

Engineering and Targeting Macrophages for Cancer Therapy

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ABSTRACT

Macrophages are tissue-resident phagocytic cells of the innate immune system that differentiate from circulating blood monocytes and play complex roles in both physiological and pathological conditions. Emerging research indicates that macrophages exhibit diverse phenotypic characteristics and considerable functional plasticity in both physiological and pathological states, including cancer. Macrophages play a complex role in the development and progression of cancer. A model has been proposed that distinguishes between M1 and M2 macrophages in cancer, with M1 macrophages exhibiting antitumor effects, while M2 macrophages have tumor-supportive and immunosuppressive functions. The selective activation of beneficial macrophage populations and the targeted inhibition or re-polarization of tumor-promoting macrophages have shown significant potential for cancer treatment. Due to their intrinsic response to inflammation and their ability to overcome physical barriers, macrophages and their derivatives, including membranes and extracellular vesicles (EVs), are considered sophisticated carriers for drug delivery, enhancing tumor targeting and therapeutic efficacy. This review details recent advances in the development of macrophages for drug delivery and their targeted use of macrophages in cancer therapy. This review aims to provide a comprehensive overview of the capabilities of macrophages in cancer treatment.

Keywords: Immunotherapy; Inflammation; Neoplasms; Tumor-associated macrophages; Tumor microenvironment

INTRODUCTION

Macrophages are white blood cells that circulate in the human body and play a crucial role in the defense

against infections. In response to inflammatory stimuli, macrophages are recruited to sites of infection, inflammation and tissue injury, where they exert antimicrobial and pro-inflammatory functions by engulfing foreign pathogens such as bacteria and activating immune response.¹ Because of their short lifespan, the biological functions of macrophages in human diseases have often been overlooked. Although the presence of macrophages in tumor tissue was

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recognized decades ago, the precise roles of these cells in cancer remained poorly understood until recently. The diverse and complex nature of macrophages in cancer is increasingly recognized, particularly the presence of different subsets of macrophages.²

There is increasing evidence that macrophages exhibit a wide range of phenotypes and perform diverse functions in cancer. Currently, a classification system divides tumor-associated macrophages (TAMs) into anti-tumorigenic M1 type and pro-tumorigenic M2 type, where M1 TAMs are defined by their ability to kill tumor cells, inhibit metastasis and stimulate immune responses. M2 TAMs, on the other hand, are characterized by their tumor-promoting, pro-metastatic and immunosuppressive properties.³ M1 macrophages exert an anti-tumor effect by releasing tumoricidal molecules such as ROS and NO, stimulating anti-tumor immune responses and promoting antibody-dependent cellular cytotoxicity (ADCC). Conversely, M2 macrophages facilitate tumor initiation, growth and progression by secreting matrix metalloproteinases (MMPs), promoting epithelial-mesenchymal transition (EMT), remodeling the extracellular matrix, establishing a pre-metastatic niche and releasing immunosuppressive factors. Distinct TAM subsets expressing markers such as SPP1 (secreted phosphoprotein 1), APOE (apolipoprotein E), LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1), and FOLR2 (folate receptor 2) have been identified across multiple tumor types and are associated with specific functional states, including immunosuppression, angiogenesis, fibrosis, and therapeutic resistance.^{4,5} Two main origins of TAMs have been reported: tissue-resident macrophages and monocyte-derived macrophages. Tissue-resident macrophages are formed during early embryogenesis by precursors, mainly those of the yolk sac or fetal liver, and can maintain their population through self-renewal in situ. Monocyte-derived macrophages develop from blood monocytes that enter tumors in response to chemotactic and inflammatory stimuli.⁶ Both groups contribute to the TAM population, but their relative contributions vary depending on cancer type and stage. Resident macrophages typically maintain homeostasis, while monocyte-derived macrophages are associated with inflammation, immunosuppression, angiogenesis, metastasis, and drug resistance.⁷

Precisely targeting the phenotype and functionality of macrophages in the tumor microenvironment (TME) represents an innovative frontier in oncology

therapeutics. For example, in advanced and metastatic tumor models, strategic targeting of cancer-associated granulocytosis and recruitment of macrophages to neoplastic sites, together with depletion of macrophages through the application of specific antibodies and inhibitors, has shown promising anti-cancer results.⁸ Conversely, targeting the pro-tumor substances secreted by macrophages through antibody blockade, pharmacological inhibition and gene interference has yielded encouraging therapeutic outcomes. Considering the molecular mechanisms that trigger pro-tumor polarization of macrophages in cancer, a strategy has been proposed to repolarize these macrophages from a pro-tumor to an anti-tumor state through direct systemic administration or by delivery of drugs and inhibitors via nanoparticles. Recently, methods using macrophage membrane mimetics and macrophage-derived extracellular vesicles (EVs) have been proposed as novel therapeutic agents and drug delivery systems.^{9,10} Parent cell-derived macrophage membranes retain the expression of targeting ligands and "don't eat me" signaling molecules on their outer surface. While significant progress has been made in macrophage-related cancer treatments, it is important to distinguish preclinical results and clinically proven approaches. Most macrophage-targeting methods, such as macrophage-mediated nanoparticles, membrane-coated nanotechnology, extracellular vesicle therapy, and macrophage reprogramming, have demonstrated effectiveness primarily in vitro and in animal models. In contrast, only a few macrophage-related interventions, such as CSF1R (Colony-Stimulating Factor-1 Receptor) inhibitors and CAR (Chimeric Antigen Receptor) macrophages, have been tested in clinical settings.^{11,12} Although early-stage clinical trials have shown promising safety results, strong evidence of clinical effectiveness has yet to be established.

In this review, we summarize the latest developments in the engineering of macrophages for drug delivery and targeted cancer therapy, focusing on macrophage-based living cell delivery systems, macrophage membrane-mimicking nanoplateforms, and macrophage-derived EVs. We also discussed future perspectives of targeting macrophages for cancer therapy.

Macrophages in Cancer: The Anti-tumor and Pro-tumor Paradigm

Recent advances in single-cell RNA sequencing (scRNA-seq), spatial transcriptomics, and multiplex imaging technologies have shown that macrophage populations within tumors are considerably more heterogeneous than previously recognized. Macrophages exhibit diverse polarization phenotypes, particularly the classical (M1) and the alternative (M2) phenotypes, in response to different signals from the TME.^{13,14} Additional populations that exhibit different phenotypes, suggesting that the M1/M2 classification is overly simplistic for these dynamic cells. The M2 phenotype refers to functional programs that are often associated with various cancer types. It can promote tumor growth while hindering effective adaptive immunity within the TME.¹⁵ Previous research has

characterized M1 and M2 macrophage phenotypes based on their morphology, stimulation/activation, expressed markers, secreted cytokines, secreted chemokines, and functional properties (Table 1). Macrophages serve a dual role in tumor development, suggesting fundamental shifts in their role (Table 2). M2 macrophages, for instance, release VEGF (Vascular endothelial growth factor), matrix metalloproteinase 2 (MMP-2) and MMP-9, all of which promote angiogenesis and suppress T cells and natural killer (NK) cells, leading to a reduced immune response.¹⁶ In contrast, M1 macrophages increase production of inducible nitric oxide synthase (iNOS), which converts L-arginine into L-citrulline and nitric oxide, substances known for their anti-cancer properties in various cancers.^{17,18}

Table 1. The distinction between M1 and M2 macrophages in cancer

Parameter	M1 (anti-tumor)	M2 (pro-tumor)
Morphology	large, rounded and flat	stretched and elongated cells
secreted cytokines	IL-23, IL-12, IL-6, IL-1 alpha	IL-10, IL-1ra, IL-4, IL-13, TGF-β
secreted chemokines	CXCL10, CCL11, CCL5, CCL8	CCL17, CCL22, CCL1, CCR2, CCL13, CCL18
phenotypic markers	CD80, CD86, CD64, CD16, CD32, iNOS	CD163, CD206, CD68, Arg1, DECTIN-1
Functional properties	inflammation, insulin resistance, tissue damage, both tumor-promoting and anti-tumor properties	Promote Th2 differentiation, increase cell growth, tissue repair, phagocytosis of apoptotic cells, angiogenesis, tumor progression
stimulation/activation	IFN-gamma, LPS	IL-4, IL-13, IL-1B, IL-1R, IL-10, IL-6

Table 2. Macrophage and tumor development: a comparison of anti-tumor and pro-tumor capabilities.

