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Heterogeneity of macrophages in PD-1/PD-L1 inhibitor therapy: a single-cell perspective

Jinjing Wu[1,2,3], Dan Zhou[3], Kunpeng Jiang[3], Zhenkui Li[3], Yudi Yao[4], Shaqi Rao[5], Xiaohong Yang[1,2,3*] and Daya Luo[1,2,3*]

*Correspondence:
Xiaohong Yang
xiaohongyang2007@ncu.edu.cn
Daya Luo
luodaya@ncu.edu.cn

Full list of author information is available at the end of the article

Abstract

Therapeutic blockade of the PD-1/PD-L1 signaling pathway is the focus of tumor immunotherapy. However, drug resistance and irAEs induced by PD-1/PD-L1 inhibitors have emerged as major limitations affecting survival outcomes in patients with cancer. Macrophages are not only the core immune cells that regulate tumor progression and metastasis, but also the key factors that affect the therapeutic effect of PD-1/PD-L1 inhibitors, thus showing significant clinical value in optimizing immunotherapy strategies. scRNA-seq technology has provided powerful analytical tools and precise biological insights to decipher macrophage heterogeneity, further elucidating disease-specific macrophage subpopulations with distinct gene signatures and functional plasticity during PD-1/PD-L1 blockade therapy. These advances have paved the way for better understanding the mechanisms underlying immunotherapy-induced toxicities and identifying novel predictive biomarkers. Herein, we comprehensively summarize the multifaceted functional roles of macrophages in PD-1/PD-L1 inhibitor-mediated antitumor efficacy, drug resistance and irAEs from a single-cell perspective, and the potential value of targeting macrophages to improve the accuracy of immunotherapy.

Keywords Macrophage, PD-1/PD-L1 inhibitor, irAEs, Drug resistance, Combination therapy

Introduction

Cancer immune checkpoint inhibitors (ICIs) represent an emerging therapeutic approach following conventional treatments including surgery, chemotherapy, and radiotherapy. The groundbreaking progress in ICI therapy has not only revolutionized the landscape of cancer treatment but also marked a paradigm shift from "attacking tumors" to "mobilizing immunity" [1]. The pivotal role of ICIs in activating immune responsiveness in early-stage tumors and improving survival outcomes in advanced cancer patients has been well-documented in numerous clinical trials [2, 3]. Programmed cell death protein 1 (PD-1) inhibitors and programmed death-ligand 1 (PD-L1) inhibitors currently constitute the most widely used class of ICIs. These therapeutics can restore the activation and cytotoxic capacity of T cells by antagonizing the negative regulatory signals resulting from PD-1/PD-L1 interaction, which ultimately potentiates

the antitumor immune response [4]. However, not all patients respond to PD-1/PD-L1 inhibitors, with only a subset deriving clinical benefit. Furthermore, immune-related adverse events (irAEs) and drug resistance associated with PD-1/PD-L1 inhibitors have become major limitations in clinical practice [5, 6]. To date, the pathological mechanisms underlying PD-1/PD-L1 inhibitor-related toxicities remain incompletely understood, and there is still a lack of reliable predictive biomarkers to guide precision medicine in clinical settings.

Macrophages represent the most plastic immune cells within the tumor microenvironment (TME), which play multifaceted roles in tumor growth, metastasis, and therapeutic response [7]. Current understanding identifies two primary origins of macrophages: tissue-resident macrophages (TRMs) and monocyte-derived macrophages (MDMs), which maintain dynamic equilibrium while exhibiting distinct functional characteristics in the TME [8]. The conventional classification system categorizes macrophages into M1-like (classically activated) and M2-like (alternatively activated) phenotypes on the basis of their responses to different stimuli. M1 macrophages demonstrate proinflammatory and antitumor properties, whereas M2 macrophages exhibit antiinflammatory and protumor characteristics [9]. However, analyses of various solid tumors reveal that the clearly demarcated M1/M2 classification in vitro fails to represent their dynamic states in vivo, as this oversimplified dichotomy cannot adequately capture the heterogeneity and complexity of tumor-associated macrophages (TAMs) [10]. Recent years have witnessed groundbreaking advances in multi-omics technologies, particularly single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics, which have enabled breakthroughs in characterizing the heterogeneity of macrophage origins [11], functional diversity [12], and spatial distribution [13]. These technologies have not only established correlations between macrophage abundance and clinical outcomes but have also elucidated causal relationships between specific TAM states and tumor progression or treatment response [14]. Research demonstrates that TAMs critically influence the efficacy of PD-1/PD-L1 inhibitors through multiple mechanisms including their high expression of immune checkpoint molecules such as PD-L1 [15, 16], regulation of PD-L1 expression on other TME components [17, 18], secretion of various cytokines to modulate crosstalk with T cells [19], and mediation of metabolic reprogramming in the TME [20].

The U.S. Food and Drug Administration has currently approved PD-L1 as a static predictive biomarker for ICIs efficacy [21]. However, the lack of correlation between PD-L1 expression and ICIs response across various tumor types suggests that predictive biomarker identification depends on the complex ecosystem of tumors [22]. At present, the research gradually reveals the key role of specific macrophage subsets in mediating ICIs drug resistance, and proves that the therapy targeting drug-resistant specific macrophage subsets can significantly improve the sensitivity of immunotherapy [23]. For irAEs, owing to its irregular occurrence, there is still a lack of effective predictive markers and therapeutic targets [24]. scRNA-seq reveals the unique genetic characteristics and functional alterations of macrophages during the treatment of PD-1/PD-L1 inhibitors at the single cell level, which provides essential insights for investigating irAE mechanisms and immunotherapy resistance while facilitating the discovery of specific biomarkers. Therefore, this review focuses on the central role of macrophages in PD-1/PD-L1 inhibitor therapy from three perspectives. First, based on single-cell transcriptomic technologies, we provide an in-depth analysis of macrophage heterogeneity,

including their ontogeny, functional diversity, metabolic profiles, and spatial heterogeneity, thereby establishing a foundation for understanding their functional versatility. Second, we systematically elucidate the multifaceted roles of macrophages in antitumor efficacy, irAEs, and mechanisms of drug resistance. Finally, building upon the aforementioned mechanisms, we explore macrophage-targeted combination therapeutic strategies, aiming to provide novel insights for the development of specific therapeutic targets and the optimization of clinical ICI treatment regimens.

Heterogeneity of macrophages in cancer from single-cell insights

Origin heterogeneity

Macrophages in TME include embryo-derived TRMs and bone marrow-derived MDMs, which have significant molecular and functional differences. TRMs demonstrate conserved expression profiles of signature genes (*LYVE1*, *HES1*, and *FOLR2*) between tumor tissues and their corresponding normal organs, while simultaneously displaying remarkable organ-specific variations across different cancer types [25]. For example, in hepatocellular carcinoma (HCC) patients, TRMs express CD68, VSIG4, MARCO, and CD163, while in murine HCC models, TRMs express F4/80, CLEC4F, and TIMD4, exhibiting a phenotype similar to Kupffer cells (KCs) [26–28]. In lung cancers, TRMs express MARCO, FABP4, MCEMP1, and PPARG, which is similar to alveolar resident macrophages [29, 30]. Inflammatory recruitment is the classic function of MDMs, and chemokine receptors CX3CR1 and CCR2 have been proven to be characteristic markers of MDMs in various tumors [31, 32]. TRMs has been colonized in specific tissues at the embryonic development stage and has the ability of self-renewal, while MDMs depends on the continuous recruitment from microenvironment. Their relative abundance exhibits significant variation across different tumor types [8]. For example, in the breast cancer model, $CCR2^+$ monocytes are the main source of TAMs, and the number of TRMs gradually decreases with the progression of the tumor. Conversely, TRMs represent the major TAMs component in pancreatic cancer, retinoblastoma, neuroblastoma, and osteosarcoma [33, 34].

Furthermore, macrophage populations of distinct origins demonstrate markedly divergent roles in tumor progression. Nasir et al. identified that antitumor macrophages within the TME primarily consist of TRMs localized perivascularly in stromal regions, including $CD169^+$ TAMs, $FOLR2^+$ $LYVE1^+$ TAMs, and $PPARG^+$ $MRC1^+$ TAMs [35]. A breast cancer study revealed that $FOLR2^+$ TRMs engage in close interactions with $CD8^+$ T cells, effectively activating cytotoxic T lymphocytes and correlating with favorable clinical outcomes [36]. However, Casanova et al. found that in the early stage of lung cancer, TRMs clustered near tumor cells to promote epithelial–mesenchymal transition and invasion of tumor cells. Meanwhile, TRMs also promotes tumor progression by inducing the recruitment and differentiation of Tregs [11]. Although the identification of TRMs function in TME is still controversial, it further suggests that TRMs presents functional heterogeneity in different spatial distribution and tumor progression stages. In contrast to TRMs, MDMs exhibit enhanced immunosuppressive capacity and protumorigenic functions [37, 38]. During the progression of glioblastoma (GBM), MDMs gradually replaced resident microglia and inhibited the proliferation of $CD3^+$ T cells. Glucose metabolism is essential for maintaining their potent immunosuppressive activity [39]. LaMarche et al. found that interleukin-4 (IL-4) is the main driving factor for the

formation of MDMs in tumors, where it guides the development of immunosuppressive myeloid cells through direct modulation of granulocyte-monocyte progenitors [40].

In summary, the origin heterogeneity of macrophages not only reflects the complexity of their developmental biology, but also provides novel insights into the dynamic regulation of cellular functions and prognostic evaluation in cancer, which holds significant implications for developing precision therapeutic strategies targeting macrophage subpopulations.

Functional heterogeneity

The scRNA-seq technology has ushered in a new era of macrophage precision biology, which is a powerful tool to transcend classical macrophage classification paradigms and establish novel categorization frameworks (Fig. 1). Current scRNA-seq analyses have identified multiple novel macrophage subpopulations in tumors through core gene signatures [41]. SPP1$^+$ TAMs have been proved to be a key marker of tumor progression and metastasis in many kinds of tumors [42, 43]. SPP1$^+$ TAMs have the characteristics of high HIF-1α, Myc, and Spi1 activities, and these genes are the core transcription factors of hypoxia and fibrosis [44]. Clinical studies consistently demonstrate that SPP1$^+$ TAMs correlate with poor patient prognosis and impaired T-cell functionality across multiple cancer types [45, 46]. C1Q$^+$ TAMs are characterized by complement activation and highly expresses C1QA and C1QC. The differentiation state of C1Q$^+$ TAMs determines its functional characteristics. Mature C1Q$^+$ TAMs express immunosuppressive and angiogenic genes, which clinically correlates with tumor progression and poor patient outcomes [47, 48], while pre C1Q$^+$ TAMs highly express genes related to phagocytosis and antigen presentation [49, 50]. This immunocompetent precursor population

