homeostasis-regulated nanoparticles for antibody-independent cancer radio-immunotherapy. *Adv. Mater*, 34(51):e2207343. http://dx.doi.org/10.1002/adma.202207343
89. Shimazaki, R., Takano, S., Satoh, M., et al., 2021. Complement factor B regulates cellular senescence and is associated with poor prognosis in pancreatic cancer. *Cell Onco*l. (Dordr.), 44(4):937-950. http://dx.doi.org/10.1007/s13402-021-00614-z
90. Shimizu, D., Yuge, R., Kitadai, Y., et al., 2024. Pexidartinib and immune checkpoint inhibitors combine to activate tumor immunity in a murine colorectal cancer model by depleting M2 macrophages differentiated by cancer-associated fibroblasts. *Int. J. Mol. Sci*, 25(13):7001. http://dx.doi.org/10.3390/ijms25137001
91. Shimoyama, S., Okada, K., Kimura, T., et al., 2023. FF-10850, a novel liposomal topotecan achieves superior antitumor activity via macrophage- and ammonia-mediated payload release in the tumor microenvironment. *Mol. Cancer Ther*, 22(12):1454-1464. http://dx.doi.org/10.1158/1535-7163.MCT-23-0099
92. Singh, S., Lee, N., Pedroza, D.A., et al., 2022. Chemotherapy coupled to macrophage inhibition induces T-cell and B-cell infiltration and durable regression in triple-negative breast cancer. *Cancer Res*, 82(12):2281-2297. http://dx.doi.org/10.1158/0008-5472.CAN-21-3714
93. Song, N., Cui, K., Zeng, L., et al., 2024. Advance in the role of chemokines/chemokine receptors in carcinogenesis: focus on pancreatic cancer. *Eur. J. Pharmacol*, 967:176357. http://dx.doi.org/10.1016/j.ejphar.2024.176357
94. Stack, E.C., Wang, C., Roman, K.A., et al., 2014. Multiplexed immunohistochemistry, imaging, and quantitation: a review, with an assessment of tyramide signal amplification, multispectral imaging and multiplex analysis. *Methods*, 70(1):46-58. http://dx.doi.org/10.1016/j.ymeth.2014.08.016
95. Stöth, M., Freire Valls, A., Chen, M., et al., 2019. Splenectomy reduces lung metastases and tumoral and metastatic niche inflammation. *Int. J. Cancer*, 145(9):2509-2520. http://dx.doi.org/10.1002/ijc.32378
96. Su, P., Wang, Q., Bi, E., et al., 2020. Enhanced lipid accumulation and metabolism are required for the differentiation and activation of tumor-associated macrophages [published correction appears in Cancer Res. 2022;82(5):945. doi:10.1158/0008-5472.CAN-21-4296]. *Cancer Res*, 80(7):1438-1450. http://dx.doi.org/10.1158/0008-5472.CAN-19-2994
97. Sui, D., Liang, K., Gui, Y., et al., 2022. Optimization design of sialic acid derivatives enhances the performance of liposomes for modulating immunosuppressive tumor microenvironments. *Life Sci*, 310:121081. http://dx.doi.org/10.1016/j.lfs.2022.121081
98. Tan, Z., Xie, N., Cui, H., et al., 2015. Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism. *J. Immunol*, 194(12):6082-6089. http://dx.doi.org/10.4049/jimmunol.1402469
99. Tang, J., Ming, L., Qin, F., et al., 2024. The heterogeneity of tumour-associated macrophages contributes to the clinical outcomes and indications for immune checkpoint blockade in colorectal cancer patients. *Immunobiology*, 229(3):152805. http://dx.doi.org/10.1016/j.imbio.2024.152805
100. Tartey, S., Neale, G., Vogel, P., et al., 2021. A MyD88/IL1R axis regulates PD-1 expression on tumor-associated macrophages and sustains their immunosuppressive function in melanoma. *Cancer Res*, 81(9):2358-2372. http://dx.doi.org/10.1158/0008-5472.CAN-20-3510
101. Tavakoli, S., Downs, K., Short, J.D., et al., 2017. Characterization of macrophage polarization states using combined measurement of 2-deoxyglucose and glutamine accumulation: implications for imaging of atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*, 37(10):1840-1848. http://dx.doi.org/10.1161/ATVBAHA.117.309494