Activity	Mechanism	Function	Tumor types	Refs
Anti-tumor	release of antimicrobial peptide cathelicidin in a vitamin D-dependent manner	improving antibody-dependent cell-mediated cytotoxicity against tumor cells	B cell lymphoma	19
Pro-tumor	release of VEGF and CCL18	promote cancer cell motility and angiogenesis	breast cancer	20
	activation of IL4/STAT6 signaling pathway	tumor cell growth and progression	lung cancer	21

Table 2. Continued...

Activity	Mechanism	Function	Tumor types	Refs
	β -Catenin-mediated CCL2 secretion	promoting stem cells properties and supporting growth and metastasis	breast cancer	22
	secretion of CCL8 and activation of ERK1/2 signaling	invasion, progression and stemness of tumor cells	glioblastoma	23
	secretion of IL-6 and activation of STAT3 pathway	promote CSC population, migration, and angiogenesis	Hepatocellular carcinoma and breast cancer	24,25
	secretion of MMP2, MMP7, and MMP9 in a TNF α -dependent manner	the breakdown of ECM proteins and thereby helping metastasis	breast cancer	26
	secretion of TGF β and activation of HIF1-TRIB3 signaling pathway	enhancing the stem-like phenotype, cell invasion, proliferation	colorectal cancer	27

The distinct functions of macrophages can be linked to their different polarization states and unique subsets within the TME. Numerous studies have identified specific macrophage subtypes that possess both pro-tumor and anti-tumor functions. Subgroups of M2 macrophages include M2a, M2b, M2c and M2d, each with unique cell markers, cytokines and functions.^{28,29} In particular, M2a promotes cell proliferation and tissue repair, whereas M2b and M2c are involved in inflammatory responses and engulfment of particles larger than 0.5 μ m in diameter, respectively. M2d macrophages, better known as TAMs, are the primary promoters of tumor progression.³⁰

Single-cell transcriptomics has advanced our fundamental understanding of macrophage biology by revealing macrophage subsets' phenotypic variability and functional range. Coulis et al investigated the molecular characteristics of dystrophic and healthy muscle macrophages and identified six novel subsets that do not conform to conventional M1 or M2 macrophage activation classifications. The gal-3+spp1+ signature represented the main transcriptional profile of macrophages from dystrophic muscle.³¹ Similarly, a study using scRNA-seq data from myeloid cells found a subset of LYVE1+ interstitial macrophages in non-cancerous tissues, alongside seven different subsets of

TAM clusters: Inhibin+ TAMs, C1QC+ TAMs, UCRP+ TAMs, PYPAF1+ TAMs, XLKD1+ TAMs and SPP1+ TAMs.³² These findings show that macrophages are mechanosensitive cells that respond to diverse biophysical cues in the tumor microenvironment, such as extracellular matrix stiffness, mechanical strain, and interstitial fluid flow. Mechanosensitive pathways, such as Piezo1 signaling, regulate macrophage polarization and pro-inflammatory behavior through NF- κ B and STAT signaling pathways.^{33,34} Moreover, spatial omics research indicates that macrophage activity depends not only on phenotype but also on localization and cellular interactions within tumor niches. Therefore, the development of immunotherapies targeting macrophages must consider macrophage heterogeneity, mechanobiology, and the spatial characteristics of the immune response.³⁵

Macrophages and Their Anti-tumor Potential

Preliminary research suggests that macrophages stimulated by bacterial components and cytokines can effectively destroy tumor cells, while macrophages from malignant, metastatic tumors can promote tumor proliferation, suggesting a dualistic relationship between macrophages and cancer.^{36,37} The anti-tumor capabilities of M1-macrophages are primarily associated with the

release of cytotoxic agents and the stimulation of anti-tumor immune responses. M1-Macrophages have the ability to eliminate tumor cells, promote antibody-dependent cellular cytotoxicity and phagocytosis, induce vascular injury and tumor necrosis, and activate innate and adaptive mechanisms mediated by lymphoid cells to combat tumors.³⁸ An elevated level of M1 macrophages has been linked to a positive outcomes for individuals diagnosed with cancer.³⁹ Factors that influence the polarization of macrophages toward the M1 phenotype include cytokines, transcription factors (TFs), proteases, exosomes, and lysosomes. Cytokines such as CXCL9/monokine induced by interferon- γ (MIG), CXCL10, IP-9, CXCR3 and IFNG can induce macrophage polarization toward the M1 phenotype. IFNG activates the Embden-Meyerhof pathway/aerobic glycolysis in macrophages, which can maintain the IFNG-induced JAK (Janus kinase)-STAT1 (Signal Transducer and Activator of Transcription 1) signaling pathway, facilitating M1 polarization in vitro.^{40,41} The interplay between TAMs and T cells is a key factor in shaping the immunological environment of the TME.⁴² The effects of TAMs can be either stimulatory or suppressive, depending on their activation state. Pro-inflammatory TAMs promote T cell recruitment through MHC-II-mediated antigen presentation, production of cytokines such as IL-12, and IFN- γ signaling with CD4⁺ and CD8⁺ T cells, thereby enhancing the anti-tumor response by T cells.⁴³ In contrast, immunosuppressive TAMs impair T cell function by secreting immunosuppressive cytokines such as IL-10 and TGF- β , expressing immunoinhibitory molecules like PD-L1, expanding T-regulatory cells, and inducing T cell exhaustion.^{43,44} Additionally, TAMs contribute to extracellular matrix remodeling, working with cancer-associated fibroblasts to create a physical barrier and biochemical factors that impede T cell migration. Interactions with tumor cells also lead to metabolic competition, resulting in nutrient depletion and accumulation of metabolic byproducts that further inhibit T cell function. Thus, bidirectional interactions between TAMs and T cells are critically important for establishing either an immunostimulatory or immunosuppressive environment in the TME.^{44,45}

PD-L1 is often expressed by TAMs, and its interaction with PD-1 on activated T cells inhibits their proliferation, cytokine production, and cytotoxicity. High PD-L1 expression in macrophages correlates with impaired anti-tumor immunity, T-cell exhaustion, poor

prognosis, and the development of resistance to immunotherapy in several cancers.⁴⁶ Recent evidence suggests that PD-L1-expressing TAMs significantly contribute to the creation of the immunosuppressive tumor microenvironment and may be more accurate predictors of responses to immune checkpoint therapy than PD-L1 expression on tumor cells. In addition, treatments targeting TAMs increase the effectiveness of anti-PD-1/PD-L1 therapies by reducing immunosuppression and activating T cells.^{47,48} M1 macrophages, which function as professional antigen-presenting cells (APCs), impede tumor proliferation by attracting CD8⁺ T and NK cells to the TME, effectively linking the innate and adaptive immune responses.⁴⁹ Considering the function of M1-macrophages as professional APCs, reprogramming M2-macrophages to the M1-macrophages could convert the immunosuppressive tumor microenvironment to a more favorable microenvironment for immunotherapy, and establish an APC niche that favors stem-like CD8⁺ T cells, representing a promising approach to improve cancer therapy.⁵⁰ M1 macrophages attract NK cells and activate CD8⁺ T lymphocytes by releasing chemokines, such as CXCL9/MIG and CXCL10, which are inducible by IFNG(51). The chemokines CXCL9 and CXCL10 bind to the CXCR3(C-X-C motif chemokine receptor 3)/GPR9/CD183 to enhance infiltration of CXCR3⁺ effector T cells into the TME. The CXCL9/CXCL10/CXCR3 pathway not only promotes the recruitment of effector T cells into the TME but also is critical for enhanced T cell activation.^{40,51,52} A recent groundbreaking cross-cancer metadata-analysis has shown that CXCL9 expression is one of the most important indicators of response to immune checkpoint inhibitors in patients with cancer and that its expression correlates with improved overall survival (OS) in patients receiving immune checkpoint therapy. This underscores the critical function of CXCL9/10, which is released by M1 macrophages in the TME.^{53,54} These cytotoxic effector cells release cytokines such as IFNG, TNF- α (Tumor necrosis factor alpha)/ TNFSF2, colony stimulating factor 2 (CSF2) and chemokines such as CCL4, CCL5 and CCL10, all of which act as positive feedback mechanisms to promote the recruitment of additional immune cells and facilitate antitumor signaling pathways.^{52,55} Activated NK cells also increase iNOS expression in M1 macrophages.⁵⁶ Nitric oxide (NO) can directly affect DNA while suppressing DNA synthesis and ribonucleotide reductase activity, which in