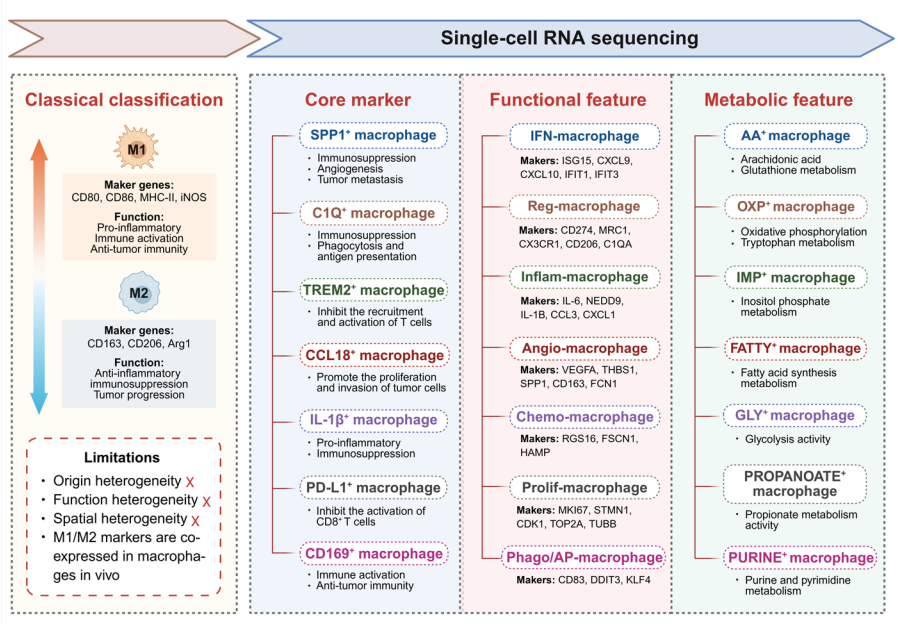

Fig. 1 Classification model of new macrophage subsets. With the development of scRNA-seq technology, the classification method of macrophages has changed from the traditional dichotomy to the classification of specific macrophage subsets. Based on different biological perspectives, new classification methods of macrophages include core gene classification, functional feature classification, and metabolic feature classification (created with BioRender.com)

has been identified as a crucial antitumor effector cell subset in both osteosarcoma and breast cancer microenvironments [51, 52]. In advanced cervical cancer, the C1QC^{high} and SPP1^{low} TAMs correlate with favorable prognosis, while C1QC^{low} and SPP1^{high} TAMs predict poorer clinical outcomes [53].

Another crucial protumorigenic macrophage subset in the TME are TREM2^{+} TAMs, which are considered to be the key factor in the formation of lipid-related macrophages [54]. These TREM2^{+} TAMs exhibit elevated expression of SPP1, C1QC, M2 macrophage markers, immune checkpoint molecules, and lipid metabolism genes [55]. They promote tumor progression by suppressing CD8^{+} T cell activity and enhancing cancer cell glycolysis [56]. In addition, CCL18^{+} TAMs highly express the characteristics of M2-type macrophages, and also have significant antiinflammatory and tumor-promoting properties [57]. CCL18^{+} TAM is differentiated from SPP1^{+} TAM, which belongs to the terminal differentiation state TAM. This group of TAM has a stronger tumor-promoting effect and is associated with a worse prognosis [49]. Within the TME, IL-1β^{+} TAMs actively recruit and modulate immune cells to coordinate inflammatory responses [52]. In pancreatic cancer, IL-1β^{+} TAMs exhibit a dual phenotype combining proinflammatory characteristics with potent immunosuppressive activity, and establish a self-reinforcing, tumor-promoting feedback loop with malignant epithelial cells through reciprocal cytokine signaling [58]. The identification of these protumorigenic TAM subsets has significantly advanced our understanding of how macrophage functional states and inflammatory programs mechanistically link to cancer progression. Furthermore, classifying macrophage subpopulations by immune checkpoint expression provides critical insights into immune microenvironment characteristics and treatment responsiveness. Both PD-1^{+} and PD-L1^{+} TAM populations typically expand in advanced tumors, where they promote M2-like polarization, inhibit CD8^{+} T cell activation, and accelerate tumor growth [59, 60]. In EBV-positive classical Hodgkin lymphoma (cHL), Hodgkin–Reed–Sternberg (HRS) cells are characteristically surrounded by PD-1^{+} immune effector cells and PD-L1^{+} macrophages, this spatial organization potentially determining treatment responses in EBV-positive cHL patients [61]. In a word, it is necessary to classify macrophage subsets on the basis of core genes to improve the accuracy of treatment. Importantly, the expression profiles of these defining markers may vary significantly across different tumor types and even during disease progression within the same malignancy.

Current research lacks consensus regarding the nomenclature of macrophage subtypes based on their most highly expressed transcripts. Only relying on key genes to divide subsets may ignore the dynamic properties of functional markers of macrophages and fail to accurately reflect the true phenotypic and functional differences of macrophage subsets [35]. Macrophage subsets identified on the basis of characteristic genes, enrichment pathways, and predictive functions provide a consensus model for the definition of functional diversity of macrophages. This approach has identified several functionally distinct TAM subsets, including: interferon-induced TAMs (IFN-TAMs), immunoregulatory TAMs (Reg-TAMs), inflammatory TAMs (Inflam-TAMs), pro-angiogenic TAMs (Angio-TAMs), proliferative TAMs (ProLiF-TAMs), chemotactic TAMs (Chemo-TAMs), and phagocytic/antigen-presenting TAMs (Phago/AP-TAMs) [62, 63]. These classifications systemically correlate the molecular heterogeneity of TAMs with their functional plasticity, elucidating the dynamic transitions of TAM functional states during tumor progression.

Metabolic heterogeneity

Metabolic heterogeneity represents a fundamental characteristic of the TME, and macrophage classification based on metabolic signatures provides novel insights for developing metabolic-modulating therapeutics. Yang et al. systematically investigated the functional significance of macrophages in distinct metabolic states within clear cell renal cell carcinoma (ccRCC), identifying seven metabolically-defined subpopulations (Fig. 1): AA^+ macrophages (associated with arachidonic acid and glutathione metabolism), OXP^+ macrophages (linked to oxidative phosphorylation and tryptophan metabolism), IMP^+ macrophages (characterized by inositol phosphate metabolism), $FATTY^+$ macrophages (showing fatty acid synthesis metabolism activity), GLY^+ macrophages (exhibiting glycolysis activity), $PROPANOATE^+$ macrophages (involved in propionate metabolism), and $PURINE^+$ macrophages (related to purine and pyrimidine metabolism) [64]. This pioneering work established a paradigm for analyzing metabolic reprogramming in tumors while identifying key metabolic targets strongly correlated with macrophage functions. In ccRCC, the metabolic heterogeneity of macrophages has also been finely delineated. Cao et al., through integration of single-cell transcriptomics and machine learning optimization, identified three lipid-associated macrophage subsets within the tumor microenvironment, characterized by ALOX5AP, HERPUD1, and PRDX1, respectively. Among these, $PRDX1^+$ lipid-associated macrophages (LAMs) were not only significantly associated with poor patient prognosis but also represented the predominant pathological subset in high-risk patients [65]. Additionally, Li et al. delineated the metabolic characteristics of macrophages in liver TME, and revealed the correlation between different metabolic patterns and the functional phenotype of macrophages. Their study revealed that glycolytic TAMs demonstrate superior phagocytic capacity, while purine-metabolizing TAMs exhibit impaired antigen presentation and pronounced protumorigenic phenotypes, which are associated with poor response to immune checkpoint blockade therapy. Notably, TAMs with purine metabolism, amino acid metabolism, and glycolytic signatures coexpress elevated levels of proangiogenic genes [66].

Furthermore, TAM classification on the basis of core metabolic gene signatures has significantly advanced our understanding of macrophage metabolic reprogramming in shaping tumor immunosuppressive microenvironments. For instance, fatty acid-binding protein 5 (FABP5) is recognized as a "lipid sensor" in tumor metabolism, and $FABP5^+$ TAM has the characteristics of enhanced fatty acid uptake and transport capacity [67]. These lipid-laden macrophages process tumor-derived unsaturated fatty acid signals to upregulate immunosuppressive molecules, ultimately impairing T-cell antitumor activity [68]. A scRNA-seq analysis of the TME in esophageal squamous cell carcinoma (ESCC) identified a distinct TAM subset characterized by high expression of phospholipase D3 (PLD3). These $PLD3^+$ TAMs exhibited dysregulated cholesterol metabolism and immunosuppressive properties, and were associated with poor prognosis [69]. In TME, the metabolic pathway of amino acids will also change significantly. Among them, indoleamine 2,3-dioxygenase 1 (IDO1) is highly expressed in many cancers, which can inhibit the antitumor immune response through tryptophan metabolism and nonenzymatic function [70]. $IDO1^+$ TAM induces immune suppression of macrophage subsets by promoting tryptophan consumption and kynurenine accumulation, thus weakening T-cell activity [71, 72].

More intriguingly, the metabolic heterogeneity of macrophages may also exhibit cross-disease commonality. Martinez-Campanario et al. found that atherosclerotic foam cells and tumor-associated LAMs share a conserved lipid-responsive transcriptional program and both rely on fatty acid oxidation metabolism, suggesting that lipid metabolic reprogramming is a conserved mechanism driving macrophage polarization. Targeting LAMs may hold potential for both antiatherosclerotic and antitumor therapy [73]. Thus, the classification of macrophages on the basis of metabolic features not only deepens our understanding of metabolic regulation within the immune microenvironment but also provides a novel "metabolic intervention" perspective for antitumor therapy and even cross-disease treatment.

Spatial heterogeneity

Spatial transcriptomics technology gradually reveals the spatial distribution of immune cells and tumor cells in different regions of tumor (tumor core, invasion margin, tumor stroma, etc.) and the complex dynamics of the interaction between different types of cells in TME [74]. The tumor core is mainly composed of malignant tumor cells, while the tumor boundary and interstitial region contain more immune cells and interstitial cells [75]. Emerging evidence demonstrates spatial partitioning of macrophage origins: embryonically derived TRMs primarily localize to peritumoral areas, while MDMs preferentially infiltrate angiogenic niches and perinecrotic regions [35, 38].

The spatial location of TAM in TME is also very important for the definition of its function. $SPP1^+$ macrophages predominantly localize to the tumor invasive front rather than tumor core. $SPP1^+$ TAM can not only recruit additional $SPP1^+$ macrophages by secreting IL-1β [76], but also promote T-cell depletion and tumor cell invasion through SPP1-CD44 and SPP1-ITGAV/ITGB1 signaling pathways, respectively [77, 78]. The spatial colocalization of $SPP1^+$ TAMs, tumor cells, and cancer-associated fibroblasts (CAFs) creates a synergistic protumorigenic niche [77].

In contrast, $CXCL9^+$ TAMs with antitumor properties are distributed in the peripheral tumor region, where they cooperate with $CD8^+$ T cells to form an immunostimulatory microenvironment [79]. Other studies have shown that immunosuppressive $PD\text{-}L1^+$ TAMs also frequently occupy peritumoral regions, where they restrict T-cell infiltration and facilitate tumor invasion [80]. In addition, the study of colorectal cancer shows that the ratio of $CD163^+/CD68^+$ in the marginal region of the tumor is significantly higher than that in the tumor core. The high ratio of $CD163^+/CD68^+$ is closely related to lymphatic vessel infiltration and tumor staging [81]. Nalio et al. quantitatively evaluated the spatial distribution of specific TAM in breast tumors, and found that $TREM2^+$ TAM infiltrated into tumor nests and tumor nest edges was associated with poorer survival, while $FOLR2^+$ TAM located in tumor stroma was associated with favorable outcomes [36]. Hypoxic and angiogenic regions within the tumor core predominantly harbor inflammatory (Inflam-TAM) and proangiogenic (Angio-TAM) subsets [35]. Pancreatic cancer demonstrates $IL\text{-}1\beta^+$ TAM accumulation in the tumor core, where they establish an inflammatory niche that enhances malignant features [58].