102. Tharp, K.M., Kersten, K., Maller, O., et al., 2024. Tumor-associated macrophages restrict CD8+ T cell function through collagen deposition and metabolic reprogramming of the breast cancer microenvironment. *Nat. Cancer*, 5(7):1045-1062. http://dx.doi.org/10.1038/s43018-024-00775-4
103. Tsai, T.L., Zhou, T.A., Hsieh, Y.T., et al., 2022. Multiomics reveal the central role of pentose phosphate pathway in resident thymic macrophages to cope with efferocytosis-associated stress. *Cell Rep,* 40(2):111065. http://dx.doi.org/10.1016/j.celrep.2022.111065

104. Tsuruta, A., Shiiba, Y., Matsunaga, N., et al., 2022. Diurnal expression of PD-1 on tumor-associated macrophages underlies the dosing time-dependent antitumor effects of the PD-1/PD-L1 inhibitor BMS-1 in B16/BL6 melanoma-bearing mice. *Mol. Cancer Res*, 20(6):972-982. http://dx.doi.org/10.1158/1541-7786.MCR-21-0786
105. Voissière A, Gomez-Roca C, Chabaud S, et al., 2024. The CSF-1R inhibitor pexidartinib affects FLT3-dependent dendritic cell differentiation and may antagonize the effect of durvalumab in patients with advanced cancers. *Sci. Transl. Med*, 16(731):eadd1834. https://doi.org/10.1126/scitranslmed.add1834
106. Wainberg, Z., Eisenberg, P., Sachdev, J., et al., 2016. Phase 1/2a study of double immune suppression blockade by combining a CSF1R inhibitor (pexidartinib/PLX3397) with an anti PD-1 antibody (pembrolizumab) to treat advanced melanoma and other solid tumors. *J. Clin. Oncol*, 34:TPS465. http://dx.doi.org/10.1200/JCO.2016.34.4_SUPPL.TPS465
107. Wan, C., Sun, Y., Tian, Y., et al., 2020. Irradiated tumor cell-derived microparticles mediate tumor eradication via cell killing and immune reprogramming. *Sci. Adv*, 6(13):eaay9789. http://dx.doi.org/10.1126/sciadv.aay9789
108. Wang, H., Liang, Y., Liu, Z., et al., 2024. POSTN+ cancer-associated fibroblasts determine the efficacy of immunotherapy in hepatocellular carcinoma. *J. Immunother. Cancer*, 12(7):e008721. http://dx.doi.org/10.1136/jitc-2023-008721
109. Wang, H., Yung, M.M.H., Ngan, H.Y.S., et al., 2021. The impact of the tumor microenvironment on macrophage polarization in cancer metastatic progression. *Int. J. Mol. Sci*, 22(12):6560. http://dx.doi.org/10.3390/ijms22126560
110. Wang, X., Zhang, S., Xue, D., et al., 2025. Metabolic reprogramming of macrophages in cancer therapy. *Trends Endocrinol*. Metab., 36(7):660-676. http://dx.doi.org/10.1016/j.tem.2024.08.009
111. Wang, Y., Wang, D., Yang, L., et al., 2022. Metabolic reprogramming in the immunosuppression of tumor-associated macrophages. *Chin. Med. J. (Engl.)*, 135(20):2405-2416. http://dx.doi.org/10.1097/CM9.0000000000002426
112. Wei, F., Wang, D., Wei, J., et al., 2021. Metabolic crosstalk in the tumor microenvironment regulates antitumor immunosuppression and immunotherapy resistance. Cell Mol. *Life Sci*, 78(1):173-193. http://dx.doi.org/10.1007/s00018-020-03581-0
113. Wei, Z., Zhang, X., Yong, T., et al., 2021. Boosting anti-PD-1 therapy with metformin-loaded macrophage-derived microparticles. *Nat. Commun*, 12(1):440. http://dx.doi.org/10.1038/s41467-020-20723-x
114. Wen, Z., Sun, H., Zhang, Z., et al., 2023. High baseline tumor burden-associated macrophages promote an immunosuppressive microenvironment and reduce the efficacy of immune checkpoint inhibitors through the IGFBP2-STAT3-PD-L1 pathway. *Cancer Commun. (Lond.)*, 43(5):562-581. http://dx.doi.org/10.1002/cac2.12420
115. Wu, S.Z., Al-Eryani, G., Roden, D.L., et al., 2021. A single-cell and spatially resolved atlas of human breast cancers. *Nat. Genet*, 53(9):1334-1347. http://dx.doi.org/10.1038/s41588-021-00911-1