turn prevents cell proliferation and triggers oxidative stress, serving as a potential means to eradicate tumor cells.⁵⁷ By increasing IL-12 production, M1 macrophages also increase IFNG production by T and NK cells, increase the cytotoxicity of NK and NKT cells and promote the development of Th1 cells and cytotoxic CD8⁺ T cells.⁵⁸ Watkins et al. demonstrated that macrophages treated with IL-12 showed reduced expression of tumor-promoting macrophage functions (IL-10, monocyte chemoattractant protein-1 (MCP-1)/CCL2, migration inhibitory factor (MIF) and TGF β production) with a simultaneous increase in pro-inflammatory and pro-immunogenic activities (TNFSF2, IL-15 and IL-18 production).⁵⁹ In addition, activation of transcription factors such as STAT1, NF- κ B (nuclear factor-kappa B), JAK3 (Janus kinase 3) and JNK (c-Jun NH2-terminal kinase) in M1 macrophages can significantly increase the production of pro-inflammatory cytokines such as IL-1, IFN beta 2, IL-12, IL-23 and TNFSF2.^{60,61} These cytokines can restrict the growth of tumor cells either by direct cytotoxic effects or indirectly by triggering the cytotoxic actions of effector immune cells against cancer cells.⁶² M1 macrophages have also been demonstrated to exhibit elevated levels of major histocompatibility complex class II (MHC II), mainly presenting exogenous antigens to CD4⁺ T cells and consequently promoting immune surveillance and antitumor immune response.⁶³ Clinical studies have shown that an increased presence of M1 macrophages is associated with prolonged survival and better clinical outcomes in a number of cancers, including lung cancer,⁶⁴ colorectal cancer,⁶⁵ ovarian cancer⁶⁶ and breast cancer(BC).⁶⁷

Macrophages and the Pro-tumor Potential

Despite the antitumor capabilities of macrophages, cancers use these cells to promote tumor development, proliferation and progression.² Numerous studies indicate that macrophages play a crucial role in numerous tumor-related processes. The most compelling evidence comes from animal studies showing that macrophage depletion can improve the efficacy of various immunotherapeutic approaches such as vaccinations⁶⁸ and checkpoint inhibitors.^{69,70} Key functional characteristics of the M2 phenotype include angiogenesis, drug resistance, immunosuppression, and tumor metastasis. In the following paragraphs, we summarized the key functional characteristics of the M2 phenotype.

Immunosuppression

Immunosuppression is a mechanism that promotes tumor growth. Cytokines and proteases produced by M2 macrophages, including TGF- β , IL-10, human leukocyte antigen G (HLA-G) and arginine amidinase, contribute significantly to immunosuppression.^{71,72} TGF- β , for example, promotes the polarization of macrophages toward the M2 phenotype, which in turn increases TGF- β production and enhances immunosuppression.⁷³ TGF- β also inhibits the cytolytic activity of NK cells expressing NKG2D, a receptor that is critical for the activation and regulation of these cells, ultimately leading to a diminished antitumor response.⁷⁴ In addition, TGF- β reduces the migration of dendritic cells (DCs) and supports the differentiation of Treg cells, interfering with the adaptive immune response.^{74,75} IL-10, known for its anti-inflammatory and immunosuppressive properties, is synthesized by various cells, including M2 macrophages, CD8⁺ T cells, and tumor cells, thereby promoting tumor evasion of immune surveillance. IL-10 produced by M2 macrophages inhibits the expression of IL-12, an important cytokine that modulates malignancy through its ability to activate both innate and adaptive immune effector cells.⁷⁶ In addition, IL-10 inhibits the maturation of DCs,⁷⁷ diminishes the activity of epidermal APCs,⁷⁸ and promotes the differentiation of macrophages, thereby reducing antigen presentation. Furthermore, this cytokine can block the secretion of IFN- γ , the primary chemoattractant for CD8⁺ T cells and a key factor in the differentiation of naïve T cells, and support immune evasion.⁷⁹ Nevertheless, some studies have indicated that IL-10 may have certain immunostimulatory effects.^{80, 81} For example, in stage I non-small cell lung cancer (NSCLC), a correlation was observed between prolonged OS and higher infiltration of CD8⁺/IL-10⁺ cells, highlighting the dual role of IL-10 in the tumor microenvironment.⁸⁰ Arginine amidinase, a specific marker of M2 macrophages, shows significant expression in tumors. This enzyme, which facilitates the conversion of L-arginine to polyamines and proline, has been shown to interfere with T cell receptor (TCR) signaling and ultimately contributes to CD8⁺ T cell insensitivity.⁸² Furthermore, activation of arginine amidinase leads to the production of hydrogen peroxide (H₂O₂) by myeloid-derived suppressor cells (MDSCs), which directly present HLA class I-linked epitopes to CD8⁺ T cells and prevent their release of IFNG through a contact-dependent production of

H₂O₂.⁸³ However, the exact mechanism by which H₂O₂ is produced after arginine amidinase activation remains unclear and may be related to the concomitant activation of a different NOS isoform.⁸²

Angiogenesis

Macrophages have been shown to release a number of pro-angiogenic factors, including acidic fibroblast growth factor (aFGF),⁸⁴ angiopoietin-2 (Ang2),⁸⁵ angiostatin,⁸⁶ IL-1 β ,⁸⁷ IL-20,⁸⁸ CCL2,⁸⁹ Thrombospondin-1 (TSP-1),⁹⁰ CXCL12, basic fibroblast growth factor (bFGF),⁹¹ vascular endothelial growth factor A (VEGFA),⁹² MMPs; particularly MMP-1, MMP-2, and MMP-9, and transforming growth factor- α /- β (TGF- α /- β).^{93,94} These factors can influence angiogenesis at different stages. For example, IL-1 β , VEGF and TGF- β , α can activate mesenchymal stromal cells, which are an important cellular component of the TME, creating favorable conditions for angiogenesis. MMPs released by macrophages influence the extracellular matrix and promote the budding cycle. This proteolytic mechanism is initiated by the infiltration of endothelial cells and facilitates the migration of other cell types. Transcription factors such as STAT-3, E2F1, Myc and others play a role in macrophage-stimulated angiogenesis. STAT-3, for example, can trigger the production of various pro-angiogenic factors such as VEGF and bFGF in macrophages.⁹⁵ Badawi et al demonstrated a significant correlation between the degree of macrophage infiltration and tumor angiogenesis and metastasis.⁹⁶ Similarly, Hwang et al found that an increase in M2 macrophage infiltration can enhance the expression of VEGF-A/C, which in turn stimulates angiogenesis in NSCLC.⁹⁷ In addition, another study showed that the increased presence of CD163+ macrophages in the central tumor region along with the infiltration of CD202B (cluster of differentiation 202B)-expressing monocytes at the tumor-infiltrating margin can promote angiogenesis and negatively affect the OS rate and recurrence-free survival rate of patients with pancreatic ductal adenocarcinoma (PDAC).⁹⁸ In addition, a study showed that M2 macrophages are positively correlated with the density of microvessels in PDAC. Extracted exosomes were rich in miR-155-5p and miR-221-5p, which enhance the angiogenic potential of endothelial cells.⁹⁹ In lung cancer, M2 macrophage-derived exosomal miR-942 modulates FKH1 protein expression by binding to the 3'-UTR regions of FKH1, thereby attenuating β -

catenin suppression in lung cancer cells and promoting angiogenesis.¹⁰⁰

Drug Resistance

M2 macrophages can interact with neoplastic cells through both direct and indirect mechanisms, thereby influencing the therapeutic tolerability of cancer treatments such as chemotherapy, targeted therapy, and immunotherapy. Potential regulatory mechanisms by which M2 macrophages influence tumor drug resistance include modulation of signaling pathways and the immunological microenvironment, secretion of various exosomes, alteration of cytokine profiles, interaction with cancer stem cells (CSCs), and promotion of tumor angiogenesis.