Furthermore, macrophages within the tumor metastatic microenvironment also exhibit phenotypic diversity and spatial organization features. Integrative studies using single-cell and spatial transcriptomics have identified macrophage subsets significantly enriched in colorectal cancer liver metastases, including $SPP1^+$ TAMs, $HLA\text{-}DRB5^+$

TAMs, CD1C$^+$ TAMs, CXCL10$^+$ TAMs, and CX3CR1$^+$ TAMs. These subsets collectively participate in the construction of the metastatic immune microenvironment through interactions with the hepatic parenchymal microenvironment. Among them, SPP1$^+$ macrophages are primarily localized to the tumor region and the tumor-stroma interface, whereas other macrophages are distributed across both the tumor area and the hepatocyte region [82, 83]. Similarly, studies on pancreatic cancer metastasis have also revealed that APOE$^+$/SPP1$^+$ macrophages are enriched in the metastatic microenvironment and promote metastatic niche formation [84]. Collectively, these findings demonstrate that distinct ecological niches within tumors shape TAM spatial heterogeneity, ultimately endowing macrophages with specialized functional properties that influence tumor progression and therapeutic responses.

The role of macrophages in the treatment of PD-1/PD-L1 inhibitors

Role of macrophages in anti-tumor efficacy of PD-1/PD-L1 inhibitors

PD-1/PD-L1 inhibitors drive dynamic evolution and functional remodeling of macrophages

During PD-1/PD-L1 inhibitor therapy, macrophages are not static but exhibit complex spatiotemporal dynamic evolution, characterized by functional evolution in the temporal dimension and migratory rearrangement in the spatial dimension. At the temporal dynamic level, in the early stage of treatment, macrophages rapidly upregulate interferon-inducible chemokines and T cell-recruiting factors, displaying activation features conducive to antitumor immunity [85]. However, as treatment progresses, macrophages gradually transition toward an immunosuppressive phenotype. In the context of combination therapy or the relapse phase, macrophages exhibit more complex adaptive remodeling patterns. This process is typically mediated by the interplay between macrophages and cancer cells, which suppresses the immune response capacity of macrophages and drives their polarization toward an M2-like phenotype [86, 87]. At the spatial dynamic level, the physical barrier formed by macrophages and tumor cells is either absent or dispersed in responders to immunotherapy, and this spatial rearrangement facilitates the initiation and maintenance of antitumor immune responses [88]. In addition, macrophages are more frequently localized in close proximity to HLA-DR$^+$ tumor cells, suggesting that macrophages may participate in antigen presentation and cooperatively activate antitumor immune responses [89].

On the basis of the aforementioned spatiotemporal dynamic evolution, the functional status of macrophages also undergoes profound remodeling, primarily manifested as dynamic adjustments in phenotypic polarization and immunoregulatory functions. Research has demonstrated that immunotherapy-mediated antitumor responses depend on the coordinated action of CD8$^+$ T cells and macrophages. After immunotherapy, activated CD8$^+$ T cells in the tumor can recruit macrophages near the tumor through CCR5 signal and reprogram them into M1-like antitumor phenotype [90]. These macrophages show similar transcription characteristics in human tumors and are related to the antitumor response mediated by ICIs. Xiong et al. reported that PD-L1 inhibitor therapy could decrease the expression of Arg1 and increase the expression of iNOS, MHCII,, and CD40 in TAM, which promoted the polarization of TAM to proinflammatory phenotype. These therapy-induced proinflammatory TAMs partially originate from newly differentiated monocyte-derived macrophages, and this process depends on IFN-γ signaling from activated CD8$^+$ T cells [91]. In tumor-bearing mice without ICI treatment,

TAMs maintain high expression of immunosuppressive markers, such as CX3CR1 and CD206. Conversely, ICIs treatment can not only induce the production of proinflammatory iNOS$^+$ macrophages with high glycolytic ability, but also promote the expression of Mertk, a mature marker of macrophages [92].

Different ICIs treatment strategies may play a similar role in remodeling the function and phenotype of macrophages. In a colorectal cancer immunotherapy study, both PD-L1 monotherapy and dual immune checkpoint blockade differentially increased intratumoral iNOS$^+$ macrophages while reducing CD206$^+$ and Tie2$^+$ macrophage populations. Notably, the study revealed that both PD-L1 inhibitor monotherapy and combination therapy significantly decreased PD-L1$^+$ macrophage infiltration [93]. Chen et al. also found that after ICIs treatment, the abundance of macrophages increased and tumor necrosis factor (TNF) signaling pathway was significantly enriched in lung squamous cell carcinoma. The enhancement of macrophage-tumor cell communication activates the inflammatory responses in the tumor, and may stimulate macrophages to migrate into the blood, increasing the risk of adverse reactions [94]. In addition, blocking PD-1/PD-L1 signaling pathway is also crucial to restore phagocytosis and intracellular killing ability of macrophages [95, 96]. In a word, the recovery of immune surveillance and tumor clearance ability of TAMs play a key role in the antitumor immune response mediated by PD-1/PD-L1 inhibitors (Fig. 2).

Role of macrophages in predicting the antitumor efficacy of PD-1/PD-L1 inhibitors

On the other hand, some specific macrophage subsets in TME can predict the response to PD-1/PD-L1 inhibitors (Fig. 2). According to the data of a pan-cancer map study, the macrophage cluster related to IFN-γ signaling is significantly enriched in ICIs treatment responders, and the highest upregulated gene in this group of macrophages is CXCL9, this is a chemokine involved in T-cell recruitment to tumors [12]. In the single cell map of patients with breast cancer treated with PD-1 inhibitor, proinflammatory CCR2$^+$ macrophages expressing CXCL9, CXCL10, and SOCS3 were positively correlated with T-cell expansion, whereas CX3CR1$^+$ macrophages were negatively correlated with T-cell expansion. This shows that the immunotherapy response to PD-1 inhibitor is closely related to the heterogeneity of macrophages [97]. Macrophages are the main source of

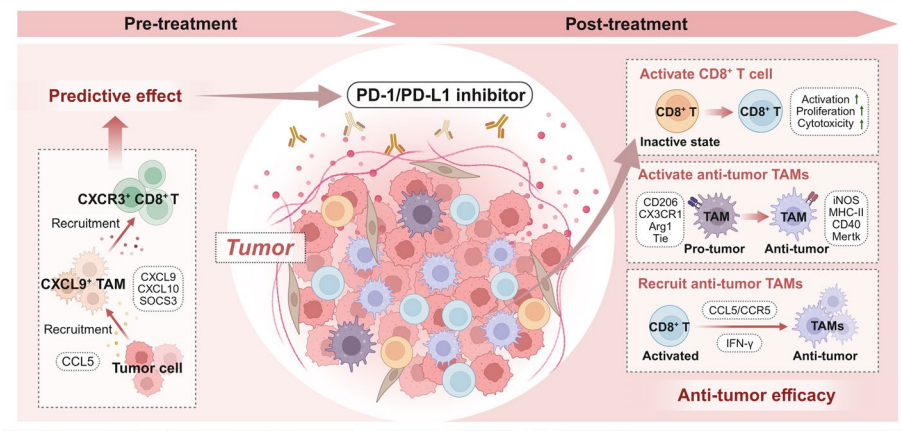

Fig. 2 Role of macrophages in antitumor efficacy of PD-1/PD-L1 inhibitor. CXCL9$^+$ macrophages in TME can predict the reactivity to PD-1/PD-L1 inhibitor. After treatment with PD-1/PD-L1 inhibitor, TAM acquired pro-inflammatory phenotype and enhanced antitumor function (created with BioRender.com)

CXCL9 in mice and patients receiving immunotherapy [98]. The research of Qu et al. proved that the proinflammatory CXCL9^{+} TAM population can drive the recruitment of CXCR3^{+} T cells, thus enhancing the responsiveness to PD-L1 inhibitors [99]. Similarly, Dangaj et al. found that the high expression of CCL5 and CXCL9 correlates with increased CD8^{+} T-cell infiltration in solid tumors. CCL5 expressed by tumor cells promotes the infiltration of CD8^{+} T cells by driving the expression of CXCL9 in TAM, thus enhancing the sensitivity of ICIs treatment. Across multiple malignancies, CCL5^{hi} CXCL9^{hi} tumors associate with prolonged survival, while CCL5 knockout accelerates tumor growth and reduces survival [100].

Role of macrophages in adverse events of PD-1/PD-L1 inhibitors

While exerting antitumor effects, PD-1/PD-L1 inhibitors may concurrently disrupt immune tolerance, trigger cross-immune responses, and release massive cytokines, leading to irAEs involving multiple organs or systems [101]. In recent years, emerging studies have found that the function of innate immune cells is regulated by immune checkpoints, which mediates the progress of organizational adverse events after ICIs administration. Macrophages have been proved to play a central pathogenic role in irAEs such as ICI-related arthritis [102], pneumonia [103] and myocarditis [104]. With the development of scRNA-seq and spatial transcriptome sequencing technology, the pathological mechanism of irAEs has been continuously explored. Here, we summarize the latest research progress of macrophages in the occurrence and development of irAEs (Fig. 3).

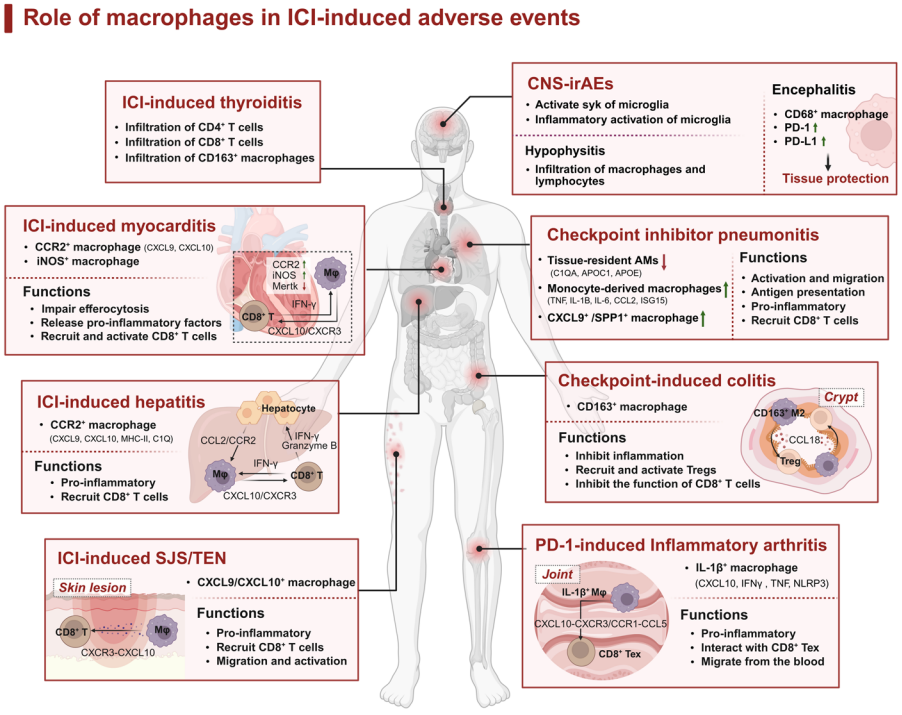

Fig. 3 Role of macrophages in adverse reactions of PD-1/PD-L1 inhibitors. Macrophages play a key regulatory role in irAEs of various tissues in the whole body. Inflammatory activation of macrophages is the key to mediate ICI-induced tissue injury. However, some macrophages may play an antiinflammatory and tissue protective role, thus alleviating ICI-induced tissue injury (created with BioRender.com)

ICI-associated myocarditis (ICI-M)

In irAEs, cardiotoxicity induced by ICIs, although uncommon, can be fatal and significantly offsets the survival benefits conferred by ICIs [105]. Current findings from multiple cardiac biopsies of ICI-M patients and animal models reveal extensive macrophage infiltration, identifying macrophages as key drivers of ICI-M pathogenesis [106–108]. Additionally, a marked increase in circulating monocytes/macrophages has been observed in the blood samples of ICI-M patients [109]. scRNA-seq analysis has demonstrated that mononuclear phagocytes are more abundant in the blood of ICI-M patients compared with dendritic cells, $CD8^+$ T cells, $CD4^+$ T cells, natural killer cells, and B cells [110]. ICI-M cases are classified into high-grade and low-grade categories. High-grade ICI-M exhibits significantly higher numbers of $CD68^+$ macrophages than low-grade ICI-M. However, low-grade ICI-M cases show a higher percentage of PD-$L1^+$ macrophages, suggesting distinct molecular profiles of macrophages across different pathological grades of ICI-M [111]. Recent histopathological studies on ICI-M have highlighted the potential diagnostic utility of macrophage-associated markers. Jimenez et al. proposed that macrophage abundance positively correlates with serum concentrations of cardiac troponin I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP). Quantitative assessment of myocardial interstitial macrophages may enhance the diagnostic accuracy of ICI-M [112].