116. Xiao, B., Li, G., Gulizeba, H., et al., 2024. Choline metabolism reprogramming mediates an immunosuppressive microenvironment in non-small cell lung cancer (NSCLC) by promoting tumor-associated macrophage functional polarization and endothelial cell proliferation. *J. Transl. Med*, 22(1):442. http://dx.doi.org/10.1186/s12967-024-05245-2
117. Xu, C., Xia, Y., Zhang, B.W., et al., 2023. Macrophages facilitate tumor cell PD-L1 expression via an IL-1β-centered loop to attenuate immune checkpoint blockade. *MedComm (2020)*, 4(2):e242. http://dx.doi.org/10.1002/mco2.242
118. Xu, L., Li, B., Pi, C., et al., 2022. Targeting CD89 on tumor-associated macrophages overcomes resistance to immune checkpoint blockade. *J. Immunother. Cancer*, 10(12):e005447. http://dx.doi.org/10.1136/jitc-2022-005447
119. Xu, S., Wang, C., Yang, L., et al., 2023. Targeting immune checkpoints on tumor-associated macrophages in tumor immunotherapy. *Front. Immunol*, 14:1199631. http://dx.doi.org/10.3389/fimmu.2023.1199631
120. Yan, H., Shi, J., Dai, Y., et al., 2022. Technique integration of single-cell RNA sequencing with spatially resolved transcriptomics in the tumor microenvironment. *Cancer Cell Int*, 22(1):155. http://dx.doi.org/10.1186/s12935-022-02580-4
121. Yang, H., Kim, C., Zou, W., 2024. Metabolism and macrophages in the tumor microenvironment. *Curr. Opin. Immunol*, 86:102491. http://dx.doi.org/10.1016/j.coi.2024.102491
122. Yang, L., Venneti, S., Nagrath, D., 2017. Glutaminolysis: a hallmark of cancer metabolism. *Annu. Rev. Biomed. Eng*, 19:163-194. http://dx.doi.org/10.1146/annurev-bioeng-071516-044546
123. Zeng, W., Li, F., Jin, S., et al., 2023. Functional polarization of tumor-associated macrophages dictated by metabolic reprogramming. *J. Exp. Clin. Cancer Res*, 42(1):245. http://dx.doi.org/10.1186/s13046-023-02832-9
124. Zhang, N., Liu, C., Jin, L., et al., 2020. Ketogenic diet elicits antitumor properties through inducing oxidative stress, inhibiting MMP-9 expression, and rebalancing M1/M2 tumor-associated macrophage phenotype in a mouse model of colon cancer. *J. Agric. Food Chem*, 68(40):11182-11196. http://dx.doi.org/10.1021/acs.jafc.0c04041
125. Zhao, K., Shi, R.C., Miao, H.M., 2021. A Review of the Lipid Metabolism Reprogramming in Tumor Associated Macrophages. *Sichuan Da Xue Xue Bao Yi Xue Ban,* 52(1):45-49 (in Chinese). http://dx.doi.org/10.12182/20210160202
126. Zhao, X., Ren, T., Li, S., et al., 2024. A new perspective on the therapeutic potential of tumor metastasis: targeting the metabolic interactions between TAMs and tumor cells. *Int. J. Biol. Sci*, 20(13):5109-5126. http://dx.doi.org/10.7150/ijbs.99680
127. Zhen, X., Li, Y., Yuan, W., et al., 2024. Biointerface-engineered hybrid nanovesicles for targeted reprogramming of tumor microenvironment. *Adv. Mater*, 36(41):e2401495. http://dx.doi.org/10.1002/adma.202401495
128. Zhou, W., Hu, G., He, J., et al., 2022. SENP1-Sirt3 signaling promotes α-ketoglutarate production during M2 macrophage polarization. *Cell Rep*, 39(2):110660. http://dx.doi.org/10.1016/j.celrep.2022.110660
129. Zhu, N., Chen, S., Jin, Y., et al., 2024. Enhancing glioblastoma immunotherapy with integrated chimeric antigen receptor T cells through the re-education of tumor-associated microglia and macrophages. *ACS Nano*, 18(17):11165-11182. http://dx.doi.org/10.1021/acsnano.4c00050
130. Zhu, Y., Yang, J., Xu, D., et al., 2019. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced

colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. *Gut*, 68(9):1653-1666. http://dx.doi.org/10.1136/gutjnl-2019-318419

131. Zuo, B., Li, T., Liu, X., et al., 2023. Dipeptidyl peptidase 4 inhibitor reduces tumor-associated macrophages and enhances anti-PD-L1-mediated tumor suppression in non-small cell lung cancer. *Clin. Transl. Oncol*, 25(11):3188-3202. http://dx.doi.org/10.1007/s12094-023-03187-5