Studies have shown that M2 macrophages, through the secretion of cytokines, chemokines, and growth factors, can over-activate the PI3K/Akt, JAK/STAT, mitogen-activated protein kinases (MAPK) and other related pathways, which all play critical roles in encouraging tumor growth and the development of drug resistance and consequently alter the TME to stimulate tumor proliferation and drug resistance.¹⁰¹ Li et al found that M2 macrophages via the CCL2/PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway increase tumor cells' capacity to resist apoptosis. These tumor cells showed increased autophagy, leading to tamoxifen resistance.¹⁰² Also, M2 macrophages can activate cancerous breast cells via the epidermal growth factor receptor (EGFR)/PI3K/Akt signaling pathway leading to increase in solute carrier family 5 member 1 (SLC5A1) and modulation of glycolysis, thereby promoting tamoxifen resistance and accelerating tumor growth.¹⁰³ Macrophage-derived exosomes have the ability not only to influence multiple signaling pathways to regulate therapeutic resistance but also to influence drug resistance by facilitating the transfer of chemotherapeutic agents away from tumor cells. Exosomes derived from macrophages by miR-365 transfer can deactivate the Gemzar reservoir and increase Gemzar resistance in PDAC cells, leading to an increase in nucleotide triphosphates and activation of cytidine deaminase in PDAC cells.¹⁰⁴ Pinar et al showed that miR-1246 stimulates P-glycoprotein by targeting the caveolin 1/P-glycoprotein/ribose phosphate pyrophosphokinase 2/M2-like macrophage signaling pathway, thereby interfering with Taxol absorption and transport in ovarian cancer.¹⁰⁵

Tumor Metastasis

Macrophages are important in the process of tumor cell metastasis. Macrophages synthesize Osteonectin (ON), a collagen-binding protein important for type IV collagen deposition. This protein enhances tumor cell invasion, and adhesion to other ECM components (such as fibronectin) and thereby promoting metastasis from the primary tumor.¹⁰⁶ Research has shown that EMT hotspots, characterized by a morphological change that facilitates metastasis, particularly at the periphery of tumor nests, represent regions that are significantly infiltrated by macrophages.¹⁰⁷ In addition, macrophages are able to form clusters in the intra-tumoral areas and invasive fronts, which serve as critical sites for metastasis and angiogenesis. Conversely, the absence of macrophages led to a significant decline in the density of vascular by 40%.¹⁰⁸ Simultaneous cultivation of hepatocellular carcinoma cell lines with macrophages resulted in a marked increase in the expression of N-cadherin and Snail, while downregulating E-cadherin expression. Similarly, this phenomenon was observed in gastric cancer and PDAC.¹⁰⁹ Reduction of macrophage populations by genetic methods¹¹⁰ or by blocking EGF¹¹¹ or CSF-1¹¹⁰ signaling in mice with breast tumors leads to a reduction in circulating tumor cells, suggesting that the paracrine interaction between these two cell types is essential for their entry into the bloodstream *in vivo*. Notably, a structure termed TMEM (tumor microenvironment of metastasis) is characterized by the co-localization of macrophages, tumor cells and endothelial cells and serves as a prognostic indicator of unfavorable survival rates in breast cancer.¹¹²

Beyond the Classical M1/M2 Paradigm: Macrophage Heterogeneity

While the M1/M2 dichotomy has been useful for studying mechanisms, increasing evidence shows that it is an oversimplification of macrophage biology, especially in the TME. The continuous adaptation of macrophages to changing stimuli such as cytokines, hypoxia, metabolism, extracellular matrix components, and interactions with tumor and stromal cells produces a wide range of activation states rather than two defined phenotypes. Single-cell RNA sequencing (scRNA-seq) has revolutionized how we think about macrophage heterogeneity, revealing that TAMs occupy a complex continuum of transcriptional and functional states rather than fitting into M1 and M2 categories.⁴ For instance, SPP1-positive macrophages are implicated in

extracellular matrix remodeling, angiogenesis, and metastatic progression,¹¹³ whereas TREM2-positive macrophages are strongly immunosuppressive and cause T-cell dysfunction, thereby contributing to resistance to immune checkpoint blockade therapy.¹¹⁴ Other macrophage populations include C1QC-positive resident macrophages,¹¹⁵ IFN-induced inflammatory macrophages,¹¹⁶ and FOLR2-positive tissue-resident macrophages that perform specific roles in antigen presentation, immune regulation, and maintenance of tissue homeostasis.¹¹⁷ Notably, these macrophage populations coexist in the same TME, highlighting the tremendous plasticity of the macrophage compartment, which is not fully captured by the classical M1/M2 classification. In addition to single-cell RNA sequencing (scRNA-seq), spatial transcriptomics has been instrumental in elucidating the spatial distribution of macrophage subsets in the TME. Unlike traditional transcriptomic techniques that require tissue dissociation, spatial transcriptomics preserves tissue architecture, allowing the study of both gene expression profiles and the spatial distribution of cells *in situ*.¹¹⁸ Different macrophage subsets have been shown to associate with specific tumor regions and to form distinct interactions with neighboring cancer cells, fibroblast cells, endothelial cells, and lymphocyte cells. For example, SPP1⁺ macrophages are abundant in hypoxic and fibrotic tumor areas, where they contribute to angiogenesis and extracellular matrix remodeling, whereas inflammatory macrophages tend to localize near T lymphocytes and tertiary lymphoid structures.¹¹⁹ This indicates that macrophage function depends on both their intrinsic transcriptional programs and their spatial localization within the tumor environment. Consequently, integrating scRNA-seq with spatial transcriptomic analysis has shifted the prevailing binary M1/M2 paradigm toward a continuous model.

Macrophage-Based Drug Delivery Systems

The application of immune cells as Trojan horses for delivering nanomedicines to tumors represents a promising approach in cancer treatment. Recently, the potential of macrophages as drug delivery vehicles in cancer therapy has garnered significant attention.¹²⁰ Unlike traditional delivery methods such as liposomes, polymeric nanoparticles, dendritic cell-derived extracellular vesicles, and synthetic nanocarriers, macrophage delivery systems offer many advantages. Macrophages have natural tumor-tropic behavior; they

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can migrate across barriers, enter hypoxic tumor zones, and avoid rapid immune clearance. Therefore, macrophage-mediated drug delivery is enhanced in target areas. In contrast, liposomes and synthetic nanocarriers mainly rely on the enhanced permeability and retention (EPR) effect to achieve passive targeting and are often hindered by opsonization and uptake by mononuclear phagocytes.^{121,122} Although polymeric nanoparticles allow for precise tuning and controllable drug release, they exhibit poor tumor-tropic ability. Dendritic cell-derived vesicles can stimulate the immune response and are effective in cancer vaccines, but their migration and cargo delivery to solid tumors are less efficient compared to macrophages. However, macrophage-based delivery systems have some disadvantages, such as donor variability, challenges in scaling up, and complex manufacturing processes.^{123,124} In this section, we will explore six key strategies that employ macrophages as drug carriers: 1) nanoparticle (NP) delivery mediated by macrophages; 2) direct drug delivery facilitated by macrophages; 3) macrophages serving as indirect drug delivery vehicles; 4) CAR-modified macrophages acting as drug delivery systems for cancer therapy; 5) macrophage membrane-mimetics functioning as drug delivery agents; and 6) macrophage-derived extracellular vesicles serving as drug delivery platforms (Figure 1).