Multiple preclinical models have revealed the specific pathological mechanisms by which macrophages contribute to the development of ICI-M (Table 1), wherein the expansion of proinflammatory macrophages induced by ICIs is a critical factor in the pathogenesis of ICI-M [113]. scRNA-seq data from a murine ICI-M model demonstrated that ICI-M is associated with the expansion and activation of monocyte-derived $CCR2^+$ ($CXCL9^+$ $CXCL10^+$) macrophages. These $CXCL9^+$ $CXCL10^+$ macrophages are recognized by activated T cells via IFN-γ signaling and further promote T-cell activation, differentiation, and recruitment through CXCR3 signaling, thereby disrupting cardiac immune homeostasis [114]. Mechanistically, PD-1 inhibitors upregulate miR-34a in macrophages, which suppresses the cardioprotective Krüppel-like factor 4 (KLF4), driving macrophage M1 polarization and inducing myocardial inflammation [115]. Additionally, following PD-1 inhibitor treatment, macrophages mediate cardiomyocyte senescence via the miR-34a-5p/protein phosphatase 1 nuclear targeting subunit (PNUTS) signaling pathway, leading to cardiac injury in mice [116]. Zhang et al. discovered that PD-1 inhibitors bind to PD-1 on macrophages, activating the MKK3/p38 MAPK pathway and promoting ADAM17-mediated shedding of Mertk. This downregulates Mertk expression on the macrophage surface, impairing efferocytosis and tissue repair functions, ultimately contributing to the development of myocarditis [117]. Another study on ICI-M revealed a significant increase in $iNOS^+$ macrophages in cardiac tissue, accompanied by mitochondrial damage and elevated expression of DNA damage markers in these macrophages. PD-1 inhibitors induce mitochondrial dysfunction in macrophages and activate the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway, driving macrophage polarization toward a proinflammatory phenotype and thereby promoting the development of ICI-M [118].

Furthermore, gut microbiota dysbiosis induced by PD-1/PD-L1 inhibitors also promotes the polarization of macrophage M1 and the release of proinflammatory factors by suppressing the butyrate/PPARα/cytochrome P450 4X1 (CYP4X1) axis, thus aggravating

Table 1 Molecular mechanism of macrophage-mediated ICI-M

Research model	Component variation	Signal axis	Potential effect	Therapy	Refs.
PD-1 inhibitor	miR-34a ↑	miR-34a/KLF4	Macrophage polarized to M1 phenotype, Increase of proinflammatory cytokines	miR-34a inhibitor	[115]
PD-1 inhibitor	miR-34a-5p ↑	miR-34a-5p/PNUTS	Prosenescent effect in cardiomyocytes	miR-34a-5p inhibitor	[116]
PD-1 inhibitor cTnI peptide	Soluble MerTK ↑ $MerTK^{+}$ macrophage ↓	PD-1/MKK3/P38 MAPK/ADAM17	Inhibition of macrophage efferocytosis, Impairment of tissue repair function	P38 MAPK inhibitor (SB203580) ADAM17 inhibitor (TAPI-0)	[117]
PD-1 inhibitor cTnI peptide	DNA damage marker γ H2AX ↑	cGAS/STING	Macrophage polarized to M1 phenotype, Mitochondrial damage of macrophages	STING inhibitor (H151)	[118]
PD-L1 inhibitor (BMS-1) Tumor-bearing mice	Gut microbiota dysbiosis	Butyrate/PPARα/CYP4X1	Macrophage polarized to M1 phenotype, cardiomyocyte apoptosis	Butyrate supplementation, Prevotella loescheii recolonization	[119]
PD-1 inhibitor cTnI peptide	L-Kynurenine ↑	JAK1/STAT3	Inhibition of macrophage polarization to M1 phenotype, decrease of proinflammatory cytokines	L-Kynurenine	[120]
PD-1 inhibitor Tumor-bearing mice	/	STAT1/NF-κ B/NLRP3	Macrophage polarized to M1 phenotype	NLRP3 inhibitor (MCC950)	[121]
$Ctla4^{\pm}Pdcd1^{-/-}$ mice MRL/$Pdcd1^{-/-}$ mice	IFN-γ signaling ↑	IFN-γ/STAT1/CXCL9/10	Promote macrophage activation (migration, cytokine production, and antigen presentation)	Anti-CSF1R Anti-IFN-γ Anti-CCR2 (MC-21)	[114] [192]

PD-1/PD-L1 inhibitor-associated cardiotoxicity [119]. Through untargeted metabolomics, He et al. identified a protective upregulation of serum L-kynurenine levels in ICI-M patients. Mechanistically, L-kynurenine ameliorates cardiac function in ICI-M primarily by inhibiting the JAK1/STAT3 signaling pathway, reducing macrophage M1 polarization and proinflammatory cytokine secretion [120]. A study on tumor-bearing mice treated with PD-1 inhibitor demonstrated that PD-1 blockade drives macrophage M1 polarization via the activation of the STAT1/NF-κB/NLRP3 signaling pathway, triggering myocardial inflammation [121].

In summary, the diverse regulatory mechanisms of macrophages provide critical insights for precision therapy in ICI-M, facilitating the development of more effective and less toxic treatment strategies. However, current animal models predominantly rely on immune checkpoint knockout-induced spontaneous myocarditis rather than tumor models. Developing ICI-M therapeutic approaches that preserve antitumor efficacy remains a key focus for future research.

Checkpoint inhibitor pneumonitis (CIP)

CIP is a common yet frequently fatal irAE; however, its pathological mechanism is still largely unknown [122]. Histopathological studies reveal that foamy macrophages and

pneumocyte vacuolization are characteristic pathological features in the lung tissues of patients with CIP [123]. In healthy individuals, nearly all macrophages in bronchoalveolar lavage fluid (BALF) are tissue-resident alveolar macrophages (AMs). In patients with CIP, there is a relative reduction in AMs (highly expressing C1QA, APOC1, and APOE) and an increase in proinflammatory monocyte-derived macrophages (highly expressing CCL2, IFITM3, and ISG15), which exhibit enhanced migratory capacity [124]. Similarly, scRNA-seq data from Franken et al. demonstrated that the BALF of patients with CIP lacks antiinflammatory resident AMs but shows significant enrichment of proinflammatory M1-like macrophages (expressing TNF, IL-1β, IL-6, IL-23A, and GM-CSF receptors CSF2RA/CSF2RB) [103]. Furthermore, Cui et al. performed comprehensive single-cell transcriptomic analysis of patient BALF, revealing disrupted differentiation trajectories of antiinflammatory macrophages in the CIP microenvironment. $CXCL9^+$ and $SPP1^+$ monocytes were significantly increased in CIP samples, while $FABP4^+$ macrophages were markedly reduced [125]. Across varying CIP severities, macrophages were notably enriched in BALF from severe CIP cases, displaying enhanced activation, migration and antigen-presenting capacity, leading to greater infiltration of $CD8^+$ T cells [126]. These findings highlight the pathogenic role of macrophage functional alterations in CIP. However, the molecular and cellular mechanisms of immune cells driving lung tissue injury in CIP microenvironment are still poorly understood.

ICI-induced hepatitis (ICIH)

Recent studies reveal that crosstalk among innate immune cells, adaptive immune cells, and hepatocytes shapes the inflammatory microenvironment in ICIH. Necroinflammatory liver lesions in ICIH consist not only of lymphocytes but also exhibit significant macrophage infiltration. Spatial analyses demonstrate that macrophages localize closer to apoptotic hepatocytes and $CD8^+$ T cells than other cell types [127]. Human studies show that monocyte-derived $CCR2^{hi}$ $CD68^+$ macrophages in ICIH liver tissues display significant proinflammatory signatures and exhibit marked colocalization with $CD8^+$/granzyme B^+ T cells [128]. Similarly, murine ICIH models by Gudd et al. revealed the expansion of $CD8^+$ T cells, $Ly6C^{high}$ inflammatory monocytes, KCs, and MDMs, identifying $CCR2^+$ monocytes and $CXCR3^+$ $CD8^+$ T cells as key drivers of the injury hepatic microenvironment [129]. Mechanistically, cells in liver tissue secrete a large amount of CCL2 in response to ICI-mediated imbalance of liver immune tolerance, recruiting activated $CCR2^+$ monocytes/MDMs to the liver. These macrophages interact with cytotoxic $CD8^+$ T cells to induce hepatocyte apoptosis and aggravate hepatitis post-ICI therapy [130]. Llewellyn et al. characterized immune signatures in murine ICIH lesions, finding MDMs highly express CXCL9, CXCL10, MHC-II, and C1Q. T cells can promote the activation/differentiation of MDMs, and regulate the immune response of MDMs through IFN-γ signaling [131].

Other ICI-related adverse events

In addition to the main types of irAEs, the current research also reports the role of macrophages in the low incidence or even rare irAEs. In central nervous system (CNS)-irAEs, microglia mediate PD-1 inhibitor-induced neurocognitive deficits. PD-1 inhibitor therapy promotes the inflammatory activation of microglia by activating spleen tyrosine kinase (Syk), thus driving the development of CNS-irAEs [132]. A retrospective

autopsy study by Maccio et al. identified lymphocyte and macrophage infiltration within inflammatory lesions of ICI-associated hypophysitis pituitary tissue [133]. scRNA-seq of patients with ICI-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) revealed that blister cells in ICI-SJS/TEN skin lesions predominantly consist of macrophages. ICIs activate TNF signaling to induce high CXCL9/CXCL10 expression of macrophages in skin lesions. These macrophages recruit $CXCR3^+$ $CD8^+$ T lymphocytes from blood to skin lesions, which leads to epidermal necrosis and exfoliation [134]. In ICI-associated arthritis, Zhou et al. identified monocyte-derived IL-$1\beta^{hi}$ macrophages as disease-specific cellular populations in PD-1 inhibitor-induced arthritis. IL-$1\beta^{hi}$ macrophages communicate with T cells through CCR1-CCL5/CCL3 and CXCL10-CXCR3 axes, which promotes the "vicious circle" of inflammation [102]. While histopathological data remain limited for ICI-related thyroiditis, some studies demonstrate substantial $CD4^+$ T cell, $CD8^+$ T cell, and macrophage accumulation in the thyroid tissue of mice with ICIs-related thyroiditis [135].