As vital elements of the innate immune system, macrophages offer a variety of benefits, such as their abundant availability, lack of immunogenicity, ease of harvest, rapid response to inflammation, and diverse

biological functions.^{125,126} The use of macrophages and their derivatives as vehicles for drug delivery is an important focus in the application of macrophages for cancer treatment. Due to their ability to cross biological barriers and accumulate in significant quantities at sites of inflammation and tumors, a "hitchhiking" approach—in which circulating macrophages are used for drug delivery—has been widely proposed. Macrophages can transport drug-loaded carriers to tumors by following a gradient of inflammatory chemokines, thus improving the specificity, efficacy, and safety of chemotherapy and immunotherapy. Several factors play crucial role in determining the efficacy of macrophage-mediated cancer therapy. First, the high heterogeneity and plasticity of macrophages within the TME can lead to unpredictable results, as therapeutic macrophages may develop pro-tumor phenotypes under the influence of tumor-derived cues.¹²⁷ Second, effective delivery, migration to the tumor, and survival of therapeutic macrophages require further investigation, especially in hypovascular and immunosuppressive tumors. Third, the interaction of macrophages with other immune cells, such as T cells, dendritic cells, and myeloid-derived suppressor cells, significantly affects therapeutic efficacy. In addition, variability in the tumor microenvironment among patients, along with manufacturing, scaling, and safety issues, may pose obstacles for translational studies.^{50, 128} Therefore, future research should address macrophage stabilization and resistance to immunosuppressive factors. Table 3 Compares macrophage-based drug delivery platforms for cancer therapy.

Table 3. Comparison of macrophage-based drug delivery platforms for cancer therapy.

Platform	Cargo	Particle size (nm)	Loading efficiency	Tumor accumulation	Therapeutic efficacy	Advantages	Limitations
Macrophage-mediated nanoparticles	PTX, DOX, Fe3O4	14–200	High	Excellent	Significant tumor inhibition	Active homing	Complex manufacturing
Direct drug-loaded macrophages	DOX, Oxaliplatin	N/A	Moderate	High	Enhanced survival	Long circulation	Drug toxicity to macrophages
CAR-macrophages	CAR construct	Cell therapy	N/A	Excellent	Promising	Phagocytosis + APC	Early clinical stage
Membrane-coated nanoparticles	PTX, Gold nanoshell	80–250	High	Excellent	Improved targeting	Immune evasion	Membrane isolation
Macrophage-derived EVs	PTX, DOX, Gemcitabine	30–150	Moderate-High	Excellent	Strong antitumor activity	BBB penetration	Standardization challenges

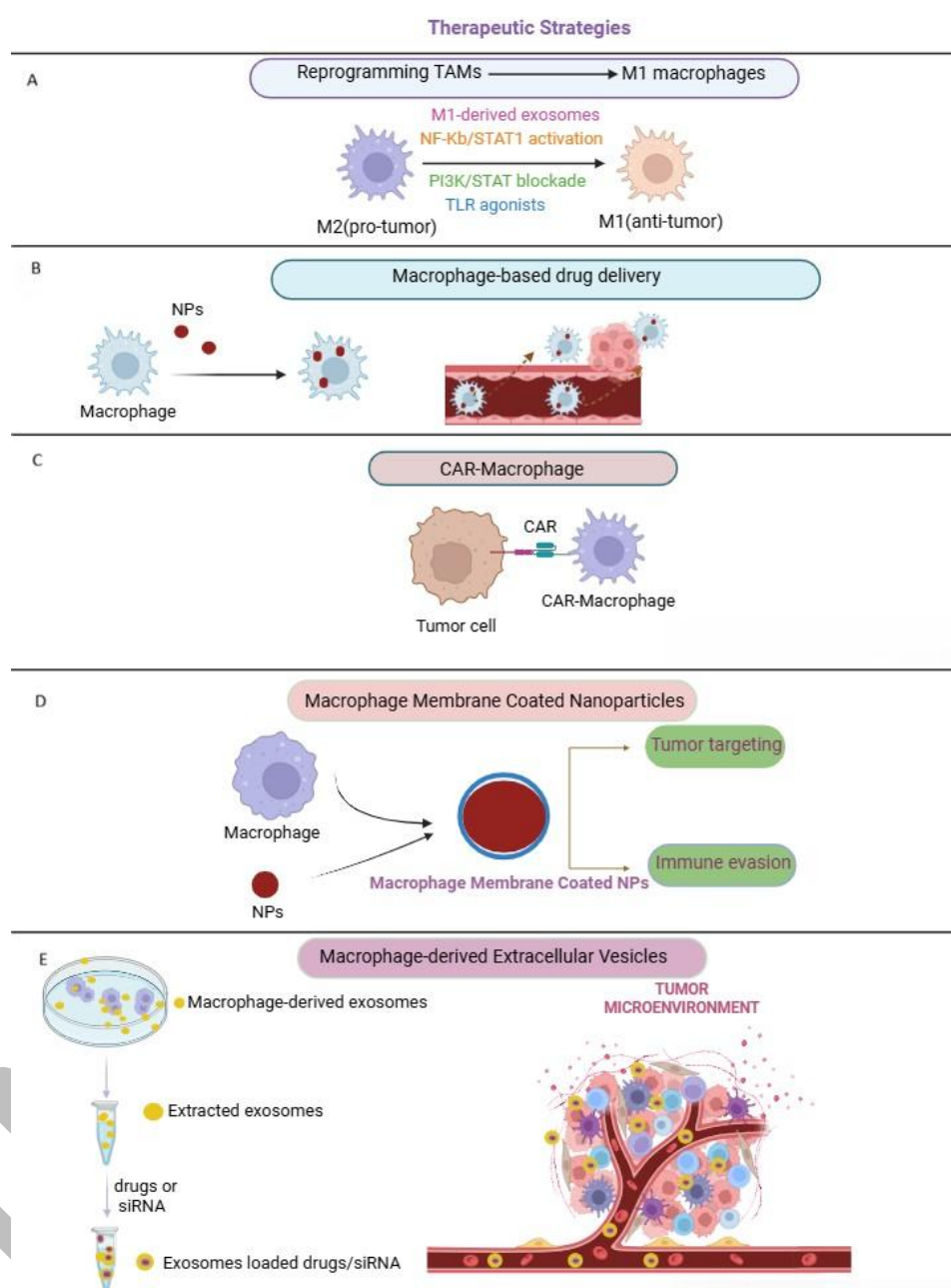


Figure 1. Integrated conceptual framework of macrophage biology, tumor microenvironment interactions, and approaches for treatment of cancer.

Macrophage-mediated nanoparticle delivery system for cancer therapy

Nanoparticles (NPs) have gained considerable importance in the field of nanomedicine, particularly in the treatment of cancer. The application of NPs offers pharmacokinetic advantages and demonstrates high efficacy, safety and targeting capabilities. Nevertheless,

challenges arise from their toxicity, rapid degradation in the bloodstream and the need for accurate characterization, which necessitates the development of NP delivery systems. Macrophages are proving to be promising vehicles for the delivery of NPs to the TME. For this therapeutic approach to be effective, it is critical that the NPs taken up by the macrophages do not

interfere with their normal functions. The origin of macrophages used for drug delivery primarily include primary macrophages derived from animals, such as bone marrow-derived macrophages, and commercially available cells, such as RAW264.7, and J774A.1 cells, or human THP-1 monocytic leukemia cells.¹²⁹ Through their $\alpha 4 \beta 1$ integrins, these macrophages can interact with VCAM-1 (Vascular Cell Adhesion Molecule 1)/CD106 on cancer cells.¹³⁰ The mechanisms by which NPs are internalized by macrophages include phagocytosis (the primary method of NP uptake), pinocytosis, and receptor-mediated endocytosis.^{131,132} The type of internalization is influenced by the properties of NPs, such as size, shape, surface chemistry, and mechanical characteristics.¹³³ Phagocytosis begins with the activation of pattern recognition receptors (such as mannose receptors, integrins, and scavenger receptors) on the macrophage by the ligands on the particle surface. The cellular cytoskeleton is then reorganized, facilitating the internalization of the particles by an actin-dependent mechanism. Subsequently, the phagosome matures into a phagolysosome, a process that depends on the interaction between the actin- and tubulin-based cytoskeleton.¹³¹ Li et al used macrophages to deliver paclitaxel (PTX) conjugated with polymeric micelles forming by N-succinyl-N'-octyl-chitosan (SOC). Their studies showed that macrophages loaded with SOC-PTX exhibited superior antitumor efficacy compared with PTX administered in isolation, effectively reducing the viability of the BC cell lines by 80% in vitro. Moreover, in mice bearing tumors, macrophages containing SOC-PTX resulted in a substantially reduced tumor size, by more than 90%.¹³⁴ A study used NPs consisting of reduced graphene oxide, branched polyethyleneimine, and polyethylene glycol monomethyl ether to incorporate doxorubicin (DOX) and facilitate its internalization into macrophages. DOX release was triggered by near-infrared (NIR) laser irradiation and led to a marked suppression of tumor development in mice bearing RM-1, a prostate cancer cell line. These NPs demonstrated efficient accumulation in the tumor region and in vitro showed toxic effects on RM-1 cells.¹³⁵ Other study demonstrated that liposome-encapsulated DOX and gold nanorods loaded into macrophages produced synergistic photothermal-chemotherapy. The results indicated a significantly diminished viability of 4T1 cells (a breast cancer cell line) following NIR irradiation. In addition,