These studies collectively demonstrate that proinflammatory macrophages play non-negligible roles in irAEs. However, macrophages may also exert tissue-protective functions in irAEs. In brain tissues of patients with PD-1 inhibitor-induced encephalitis, infiltrating $CD68^+$ macrophages exhibit high PD-1/PD-L1 expression, suggesting their potential role in maintaining tissue homeostasis during irAEs [136]. Thyroid fine-needle biopsies from ICI-associated thyroiditis reveal extensive infiltration of necrotic cells, lymphocytes and $CD163^+$ macrophages [137]. In addition, the data of single cell and spatial transcriptome of ICIs-related colitis show that $IDO1^+$ cells are usually adjacent to the macrophage layer at crypt tops, which may regulate Treg through kynurenine pathway. These macrophages, defined as a colitis-specific $CD163^+$ M2 subtype, further promote Treg recruitment and differentiation through CCL18 expression. This suggests that individual crypts and neighboring macrophages suppress $CD8^+$ T cell-mediated inflammation by enhancing Treg polarization/recruitment, though this immunosuppressive pathway remains insufficient to fully mitigate local inflammatory responses in colonic crypts [138]. In a word, these data reveal that the balance between the damage effect and repair function of macrophages may determine the progress and outcome of ICI-related adverse reactions. At present, the functional changes and mechanism of macrophages in the development of different types of irAEs are still unclear. Future investigations should emphasize: (1) establishing more relevant preclinical models that better recapitulate human irAEs pathogenesis; (2) using integrated multiomics approaches to decipher the molecular networks underlying irAEs development; and (3) identifying novel macrophage-centric therapeutic targets for irAEs management.

Role of macrophages in drug resistance of PD-1/PD-L1 inhibitors

M2-like TAM

ICIs targeting the PD-1/PD-L1 pathway have transformed the landscape of cancer therapy, yet a significant proportion of patients exhibit either primary resistance or acquired tumor recurrence following initial response [139, 140]. The emergence of resistance poses a major clinical challenge for PD-1/PD-L1 blockade therapy. Accumulating evidence from both clinical specimens and preclinical models consistently demonstrates that macrophage infiltration, particularly immunosuppressive M2-like polarized macrophages, is strongly correlated with therapeutic resistance to PD-1/PD-L1 inhibitors

[141–144] (Fig. 4). Comparative analyses reveal that PD-1 inhibitor-sensitive murine models show marked expansion of M1-like macrophages ($CD11c^+CD206^-$) with enhanced antigen-presenting capacity [145], whereas resistant models display predominant M2-like macrophage infiltration characterized by impaired antigen presentation and upregulated expression of immunosuppressive genes (SPP1, Arg1, CCL2, and Fn1) [146]. Similarly, Etzerodt et al. identified that M2-like $CD163^+$ TAMs specifically sustain an immunosuppressive microenvironment in PD-1 inhibitor-resistant melanoma. The specific depletion of $CD163^+$ macrophages promotes the activation of T cells and helps to overcome the drug resistance of immunotherapy [147]. Tumor cells are an important factor in inducing immunosuppression phenotype of macrophages. In HCC tissues from PD-1 inhibitor-resistant patients, tumor cell-derived protein kinase Cα (PKCα) drives macrophage polarization toward the M2 phenotype through CSF1 activation, thereby promoting immune evasion and conferring treatment resistance [148]. Similarly, in hepatic metastases, WNT11-overexpressing tumor cells potentiate macrophage immunosuppression through the CAMKII/NF-κB/IL-17D signaling axis, ultimately compromising PD-1 inhibitor efficacy [149]. A study utilizing spatial functional genomics technology in ovarian cancer further revealed that tumor cell-derived IL-4 mediates resistance to PD-1 inhibitors by inducing macrophage polarization toward the M2 phenotype, thereby remodeling the immunosuppressive tumor microenvironment [150].

The primary objective of PD-1/PD-L1 blockade therapy is to restore T cell functionality and promote T cell activation [4]. Within the TME, TAMs serve as critical determinants of T cell distribution and effector function. TAMs impair $CD8^+$ T cell motility in the TME through sustained cellular interactions, thereby preventing T cell infiltration

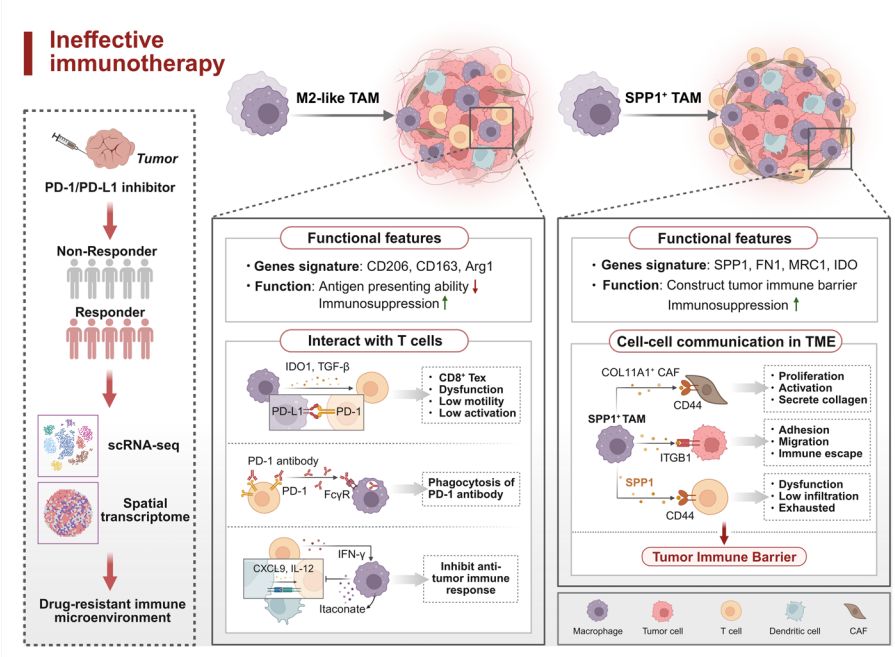

Fig. 4 Role of macrophages in drug resistance of PD-1/PD-L1 inhibitors. In the drug-resistant immune microenvironment of PD-1/PD-L1 inhibitor therapy, M2-like macrophages mediate immunosuppression and T cell dysfunction through various mechanisms. Single cell transcriptome and spatial transcriptome sequencing revealed that $SPP1^+$ macrophage was the main ICIs-resistant cell subset. It can not only promote the adhesion and migration of tumor cells, but also form a tumor immune barrier through the interaction with CAFs, thus hindering the infiltration of T cells and leading to functional exhaustion of T cells (created with BioRender.com)

into tumor regions. This feature is called "immune exclusion" [151]. Quaranta et al. demonstrated that macrophage-derived granulin mediates immune exclusion of cytotoxic $CD8^+$ T cells in metastatic pancreatic ductal adenocarcinoma (PDAC). Targeting granulin protein may be a potential strategy to restore the infiltration of $CD8^+$ T cells in metastatic tumors and overcome the drug resistance of PD-1 inhibitors [152]. Furthermore, M2-polarized TAMs suppress PD-1/PD-L1 inhibitor efficacy through multiple mechanisms: secretion of immunosuppressive factors (IDO1, TGF-β), exosome-mediated signaling, and induction of T cell exhaustion/dysfunction [153, 154]. Intravital imaging studies of PD-1 inhibitor activity revealed that while anti-PD-1 antibodies initially bind effectively to tumor-infiltrating PD-1^+ $CD8^+$ T cells, this interaction is transient. Fcγ receptor (FcγR)-expressing TAMs capture PD-1 inhibitors through glycan-mediated binding to the antibody Fc region, sequestering the inhibitors from T cell surfaces and contributing to treatment resistance [155]. Notably, FcγR blockade prior to PD-1 inhibitor administration significantly prolongs drug binding to tumor-infiltrating $CD8^+$ T cells and enhances immunotherapy-induced tumor regression in murine models [155, 156].

In addition, the expression of PD-L1 usually induces the immunosuppression phenotype of macrophages. A growing number of studies have proven that PD-L1 expression in macrophages can negatively regulate the antitumor immunity of T cells and promote tumor immune escape and immunotherapy resistance [157, 158]. In GBM patients receiving neoadjuvant PD-1 blockade therapy, $CD206^+$ TAMs exhibit significantly upregulated PD-L1 expression, suggesting these immunosuppressive TAMs may counteract therapeutic efficacy [159]. HCC models reveal that TAM-derived exosomes express substantially higher PD-L1 levels than tumor cells, with this macrophage-specific PD-L1 contributing to ICIs resistance [16]. Recent investigations show that during PD-1 inhibitor treatment, IFN-γ produced by $CD8^+$ T cells activates the JAK/STAT1 signaling pathway in TAMs, stimulating itaconate production. This immunometabolite subsequently impairs dendritic cell antigen presentation and cross-priming capacity, ultimately suppressing $CD8^+$ T cell antitumor responses and driving treatment resistance [160]. These findings demonstrate that immunometabolic dysregulation mediated by the crosstalk between adaptive and innate immunity represents a critical mechanism underlying resistance to PD-1/PD-L1 inhibitors therapy.

Drug resistance-associated TAM subsets identified by scRNA-seq

Although macrophages are conventionally classified according to the classical M1/M2 paradigm, growing evidence indicates that this polarization framework fails to adequately reflect the functional heterogeneity of macrophages across diverse pathological contexts [161]. The scRNA-seq study of PD-1/PD-L1 inhibitors in the treatment of drug-resistant patients and animal models revealed the characteristic macrophage subsets related to drug resistance (Table 2).

Paczkowska et al. performed scRNA-seq on patients with classical Hodgkin lymphoma who were insensitive to treatment or acquired resistance to PD-1 inhibitors, and found that the expansion of IL-$1\beta^+$ monocytes/macrophages was significantly related to drug resistance. These IL-$1\beta^+$ cells were spatially proximal to HRS cells and exhibited elevated expression of both proinflammatory and immunosuppressive genes including IL1β, CXCL8, CXCL2, CXCL3, CCL2, and PD-L1 [162]. In lymph node metastases of oral squamous cell carcinoma (OSCC), $PERK^+$ macrophages interacted with CAFs to

Table 2 Significant markers and functions of drug resistance-related macrophage subsets

Cancer type	Species	Method	Subtype	Gene profile	Function	Refs.
Glioblastoma	Human	CyTOF scRNA-seq mIF	CD206$^+$ TAM	MRC1, ANGPTL4, GPNMB	Immunosuppression	[159]
Classic Hodgkin lymphoma	Human	scRNA-seq	IL-1β$^+$ TAM	IL1B, CXCL8, CXCL2, CXCL3, CCL2, CD274, SIRPA	Immunosuppression, promote inflammation	[162]
Oral squamous cell carcinoma	Human	scRNA-seq ST mIHC	PERK$^+$ TAM	Genes associated with UPR	Promote the polarization and proliferation of M2 macrophages	[163]
Breast cancer	Human	scRNA-seq	MGP$^+$ TAM	MGP, SCGB2A2, FABP4, RGS5, KRT15, TFF3	Secrete tumor-promoting growth factors	[164]
			IDO$^+$ TAM	IDO1, PPA1, WARS		
Triple-negative breast cancer	Human	scRNA-seq	APOE$^+$ TAM	APOE, CTSD, PLD3, HLA-C, PSAP, FTL, C1QB	Interact with CD8$^+$ exhausted cells	[165]
Breast cancer	Human	scRNA-seq mIF	CCL18$^+$ TAM	CCL18, C1QA, C1QB, APOE, MARCO	Immunosuppression	[166]
Pancreatic ductal adenocarcinoma and melanoma	Human Mouse	bulk RNA-seq scRNA-seq	P2Y6$^+$ TAM	P2y6	Inhibit the recruitment and activation of CD8$^+$ T cells	[167]
Gastric cancer	Human	scRNA-seq	USP14$^+$ TAM	USP14, MRC1, CD163, MARCO	Promote the recruitment of MDSCs to TME	[168]
Colon carcinoma	Mouse	bulk RNA-seq scRNA-seq TCR-seq	Tim-4$^+$ cavity-resident macrophage	Tim-4, Vsig4, Gata6, Adgre1, Icam2	Impair the proliferation of cytotoxic antitumor CD8$^+$ T cells	[169]
Glioblastoma	Human	bulk RNA-seq scRNA-seq ST mIF	VSIG4$^+$ S100A10$^+$ TAM	VSIG4, S100A10	Inhibit the function of T cells	[170]
Non-small cell lung cancer and melanoma	Human	scRNA-seq	TREM2$^+$ TAM	SPP1, RNASE1, MT1G, SEPP1, FOLR2, NUPR1, KLHDC8B, CCL18, MMP12, APOC2, C1QA, C1QB, C1QC	Secrete immunosuppressive factors and potentiate T cell dysfunction	[55] [172]
Glioblastoma, esophageal squamous cell carcinoma, colorectal cancer, prostate cancer, hepatocellular carcinoma and non-small-cell lung cancer	Human	scRNA-seq ST mIHC	SPP1$^+$ TAM	SPP1, FN1, MRC1, IDO, PPARG, MARCO	Impair the anti-tumor activity of CD8$^+$ T cells, promote the construction of tumor immune barrier	[75] [174, 175, 177–179]

CyTOF, Cytometry by Time-of-Flight; ST, Spatial Transcriptomics; mIF, multiplex immunofluorescence; mIHC, multiplex immunohistochemistry; UPR, unfolded protein response; TCR, T cell receptor

promote an extracellular matrix (ECM)-rich microenvironment that impaired T cell-mediated cytotoxicity and conferred immunotherapy resistance [163]. Transcriptomic analysis of patients with breast cancer identified MGP^+ and IDO^+ TAMs that diminished PD-1 inhibitor efficacy through upregulation of protumorigenic immunosuppressive factors associated with treatment resistance [164]. Triple-negative breast cancer (TNBC) nonresponders to ICIs showed significant enrichment of $APOE^+$ macrophages, which mediated resistance through interactions with exhausted $CD8^+$ T cells; combination therapy with APOE inhibitors and ICIs demonstrated superior efficacy in a tumor-bearing mouse model [165]. Additional studies in patients with breast cancer with hepatic/brain metastases suggested $CCL18^+$ immunosuppressive macrophages may contribute to poor response rates to PD-1/PD-L1 inhibitor [166]. In drug-resistant PDAC and melanoma models, it was also found that tumor cells established communication with $P2Y6^+$ macrophages through nucleotide metabolism, thus maintaining the immunosuppressive characteristics of macrophages and hindering T cell recruitment, resulting in tumor resistance to PD-1 inhibitors [167]. Patients with gastric cancer (GC) resistant to PD-1 inhibitors exhibited increased $USP14^+$ TAMs that recruited myeloid-derived suppressor cells (MDSCs) to the tumor microenvironment via enhanced CXCL2 secretion, ultimately compromising immunotherapy efficacy [168].

Beyond the tumor tissue, TAMs in other anatomical sites may serve as a critical factor impeding the responsiveness of metastatic tumors to ICIs. A study on tumor serosal cavity metastasis revealed that $Tim\text{-}4^+$ cavity-resident macrophages, rather than monocytes or tumor-infiltrating macrophages, could functionally sequester highly cytotoxic $CD8^+$ T cells via ligand engagement while suppressing their proliferation, thereby hindering the therapeutic efficacy of ICIs [169]. In GBM, $VSIG4^+$ $S100A10^+$ TAMs represent the predominant cellular subset responsible for resistance to anti-PD-1 therapy. This subpopulation exhibits elevated M2 polarization level, reduced M1 signatures, and impaired IFN-γ and MHC-II antigen presentation pathways. Spatially, $VSIG4^+$ $S100A10^+$ TAMs colocalize with T cells and suppress their immune activation, which are key determinants of anti-PD-1 treatment resistance [170].

A pan-cancer atlas study revealed that most immunotherapy-resistant cancers exhibit a gene signature characterized by high expression of SPP1, RNASE1, NUPR1, and TREM2. Additionally, an ECM-associated macrophage cluster was significantly enriched in nonresponders to ICIs, likely owing to its association with ECM-mediated tumor immune exclusion [12]. TREM2 has been identified as a key regulator of the immunosuppressive function of TAMs [171]. In non-small cell lung cancer and melanoma samples treated with ICIs, the proportion of $TREM2^+$ TAMs was markedly increased in nonresponding patients. Patients with low $TREM2^+$ TAMs infiltration exhibited higher response rates to PD-1 inhibitors, whereas those with high infiltration tended to experience tumor progression after treatment. $TREM2^+$ TAMs not only enhance immunosuppression by upregulating immune checkpoint genes, complement pathway-related genes, and M2-associated genes, but also promote tumor immune evasion by exacerbating T-cell dysfunction and facilitating the differentiation of $FOXP3^+$ Tregs [55, 172].

The SPP1 signaling pathway serves as a critical communication axis between macrophages and tumor cells, and the expression of SPP1 enhances the tumor-promoting ability of TAMs [173]. Currently, $SPP1^+$ macrophages have been identified as not only the predominant ICI-resistant cellular subset but also a potential biomarker for

immunotherapy resistance across various malignancies, including GBM [174], esophageal squamous cell carcinoma [175], and metastatic prostate cancer [176] (Fig. 4). Spatial transcriptomic data in colorectal cancer (CRC) revealed significant colocalization between $SPP1^+$ macrophages and CAFs, forming a mutually reinforcing interaction network that drives fibrotic niche formation and immune-excluded microenvironments in the TME [177]. The tumor immune barrier (TIB) composed of $SPP1^+$ macrophages and CAFs may be a key determinant of anti-PD-1/PD-L1 resistance in HCC. In ICIs responders, tumors exhibit fewer $SPP1^+$ macrophages and higher $CD8^+$ T-cell infiltration, whereas in nonresponders, $SPP1^+$ macrophage infiltration is markedly increased, with $CD8^+$ T cells excluded from the tumor core and instead localized outside the TIB [178]. Mechanistically, $SPP1^+$ macrophages and CAFs (especially $COL11A1^+$ CAFs) predominantly accumulate at the tumor-stroma interface, where they engage in physical interactions and signaling crosstalk via specific receptor-ligand pairs (such as SPP1–CD44). These interactions drive CAFs activation and collagen deposition, reinforcing the immune-excluding barrier [75]. The above mechanisms reveal that the interaction between macrophages and CAFs may constitute a critical fibrotic–immunosuppressive positive feedback loop within the TME. Notably, this axis is not limited to the SPP1 signaling pathway but more broadly reflects the central regulatory role of macrophages in tumor-associated fibrosis. Given the prevalence of fibrosis across various solid tumors, antifibrotic strategies targeting this axis in combination with immunotherapy may represent a promising approach to overcome immune resistance.

More interestingly, Bill et al. defined macrophage polarity on the basis of CXCL9 and SPP1 (CS) expression, which is independent of cellular differentiation, exhibited strong prognostic relevance: $SPP1^{hi}$ TAMs correlated with poor clinical outcomes, whereas $CXCL9^{hi}$ TAMs were associated with favorable responses. Notably, in pretreatment samples, nonresponders to PD-1 blockade displayed low CS TAM polarity, whereas responders exhibited high CS polarity [179]. These findings further underscore the clinical significance of $CXCL9^+$ and $SPP1^+$ macrophages in predicting the efficacy of PD-1/PD-L1 inhibitors.

Combination therapy to improve the therapeutic effect of PD-1/PD-L1 inhibitors

Existing studies have reported that therapies targeting TAMs exhaustion [151, 180, 181] and M1/M2 phenotypic transformation [182, 183] can restore the migration and infiltration of T cells to tumor areas and enhance the antitumor activity of T cells, thus playing a synergistic role in the treatment of ICIs. Nevertheless, TAMs exhaustion struggles to achieve a complete and accurate effect, and its therapeutic durability is still lower than expected. In fact, the M1/M2 macrophages clearly distinguished in vitro do not represent their state in vivo. M1/M2 macrophages in vivo usually have overlapping gene expression profiles and heterogeneous patterns, rather than existing as mutually exclusive populations [161, 184]. Consequently, labeling M2 TAMs as the definitive "villains" in the TME remains controversial [185]. Beyond TAMs depletion and repolarization, targeting TAMs receptors has also shown promise in improving ICIs efficacy. Peters et al. reported that TAMs receptor signaling (Tyro3, Axl, MerTK) negatively regulates immune responses, contributing to an immunosuppressive, protumorigenic TME. Preclinical models and early clinical data suggest that TAMs receptor inhibition

can dismantle resistance-associated TME barriers induced by multiple mechanisms. While combination therapy with TAMs receptor inhibitors and ICIs has shown potential in overcoming resistance and improving patient outcomes, concerns remain regarding potential toxicity [186]. Thus, enhancing treatment precision, stability, and safety is crucial for optimizing TAM-targeted therapies. Building on recent advances, we present three combinatorial treatment approaches derived from contemporary evidence (Fig. 5).

Targeting specific macrophage subsets

Targeting specific macrophage subsets provides a multi-dimensional approach to enhance therapeutic precision. In preclinical HCC models, blocking SPP1 or macrophage-specific SPP1 deletion disrupts the TIB, thereby increasing tumor sensitivity to PD-1 inhibitors [178]. In pituitary neuroendocrine tumors, $SPP1^+$ TAMs promote tumor cells invasion via the SPP1-ITGAV/ITGB1 signaling axis, and ITGB1 knockdown can reverse the tumor-promoting effects of SPP1 [78]. In prostate cancer, $SPP1^+$ TAMs upregulate adenosine-related genes under hypoxic conditions, and their adenosine secretion further reinforces the immunosuppressive TME. Notably, A2A receptor (A2AR) inhibitors significantly reduce $SPP1^+$ TAM infiltration and synergize with PD-1 inhibitors to enhance antitumor efficacy [187]. Moreover, anti-TREM2 therapy in tumors with high $TREM2^+$ TAM infiltration reduces $TREM2^+$ TAM abundance, activates $CD8^+$ T cells, and drives potent antitumor immunity, thereby improving PD-1 inhibitor sensitivity [188]. Luccia et al. demonstrated that TREM2 blockade or genetic deletion in macrophages reprograms intestinal macrophages and microbiota, slowing tumor progression and augmenting the antitumor effects of PD-1 inhibitors [189].