macrophages accumulated in the tumor region and reduced tumor proliferation in mice bearing 4T1 compared with the other groups after NIR exposure.¹³⁶ Similarly, a macrophage-mediated approach for the delivery of small Fe@Fe₃O₄-DHCA NPs (~14 nm) was developed to enhance magnetic resonance imaging (MRI) and magnetothermal therapy in solid tumors.¹³⁷ The results showed that Fe@Fe₃O₄-DHCA NPs accumulated in cells, leading to improved MRI and magnetothermal efficacy in vitro. Moreover, the efficacy of magnetothermal therapy using macrophages loaded with Fe@Fe₃O₄-DHCA outperformed that of Fe@Fe₃O₄-DHCA alone in vivo, which was attributed to the tumor-targeting capabilities of macrophages. This represents a novel strategy to improve the treatment efficacy of magnetothermal therapy using Fe@Fe₃O₄-DHCA NPs.¹³⁷

Macrophage-mediated direct drug delivery

Macrophages can directly absorb drugs through phagocytosis. Introducing drugs into the cell has numerous benefits. The retention time of the drug is prolonged because the entrapped drug is not removed by hepatic metabolism, is not identified by the immune system, and is not eliminated by the body's phagocytic cells. Nevertheless, drugs absorbed directly into macrophages are highly toxic to these cells.¹³⁸ In addition to the direct delivery of drugs into macrophages, it is also feasible for drugs to bind to the outer membrane of macrophages, as the cell membrane consists of proteins, lipids, and carbohydrates that feature charged or reactive groups that promote the attachment of drugs to the cell surface through either covalent or non-covalent interactions. These bonds improve the stability of macrophage-drug associations and prevent the release and absorption of drugs in non-target organs.¹³⁹ Nevertheless, there are obstacles to incorporating drugs onto macrophage membranes, including negative effects on cellular signal transduction, adhesion, and transendothelial migration, as well as heightened immunogenicity.¹⁴⁰ In one study, DOX-loaded RAW264.7 macrophages were shown to have tumor-tropic capacity toward tumor cells and to preserve their tumorigenic capabilities while targeting metastatic carcinoma cells, thereby prolonging the survival of mouse.¹⁴¹ Another study showed that modified macrophages loaded with nanodrugs of oxaliplatin prodrugs and photosensitizers acted as drug carriers triggered by near-infrared light and were able to

promote M1-type polarization *in vivo*.¹⁴² These modified macrophages exhibited an anti-tumor M1 phenotype and showed remarkable infiltration into both primary and bone metastatic tumors, enabling light-guided drug release and a combination of chemophotodynamic therapy that not only ensures targeted drug release and increases drug delivery efficiency but also induces immunogenic cell apoptosis and contributes to anti-tumor activity. Integration of these modified macrophages with anti-PD-L1 demonstrated a significant ability to eradicate both primary and osseous metastatic neoplasms while enhancing infiltration of cytotoxic CD8⁺ and CD4⁺ T lymphocytes with minimal systemic side effects.¹⁴² Most anticancer drugs within a certain dosage range, such as fluorouracil, camptosar, cisplatin and gemzar, which can be combined with chemotherapy, radiotherapy and immunotherapy, have shown no negative effects on macrophage survival.¹⁴³

Overall, direct drug administration improves concentration of the drug at the target site while minimizing systemic side effects. It also helps to overcome the immune barrier in the TME and thus represents an innovative approach to fighting diseases such as cancer.¹⁴⁴

Chimeric Antigen Receptor-Modified Macrophages for Cancer Therapy

While Chimeric antigen receptor (CAR) T cell therapy has had a significant impact on the management of hematological cancers, its use in treating solid tumors has been limited by poor tumor infiltration, T-cell exhaustion, and the immunosuppressive tumor microenvironment.¹⁴⁵ Although CAR-NK cells have shown potential as safer alternatives, with lower risks of cytokine release syndrome and neurotoxicity, they're *in vivo* half-life is relatively short.¹⁴⁶ In contrast, CAR macrophages offer several advantages, including effective infiltration of solid tumors, phagocytosis, antigen presentation, and remodeling of the tumor microenvironment (Table 4).^{147,148}

In recent decades, various techniques have been used to utilize macrophages, and these have achieved remarkable results in the laboratory. However, these methods did not yield significant results in clinical trials, indicating a need for innovative approaches to improve the efficacy of macrophages. By modifying macrophage cells to express CAR, it is possible to generate CAR macrophages, which can be used as a novel therapeutic

method and could represent a promising avenue for overcoming the challenges of solid tumors. CAR macrophages can modulate immunological reactions and thereby improve the efficacy of cancer therapies. CAR macrophages can modify the TME by activating proinflammatory pathways, and MHC genes, in addition to promoting the conversion of M2 macrophages to the M1 phenotype, which is essential for the treatment of solid tumors given the substantial presence of TAMs.¹⁴⁹ Remarkably, CAR macrophages are not affected by the immunosuppressive properties of M2 macrophages, allowing them to maintain their anti-tumor efficacy in a challenging TME. CAR macrophages can selectively target tumor cells through the use of CARs and exhibit antigen-specific phagocytic activity and cytotoxicity, which is mainly facilitated by the release of TNF- α , thereby exerting direct cytotoxic effects on tumor cells similar to those of native macrophages. CAR macrophages show profound interactions with various types of immune cells, including T cells. Research has shown that CAR macrophages enable an enhanced chemotactic response of both quiescent and activated T cells to neoplastic sites compared with non-transduced macrophages in humanized mouse models.¹⁵⁰ CAR structures delivered via adenovirus vectors can convert M2 macrophages to the M1 phenotype (primarily via interferon signaling, iNOS signaling, and the Th1 pathway) and stimulate them to release pro-inflammatory cytokines and co-stimulatory molecules, thereby enhancing the antitumor responses of both native macrophages and other immune cells.^{150, 151} In addition, CAR macrophages have significantly increased levels of CD80 (B7-1) and CD86 (B7-2), which are type I membrane proteins belonging to the immunoglobulin superfamily, compared with non-transduced macrophages, thereby enhancing both the activity and the infiltration and durability of the anti-tumor T cell response.¹⁵⁰

Macrophage Engineering for Cancer Therapy

Table 4. Comparison of CAR-T Cells, CAR-NK Cells, and CAR-Macrophages for Cancer Therapy

Feature	CAR-T Cell	CAR-NK Cell	CAR-Macrophages
Cell source	Autologous or allogeneic T lymphocytes	Peripheral blood NK cells, cord blood, NK cell lines, iPSC-derived NK cells	Peripheral blood monocytes, iPSC-derived macrophages
Primary mechanism of action	Antigen-specific cytotoxicity through perforin/granzyme release	Direct cytotoxicity via perforin/granzyme and natural cytotoxic receptors	Antigen-specific phagocytosis, cytokine secretion, antigen presentation
Antigen presentation capacity	Limited	Limited	High; professional antigen-presenting cells
Ability to remodel TME	Moderate	Moderate	High; can repolarize TAMs and promote pro-inflammatory TME
Tumor infiltration	Often limited in solid tumors	Better than CAR-T but still restricted	Excellent; naturally infiltrate solid tumors
Efficacy in hematological malignancies	Highly effective; several approved products	Promising clinical activity	Early-stage development
Efficacy in solid tumors	Generally limited	Moderate	Particularly promising due to strong tumor infiltration
Persistence in vivo	Long-term persistence	Relatively short persistence	Moderate persistence
Risk of CRS	High	Low to moderate	Low
Risk of neurotoxicity (ICANS)	Significant	Low	Minimal reported to date
Susceptibility to immunosuppressive TME	High	Moderate	Relatively resistant
Manufacturing complexity	High	Moderate	Moderate to high
Clinical development stage	Clinically established	Multiple ongoing clinical trials	Early clinical trials (e.g., CT-0508, MCY-M11)
Major limitations	Poor trafficking, exhaustion, severe toxicities	Limited persistence, manufacturing challenges	Limited clinical data, manufacturing standardization

In vitro and in vivo studies have shown significant results in hematological and solid tumors associated with CAR macrophage cells. The anti-cancer properties of CAR macrophages outperform those of control macrophages or alternative macrophage-based therapies. A number of antigens, including ganglioside GD2, EGFR III, T-Cell Surface Antigen Leu-12/CD19, MUC-1, CD340 (cluster of differentiation 340) and mesothelin/MSLN, elicited encouraging cytotoxic effects in CAR macrophages. For example, CAR macrophages targeting MSLN have shown anti-tumor effects in vivo in leukemia cells or the ovarian cancer cell line HO8910, which has high MSLN levels.¹⁵² CAR macrophages targeting MUC-1 showed a strong anti-tumor effect against MUC1-expressing lung tumors. The mechanism by which MUC-1-targeted CAR macrophages act is phagocytosis and the release of pro-inflammatory cytokines, including IL-1 β , IL-8, and TNF α .¹⁵³ In addition, HER2-specific CAR macrophages phagocytosed and eradicated HER2⁺ ovarian cancer cells, resulting in a significant reduction in tumor burden and improved survival in mouse models.¹⁴⁹ To date, several clinical studies have investigated the efficacy of CAR macrophages. In one clinical trial (NCT03608618), the drug MCY-M11 (PBMCS expressing anti-mesothelin CAR) was evaluated in individuals with advanced ovarian cancer and peritoneal mesothelioma. MCY-M11 had minimal side effects and patients tolerated the infusion well with no safety issues. In terms of efficacy, patients showed stable disease progression after a single treatment cycle; however, the final results have yet to be published.¹⁵⁴ In addition, CT-0508 (anti-HER2 CAR macrophages) was administered to patients with recurrent tumors characterized by overexpression of CD340. The infusion was well tolerated with minimal adverse effects that could be effectively treated without the need for tocilizumab (Actemra). Of the patients who completed the 8-week evaluation, 3 achieved stable disease progression, while 1 experienced disease progression. CT-0508 exhibited rapid extravasation from the bloodstream, facilitated myeloid cell activation and enhanced T cell functions, including proliferation, activation and infiltration, thereby promoting the expansion of tumor-infiltrating T cells.¹⁵⁵ While there have been encouraging results for CAR-macrophages both preclinically and clinically in early stages, it is important to highlight certain limitations that must be addressed to ensure successful clinical implementation. During manufacturing, the

CAR-macrophages production process presents significant challenges due to the short lifespan and limited proliferative capacity of primary macrophages. This makes it difficult to achieve sufficient production scale compared to CAR-T cells.¹⁵⁶ Current methods use either monocyte-derived macrophages or immortalized cell lines, both of which result in inconsistency in differentiation, transduction, and functional stability, making it challenging to ensure GMP compliance and scalable production. Additionally, genetic modification of macrophages is difficult because of their relative resistance to transduction and sensitivity to manipulation ex vivo.^{156,157}

Macrophage Membrane-Mimetics as Drug Delivery Vehicles

The recent success of NPs in combating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spurred initiatives to expand the use of NPs in the treatment of a variety of diseases, including cancer.¹⁵⁸ However, few nanotherapies have entered clinical trials. Issues such as interception by host immune cells lead to suboptimal pharmacokinetics and insufficient drug concentrations in tumors, so a different strategy is needed to evade immune cells and improve the therapeutic efficacy of drugs.¹⁵⁹ Recently, the encapsulation of NPs into the natural cell membranes of immune cells, commonly referred to as cell membrane cloaking, has attracted great interest. These natural cell membranes have 'self' markers, biological targeting capabilities, and immune compatibility, allowing NPs to achieve prolonged circulation, precise recognition, and deep tumor penetration.¹⁶⁰ Membrane mimetics are usually defined by a thin layer of cell membrane surrounding the NPs, forming a structure in which the NPs act as the core while the cell membrane acts as the outer envelope.¹⁶¹ The preparation of macrophage membrane-coated NPs usually involves (1) isolation of the macrophage cell membrane (mainly by hypotonic treatment, freeze-thaw cycles, and sonication), (2) formation of the NP core, and (3) integration of the membrane-coated NPs (by four methods: Incubation, membrane extrusion, sonication or electroporation).^{162,163} Macrophage membrane mimetics, a biomimetic platform, represent considerable therapeutic potential. NPs coated with macrophage membranes have already been used in the treatment of numerous diseases, including cancer (especially breast, skin, lung, and colon cancers) and cardiovascular

diseases.¹⁶⁴ Macrophage-derived membranes have the potential to enhance anti-cancer immune responses and have cellular self-recognition mechanisms that facilitate evasion of phagocytosis by other immune cells, and membrane-derived ligands can also bind to receptors located at pathological sites. At the same time, NPs coated with macrophage membranes show the ability to identify and respond to various pathogens and immune signals such as bacterial toxins, and inflammatory cytokines.¹⁶⁵ With regards to BC, a major public health challenge for women worldwide, macrophages, which are the main cellular components of TME in BC, play an important role in metastasis. In their research, Zhang et al developed cskc-PPiP/PTX@Ma, NPs loaded with paclitaxel coated with macrophage membranes, which indicated enhanced therapeutic effectiveness in the treatment of BC. Due to the macrophage membrane, cskc-PPiP/PTX@Ma displayed improved localization to the tumor, and the associated membrane proteins acted as a shield against clearance by the phagocytic cells while also serving as a navigational tool for tumor targeting.¹⁶⁶ In a similar study, saikosaponin D was encapsulated in a polymer matrix of PLGA, which was subsequently coated with macrophage membranes hybridized with T7 peptide, a ligand known for its affinity for transferrin receptors that are abundantly expressed on neoplastic cells. Both in vitro and in vivo, these biomimetic NPs were shown to target neoplastic cells while providing immune defense against phagocytic cell populations. In vivo evaluation of anti-tumor efficacy showed that these biomimetic NPs significantly restricted tumor proliferation in contrast to the control cohorts.¹⁶⁷ In a mouse model of skin carcinogenesis, Parodi et al showed that NPs enveloped by leukocyte membranes have a longer circulation time and greater accumulation in the tumor.¹⁶⁸ In addition, in a separate study, albumin-based NPs coated with macrophage membranes and containing paclitaxel were developed for the therapeutic treatment of melanoma. These NPs localized to the TME, while the presence of macrophage membranes attenuated the clearance of albumin by phagocytic cells and enhanced the efficacy of tumor.¹⁶⁹ A study developed gold nanoshells coated with macrophage cell membranes that showed enhanced localization at the tumor region. When MPCM AuNSs were incubated with 4T1 cells (a BC cell line), more than 80% of MPCM AuNSs were absorbed by the cells, whereas only about 40% of AuNSs were absorbed. In addition, the MPCM-AuNSs showed significantly

improved blood circulation compared with the uncoated AuNSs. The local heat generated by the MPCM AuNSs under irradiation effectively suppresses tumor proliferation and selectively eradicate cancer cells.¹⁷⁰