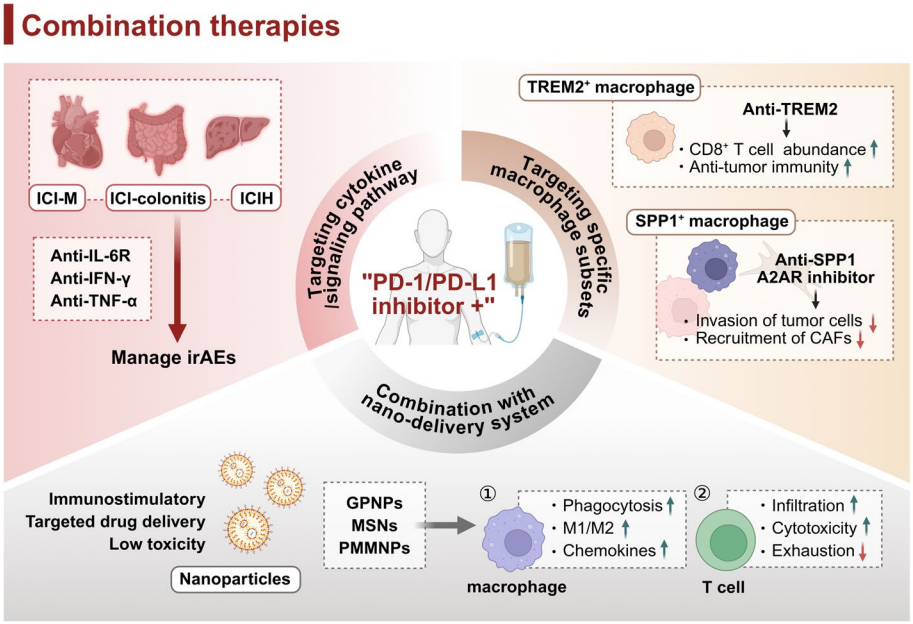

Fig. 5 Combination regimens involving PD-1/PD-L1 inhibitors. Subset-specific macrophage-targeted therapies, cytokine-directed therapies, and nanodelivery systems mitigate treatment-related side effects of PD-1/PD-L1 inhibitors through distinct mechanisms while synergistically enhancing their antitumor efficacy (created with BioRender.com)

Targeting cytokine/signaling pathway

Currently, cytokine-targeted therapies have demonstrated promising potential in mitigating ICI-related inflammatory responses [119, 190]. In ICI-M, tocilizumab alleviates M1-polarized macrophage-mediated myocardial injury by inhibiting the IL-6/JAK2/STAT3 pathway, while simultaneously exerting synergistic antitumor effects [191]. Huang et al. reported that blocking CXCR3 and CXCL10 reduces $CD8^+$ T cell migration toward macrophages, thereby attenuating the myocarditis phenotype [192]. Additionally, the JAK inhibitor baricitinib suppresses M1 macrophage polarization and proinflammatory cytokine release by targeting the JAK1/STAT3 pathway, consequently improving ICI-induced cardiac dysfunction and myocardial inflammation [193]. IFN-γ is a key mediator in recruiting pathogenic cardiac macrophages in ICI-M, and IFN-γ blockade significantly decreases $CXCL9^+$ $CXCL10^+$ macrophage infiltration in murine hearts while prolonging survival [114].

Therapies targeting TNF-α signaling have also demonstrated significant potential in ameliorating pathological damage from irAEs. In the gastrointestinal tissues of patients with melanoma with immune-related colitis, TNF-α signaling is markedly upregulated in macrophages [194]. Perez-Ruiz et al. demonstrated in xenograft mouse models that prophylactic TNF blockade could enhance antitumor efficacy while mitigating colitis and hepatitis induced by dual ICIs therapy, providing a clinically feasible combination strategy [195]. Similarly, preliminary clinical data suggest that TNF-α blockade effectively treats corticosteroid-refractory immune-related colitis [196]. These findings indicate that cytokine-targeted therapies can alleviate tissue inflammatory damage through precise modulation of specific immune pathways, offering new therapeutic options for steroid-refractory irAEs. However, owing to limitations in preclinical model systems, the clinical applicability of these therapies requires further evaluation.

Combination with nano-delivery system

Conventional ICIs therapy usually lacks the ability of tumor targeted delivery, and high dose ICIs is needed to ensure the therapeutic effect [197]. In contrast, nanoparticle delivery systems enable cell-specific targeting and phenotypic modulation, representing a paradigm shift from passive to active targeting that holds great promise for precision medicine [198–200]. Yen et al. developed gelatinase-responsive nanoparticles (GPNPs) codelivering PD-1 inhibitors and TGF-β receptor I inhibitors. GPNPs effectively promoted tumor-infiltrating lymphocyte activation and increased the M1/M2 macrophage ratio, reversing the "immune-excluded" phenotype and offering novel solutions for immunotherapy resistance [201]. In multiple PD-1 inhibitor-resistant tumor models, mesoporous silica nanoparticles (MSNs) overcame therapeutic resistance by directly engaging TLR4 receptors on TAMs, activating NF-κB signaling to drive T-cell infiltration and activation, thereby rapidly establishing an immunosuppression-resistant TME during combinatorial PD-1 blockade therapy [202]. In addition, Moon et al. engineered PD-1-transfected macrophage membrane-derived nanoparticles (PMMNPs), which blocked CD47 and PD-L1 on tumor cells by targeted delivery of SIRPα and PD-1, respectively. This strategy induced macrophage repolarization toward an antitumor phenotype, enhanced phagocytic capacity, and boosted T-cell activity. Notably, PMMNPs achieved superior antitumor efficacy with reduced side effects compared with nontargeted low-dose ICIs [197]. These advances highlight the enormous potential of nanocarriers in

Table 3 Functional reprogramming of macrophages in commonly used clinical combination regimens

Combination therapy	Cancer type	Subtype	Functional changes	Impact on treatment efficacy	Method	Refs.
Radiotherapy combined with immunotherapy	ESCC	M2 TAM	Reduced accumulation	Synergistically enhance antitumor immunity	Transcriptomic analysis (mouse)	[203]
	Multiple solid tumors	M1 TAM	Enhance the capacity for immune activation and interaction with $CD8^+$ T cells	Enhance long-term immune memory	scRNA-seq (mouse)	[204]
	TNBC	M1 TAM (intermediate doses)	Immune activation effect	Synergistically enhance antitumor immunity	mRNA-seq (mouse)	[206]
		M2 TAM (higher doses)	Immunosuppressive effect	Abrogate the efficacy of immunotherapy		
Chemotherapy combined with immunotherapy	NSCLC	$FABP4^+$ $C1q^+$ TAM	Enhanced proinflammatory function and increased phagocytic capacity	Correlated with major pathological response	scRNA-seq (human)	[208]
	Cervical cancer	$CD74^+$ TAM	Enhanced interaction with cancer cells, reduced phagocytic capacity, and promoted M2 polarization	Impairs the efficacy of combination therapy	scRNA-seq (human)	[86]
Antiangiogenic therapy combined with immunotherapy	Multiple solid tumors	M1 TAM	M2-to-M1 polarization shift, with reduced immunosuppression	Synergistically enhance antitumor immunity	/	[209]
	NSCLC	Monocyte-derived and alveolar-derived TAMs	Construct a microenvironment that supports Tregs	Mediate resistance and even accelerate tumor progression	scRNA-seq (mouse)	[210]
	HCC	$SPP1^+$ TAM	Recruit Tregs and maintain their function	Mediate resistance	scRNA-seq (human)	[211]

improving immunotherapy precision and safety. Current multi-omics technologies have unveiled the heterogeneity and subtle functional distinctions among macrophage subsets across diseases, complicating therapeutic strategies while enabling precision medicine. Future research should leverage bioengineering approaches to develop subset-specific therapies and combination strategies on the basis of transcriptomic signatures and functional profiles of pathogenic macrophage populations.

Optimization strategies from combination therapy to triple therapy

In addition to the aforementioned strategies that directly target macrophages or their signaling pathways, widely used clinical combination therapies, such as radiotherapy combined with immunotherapy, chemotherapy combined with immunotherapy, and antiangiogenic therapy combined with immunotherapy, primarily act on tumor cells. However, these regimens can also indirectly modulate the phenotype and function of macrophages by remodeling the tumor microenvironment (Table 3). Single-cell transcriptomics provides a key technological foundation for dissecting the dynamic responses of macrophages in the context of these combination therapies. In the context

of radiotherapy, the combination of radiotherapy with ICIs has been shown to reduce the accumulation of M2-like TAMs, promote the secretion of chemokines and inflammatory cytokines by macrophages, and establish an immune-inflamed TME [203, 204]. Mechanistically, radiotherapy not only upregulates PD-L1 expression on tumor cells but also enhances PD-L1 expression on MDSCs and macrophages within the TME, which serves as one of the theoretical foundations for the synergistic effect of radiotherapy combined with ICIs [205]. Additionally, a nonlinear relationship exists between radiotherapy dose and macrophage polarization. Intermediate dose radiotherapy combined with PD-1 inhibitors induces macrophage conversion to an M1-like phenotype, whereas higher doses revert them to an M2-like state [206]. In chemo-immunotherapy, macrophages generally polarize toward a proinflammatory M1-like phenotype and show enhanced cross-presentation of tumor antigens [207]. Nevertheless, macrophage heterogeneity leads to diverse and even opposing roles. Neoadjuvant chemotherapy enriches a subset of $FABP4^+$ $C1q^+$ macrophages with proinflammatory functions, which is associated with favorable outcomes [208]. Conversely, $CD74^+$ macrophages interact with the most immunoreactive epithelial subpopulation 3 cancer subgroup to drive immunosuppression. CD74 blockade can improve macrophage phagocytosis and reduces tumor cell viability [86]. Similarly, antiangiogenic therapy combined with immunotherapy can induce the conversion of the immunosuppressive tumor microenvironment by inhibiting M2-like macrophage polarization and promoting M1-like infiltration [209]. However, TAMs, particularly the $SPP1^+$ TAM subset, can form a synergistic network with Tregs, sustaining Treg infiltration and function, thereby constituting a major resistance barrier to antiangiogenic therapy combined with PD-1 blockade [210, 211]. In summary, macrophages serve as a critical regulatory node connecting various combination therapies with immune responses, and their functional heterogeneity profoundly influences the therapeutic outcomes of combination regimens.

Given the critical regulatory role of macrophages in various combination therapies, combining macrophage-targeted therapy with chemotherapy, radiotherapy, or antiangiogenic agents plus PD-1/PD-L1 inhibitors to form a triple combination regimen may achieve synergistic efficacy through multiple mechanisms, thereby overcoming the limitations of current dual combination approaches. Tang et al. showed that in a non-small cell lung cancer (NSCLC) model, the autophagy inhibitor chloroquine induced M2-like macrophage apoptosis to promote $CD8^+$ T cell infiltration, thereby enhancing the efficacy of anlotinib combined with PD-1 inhibitor therapy [212]. Moreover, dual targeting of monocyte-derived and alveolar-derived TAMs using a CSF1R inhibitor plus cisplatin, or targeting of $SPP1^+$ TAMs, effectively eliminated Tregs and significantly improved the efficacy of antiangiogenic therapy combined with immunotherapy [210, 211]. O'Connell et al. also reported that Eganelisib, a PI3K-γ inhibitor, in combination with PD-1/PD-L1 inhibitors and chemotherapy exhibited synergistic efficacy in first-line metastatic triple-negative breast cancer, with the underlying mechanism involving reprogramming of macrophage phenotype from immunosuppressive M2-like to proinflammatory M1-like [213]. The aforementioned studies indicate that triple combination therapies incorporating macrophages as a "third target," whether by inducing apoptosis of M2-like macrophages, eliminating TAMs to relieve immunosuppression, or reprogramming macrophage phenotypes to activate antitumor immunity, can synergistically remodel the

tumor immune microenvironment, thereby providing a feasible strategy for optimizing combination therapies.

Conclusion and perspectives

PD-1/PD-L1 inhibitors have demonstrated significant efficacy in various malignant solid tumors. However, challenges such as irAEs, drug resistance, low response rates, and pseudoprogression/hyperprogression persist during treatment [5, 6, 214, 215]. Currently, clinically utilized predictive biomarkers for ICIs efficacy include PD-L1 expression, tumor mutational burden, tumor-infiltrating lymphocytes, and circulating tumor DNA. Nevertheless, patient heterogeneity across different anatomical sites and time points, as well as intertumoral and intratumoral variability, pose significant challenges to static biomarkers [216]. Identifying dynamic biomarkers and tumor-specific biomarkers associated with irAEs and drug resistance constitutes a pressing challenge that must be addressed in the era of precision medicine.

Macrophages are pivotal orchestrators of the cytokine network and cellular communication within the TME, serving as key regulatory targets of PD-1/PD-L1 inhibitors [217]. The classical M1/M2 dichotomy in macrophage classification has significantly limited our understanding of their complex functional phenotypes and activation states. scRNA-seq data reveal that M1 and M2 signatures in TAMs are not mutually exclusive. For instance, proinflammatory $CCL20^{hi}/CD163^{lo}$ macrophages still express moderate levels of M2 markers (CD163 and MRC1) [218]. With advancing insights into macrophage biology and the development of novel technologies, growing research has uncovered the single-cell and spatial heterogeneity of macrophages during ICIs therapy.

Although substantial scRNA-seq data have unveiled critical pathogenic macrophage subpopulations in irAEs, current research still faces several limitations. First, the functional characterization of macrophages in injured tissues remains oversimplified. While the identified pathogenic macrophages exhibit enhanced proinflammatory and chemotactic properties, emerging evidence suggests that immunosuppressive macrophages may play a tissue-protective role in mitigating ICI-induced inflammatory damage [136, 138]. This implies that maintaining a balance between proinflammatory pathogenic macrophages and immunosuppressive protective macrophages within tissues is crucial for counteracting irAEs. Second, spatial localization studies of pathogenic macrophages in injured tissues remain inadequate. Pathological findings from ICIH specimens reveal extensive inflammatory cell infiltration in both centrilobular and periportal regions [219]. These hepatic necroinflammatory foci include not only lymphocytes, but also macrophages [127]. However, comprehensive spatial mapping data characterizing immune cell features across distinct hepatic zones are currently lacking. A spatiotemporal transcriptomic analysis of fulminant viral myocarditis demonstrated that inflammation initially emerges near the pericardium before disseminating throughout the myocardium, this a process mediated by mesothelial–macrophage interactions at the cardiac periphery [220]. Therefore, future investigations should employ spatial transcriptomics to elucidate the relationship between functional characteristics and spatial distribution of pathogenic macrophages in irAEs, which is essential for deciphering the immunoregulatory mechanisms underlying irAE pathogenesis. Thirdly, the cellular/molecular targets of ICIs-related adverse reactions lack the evaluation of antitumor effect. $CCR2^{+}$ ($CXCL9^{+}$ $CXCL10^{+}$) macrophages have been implicated in mediating

multi-organ irAEs. However, studies demonstrate that the therapeutic efficacy of PD-1/PD-L1 inhibitors in murine tumor models depends on CXCL9, as CXCL9 blockade converts treatment responders into nonresponders and diminishes anti-tumor activity [99]. Most current animal models utilize immune checkpoint knockout-induced spontaneous irAE models rather than tumor-bearing systems. The development of irAEs therapeutic targets that preserve antitumor efficacy remains a critical research priority. Fourth, the identification of temporally specific macrophage subpopulations across different stages of irAEs progression remains unexplored, and there is a notable absence of dynamic predictive biomarkers.

Research on PD-1/PD-L1 inhibitor resistance has identified multiple novel macrophage subpopulations associated with treatment resistance, providing new potential targets to enhance the efficacy of PD-1/PD-L1 blockade. However, two critical issues warrant further attention. First, the functional validation of these newly identified resistance-associated macrophage subsets remains incomplete. Sole reliance on scRNA-seq for macrophage subtyping may lead to discrepancies between transcriptional profiles and actual biological functions, necessitating further verification through conditional gene knockout models or specific cell depletion approaches to elucidate their precise roles. Second, the functional difference between TRMs and MDMs requires clarification. A study in HCC revealed that KCs (liver TRMs) and infiltrating macrophages (InfMs) exert opposing effects on local $CD8^+$ T-cell activation. While $CD80^+/CD86^+$ InfMs activate neighboring $CD8^+$ T cells via the CD28 costimulatory pathway, $PD\text{-}L1^+$ KCs inactivate adjacent $CD8^+$ T cells through CD28 dephosphorylation. Depletion of KCs was shown to increase the proportion of activated T cells and enhance tumor response to PD-1 inhibitor therapy, suggesting that selective targeting of TRMs rather than the entire myeloid compartment may represent a novel immunotherapeutic strategy for HCC [221].

It should be noted that relying solely on scRNA-seq data may not fully capture the functional status of macrophages in authentic clinical specimens. scRNA-seq measures mRNA expression at the transcriptomic level, whereas immunohistochemistry, which is widely employed in clinical practice, detects protein expression and spatial localization. From a clinical practice perspective, transitioning from "single-cell transcriptomic discovery" to "clinical pathological validation" is essential, requiring integration of protein expression levels and spatial distribution information. For instance, multiplex immunofluorescence has been successfully applied to quantify $CD68^+$ $SHP2^+$ macrophage subsets in NSCLC tissue samples, where high infiltration of this subset in the tumor region was significantly associated with poor overall survival [222]. Similarly, spatial proteomic technology applied in head and neck squamous cell carcinoma has enabled simultaneous detection of 580 proteins and successfully identified region-specific protein biomarkers associated with patient survival [223]. Of note, the rapid advancement of single-cell proteomics technologies also provides novel tools to overcome the aforementioned limitations. Karagach et al. utilized a high-throughput single-cell mass spectrometry-based proteomics platform to analyze a mouse model of lung cancer metastasis, identifying over 1700 proteins per cell in tumor macrophages, including key macrophage markers and more than 500 proteins differentially expressed between tumor and control macrophages [224]. These technologies provide a technical foundation for multimodal analysis of functional macrophage subsets. Furthermore, future studies should further leverage

single-cell multi-omics technologies to systematically elucidate the dynamic evolution of macrophages in the context of different combination therapies, with the aim of identifying biomarkers predictive of combination therapy response and developing novel macrophage-targeted combination therapeutic strategies.

In summary, multi-omics technologies serve as powerful tools for the precise identification of disease-specific macrophage subpopulations. The characterization of novel macrophage subsets during PD-1/PD-L1 inhibitor therapy contributes to enhanced therapeutic efficacy while minimizing unnecessary adverse effects, thereby advancing the development of precision treatment strategies. The combined application of novel macrophage-targeted therapies with immunotherapy has demonstrated synergistic effects ("1 + 1 > 2") across multiple tumor types. This therapeutic approach significantly improves the potential for achieving both "tumor-bearing survival" and even "tumor-free cure" outcomes in clinical practice.

Abbreviations

A2AR	A2A receptor
AA	Arachidonic acid
AMs	Alveolar macrophages
APOE	Apolipoprotein E
BALF	Bronchoalveolar lavage fluid
C1Q	Complement c1q
CAFs	Cancer-associated fibroblasts
ccRCC	Clear cell renal cell carcinoma
cGAS	Cyclic GMP-AMP synthase
cHL	Classical hodgkin lymphoma
CIP	Checkpoint inhibitor pneumonitis
CNS	Central nervous system
COL11A1	Collagen type XI alpha 1 chain
CRC	Colorectal cancer
cTnI	Cardiac troponin I
CYP4X1	Cytochrome P450 4X1
EBV	Epstein-barr virus
ECM	Extracellular matrix
ESCC	Esophageal squamous cell carcinoma
FABP4	Fatty acid binding protein 4
FABP5	Fatty acid-binding protein 5
FATTY	Fatty acids
FcγR	Fcγ receptor
FOLR2	Folate receptor beta
FOXP3	Forkhead box P3
GBM	Glioblastoma
GC	Gastric cancer
GLY	Glycolysis
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GPNPs	Gelatinase-responsive nanoparticles
HCC	Hepatocellular carcinoma
HRS	Hodgkin-Reed-Sternberg
ICIH	ICI-induced hepatitis
ICI-M	ICI-associated myocarditis
ICIs	Immune checkpoint inhibitors
IDO1	Indoleamine 2,3-dioxygenase 1
IL-4	Interleukin-4
IMP	Inositol phosphate metabolism
InfMs	Inflammatory monocytes
iNOS	Inducible nitric oxide synthase
irAEs	Immune-related adverse events
KCs	Kupffer cells
KLF4	Krüppel-like factor 4
LAMs	Lipid-associated macrophages
Ly6C	Lymphocyte antigen 6 complex locus C
LYVE1	Lymphatic vessel endothelial hyaluronan receptor 1
MDMs	Monocyte-derived macrophages
MDSCs	Myeloid-derived suppressor cells
MGP	Matrix gla protein
MRC1	Mannose receptor c-type 1

MSNs	Mesoporous silica nanoparticles
NSCLC	Non-small cell lung cancer
NT-proBNP	N-terminal pro-B-type natriuretic peptide
OSCC	Oral squamous cell carcinoma
OXP	Oxidative phosphorylation
P2Y6	Purinergic receptor p2y6
PD-1	Programmed cell death protein 1
PDAC	Pancreatic ductal adenocarcinoma
PD-L1	Programmed death-ligand 1
PLD3	Phospholipase D3
PMMNPs	PD-1-transfected macrophage membrane-derived nanoparticles
PNUTS	Protein phosphatase 1 nuclear targeting subunit
PPARG	Peroxisome proliferator-activated receptor gamma
S100A10	S100 calcium binding protein A10
scRNA-seq	Single-cell RNA sequencing
SJS/TEN	Stevens-johnson syndrome/toxic epidermal necrolysis
SPP1	Secreted phosphoprotein 1
STING	Stimulator of interferon genes
Syk	Spleen tyrosine kinase
TAMs	Tumor-associated macrophages
TIB	Tumor immune barrier
Tie2	Tek receptor tyrosine kinase
TME	Tumor microenvironment
TNBC	Triple-negative breast cancer
TREM2	Triggering receptor expressed on myeloid cells 2
TRMs	Tissue-resident macrophages
USP14	Ubiquitin specific peptidase 14
VSIG4	V-set and immunoglobulin domain containing 4

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Declarations

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The authors declare no competing interests.

Author details
[1]The MOE Basic Research and Innovation Center for the Targeted Therapeutics of Solid Tumors, Jiangxi Medical College, Nanchang University, Nanchang 330031, China
[2]Jiangxi Provincial Key Laboratory of Tumor Biology, Nanchang University, Nanchang 330031, China
[3]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Jiangxi Medical College, Nanchang University, Nanchang 330031, China
[4]Department of Pathology, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, China
[5]Department of Cardiology, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330031, China

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