Macrophage-Derived EVs as New Drug Delivery Platforms

Recently, extracellular vesicles (EVs) derived from the immune system have attracted interest due to their function in the transport of bioactive substances that can influence the properties and activities of target cells.¹⁷¹ Standardized protocols for the isolation, loading, storage, and characterization of EVs derived from macrophages are essential for successful clinical translation. Common methods for EV isolation include differential ultracentrifugation, density gradient ultracentrifugation, size-exclusion chromatography (SEC), ultrafiltration, tangential flow filtration (TFF), polymer-based precipitation, and immunoaffinity isolation.^{172,173} Differential ultracentrifugation is the most widely used technique, while SEC and TFF offer advantages in purity, scalability, and preservation of EV integrity. Passive cargo loading involves exposing donor cells or isolated EVs to therapeutic compounds, whereas active loading methods include electroporation, sonication, extrusion, freeze-thaw cycles, and saponin permeabilization. Storage is crucial for maintaining EV stability and activity. Storage at -80°C reduces EV degradation, but repeated freeze-thaw cycles can compromise membrane integrity and alter cargo profiles.¹⁷⁴ Macrophage-derived EVs offer several biological advantages over liposomes and synthetic NPs. Liposomes and synthetic nanocarriers provide tunable physical properties, high-capacity large-scale production, and established manufacturing platforms; however, their therapeutic potential may be limited by rapid clearance, opsonization, and poor tumor penetration.¹⁰ In contrast, macrophage-derived EVs possess biological characteristics that enable them to interact with the inflammatory microenvironment and deliver therapeutic compounds to tumors. Nevertheless, EV-based technologies have inherent limitations, including heterogeneity, low production yield, complex purification procedures, and a lack of standardized manufacturing protocols.^{175,176}

M1 macrophage-derived EVs have immunomodulatory properties and can trigger a positive immune response. Due to their capacity to transport therapeutic agents to difficult-to-access locations,

including the central nervous system and tumor sites, along with their minimized impact on healthy tissues, M1 macrophage-derived EVs present a promising option for treating a range of conditions, including cancer, autoimmune disorders, neurodegenerative diseases, and infections.¹⁷⁷ A recent study aimed at improving the delivery of PTX and DOX in triple-negative breast cancer (TNBC) utilized macrophage-derived EVs in the tumor microenvironment. PTX and DOX were encapsulated by methods such as passive incubation and sonication or by introducing a chemotherapeutic agent into the cells (only tested for DOX). In vitro, EVs were internalized to a significantly greater extent than liposomes, resulting in a substantial increase in intracellular drug accumulation, which was reported to be 150-fold for DOX and 90-fold for PTX.¹⁷⁸ In a mouse model of TNBC, macrophage-derived EVs showed superior ability to target the TME compared with liposomal delivery systems in response to chemotaxis and through efficient evasion of the immune system. In addition, these EVs showed selective interaction with neoplastic cells and resulted in enhanced therapeutic efficacy of drug-loaded EVs in vivo compared with their free drug counterparts.¹⁷⁸ A study has also shown that macrophage-derived EVs encapsulating PTX have the ability to attenuate the occurrence of multidrug resistance in P-glycoprotein (Pgp)-expressing Madin-Darby canine kidney (MDCK) cells, demonstrating anticancer efficacy of a payload 50-fold greater than that of free drugs.¹⁷⁹ The in vivo efficacy of macrophage-derived EVs in suppressing multidrug resistance was investigated by administering 3LL-M27 cells (which overexpress the multidrug resistance 1 gene, leading to increased Pgp levels) to mouse models of metastatic lung disease. These macrophage-derived EVs exhibited a 97% co-localization rate with lung metastases. Due to this selectivity, administration of PAX-loaded EVs resulted in a significant inhibition of metastatic growth compared with Taxol, culminating in a reduction in metastatic burden of approximately one-third following treatment.¹⁷⁹

Interestingly, the surface architecture of EVs mirrors that of their native parent cells, suggesting that the isolation of EVs from M1-like macrophages may play a critical role in endowing nanovesicles with similar targeting capabilities and inherent tumor-suppressive properties.¹⁸⁰ Zhao et al extracted EVs from M1

macrophages and simultaneously introduced Gem and deferasirox/exjade (an iron chelator that blocks ribonucleotide reductase) into the EVs before examining their efficacy against a GEM-resistant PANC-1 cell line (a human pancreatic cancer cell line).¹⁸¹ In vitro, the EVs loaded with 2 drugs were shown to have a better cytotoxic effect than the combination of free drugs (cell viability rates of 29% and 55%, respectively). Furthermore, hENT1 (Human Equilibrative Nucleoside Transporter 1) exhibited significant activation, and the retention levels of calcein AM was elevated following treatment with dual-loaded EVs (80% in comparison to the positive control Verapamil, a Pgp inhibitor).¹⁸¹

Targeting Macrophage for Cancer Therapy

Recent advances in the understanding of the biological properties, functional dynamics, and heterogeneity of macrophages have led to the credible hypothesis that inhibiting the pro-tumor activities of macrophages while enhancing their anti-tumor properties can lead to remarkable therapeutic results in the context of oncological treatment. Consequently, a variety of strategies have been proposed and tested in various preclinical studies, including modulation of macrophage recruitment to neoplastic sites and reprogramming of M2 macrophages from a pro-tumor to an anti-tumor phenotype. Although preclinical results have been promising, the translation of macrophage therapy to the clinic has only recently begun. Multiple macrophage-targeting approaches are under clinical investigation for both solid and hematologic tumors, including CAR macrophages, CSF1R inhibitors, CD47-SIRPα blockade, and CCR2/CCL2 axis inhibitors. Selected ongoing clinical studies are listed in Table 5.

Macrophage Engineering for Cancer Therapy

Table 5. Ongoing clinical studies of macrophage-targeting approaches

<i>Therapeutic Strategy</i>	<i>Target/Agent</i>	<i>Cancer Type</i>	<i>Clinical Phase</i>	<i>Current Status</i>	<i>Key Findings</i>	<i>ClinicalTrials.gov ID</i>
CAR-Macrophages	CT-0508 (HER2 CAR-M)	HER2-positive solid tumors	Phase I	Completed	Demonstrated acceptable safety and evidence of macrophage persistence with preliminary antitumor activity	NCT04660929
CAR-Macrophages	CT-0525	Solid tumors	Phase I	Ongoing	Safety and preliminary efficacy under evaluation	NCT05631899
CSF1R inhibitor	Pexidartinib	TGCT	Approved / Phase III	Approved	Significant reduction of macrophage infiltration and tumor burden	NCT02371369
CSF1R inhibitor	Cabiralizumab	Pancreatic cancer	Phase II	Completed	Limited efficacy as monotherapy; improved outcomes when combined with nivolumab	NCT03336216
CSF1R inhibitor	Emactuzumab	Solid tumors	Phase I	Completed	Efficient depletion of TAMs but limited objective responses	NCT01494688
CCR2 inhibitor (TAM-targeting)	PF-04136309	Pancreatic cancer	Phase Ib	Completed	Reduced inflammatory monocytes and enhanced chemotherapy response	NCT01413022
CD47-SIRPα blockade	Magrolimab	AML/MDS & solid tumors	Phase III / II	Ongoing	Promotes macrophage-mediated phagocytosis; promising early clinical responses	Multiple trials
CD47-SIRPα blockade	Evorpcept (ALX148)	HER2-positive tumors	Phase II	Ongoing	Enhanced efficacy when combined with trastuzumab	NCT05002127

Inhibition of macrophage recruitment

Given the crucial role of macrophages in promoting cancer growth and metastasis, targeting TAMs may represent a new therapeutic approach. CXCR2 is an important chemokine receptor that controls macrophage recruitment and polarization. Inhibition of the CXCLs/CXCR2 signaling pathway has been explored as an effective strategy in therapies focused on targeting macrophages for cancer treatment. Previous research has shown that CCR2 antibodies in conjunction with polyvalent NPs can significantly inhibit macrophage recruitment and facilitate repolarization of M2 macrophages to the M1 phenotype.¹⁸² In BC, inhibition of CCL2 can effectively sequester monocytes in the bone marrow and significantly reduce metastasis.

However, if CCL2 inhibition is stopped, monocytes can infiltrate the bone marrow and migrate to a tumor. In combination with an increased IL-6 level, this leads to an increased release of VEGF-A and increased angiogenesis, which in turn leads to rapid metastasis.¹⁸³ This observation suggests that when using macrophage recruitment-inhibition strategies by modulating CCL2 or CCR2, the rapid recurrence of metastases after cessation of anti-CCL2 treatment must be taken into account. In addition, the integration of this strategy with nanomultivalent targeting should be further explored to improve the efficacy of macrophage recruitment inhibition via the CCL2-CCR2 axis. The macrophage colony-stimulating factor (M-CSF) and colony-stimulating factor 1 receptor (CSF1R) pathway is

another promising method for modifying macrophage recruitment. Turalio (PLX3397), which inhibits CSF1-CSF1R signaling, reduces macrophage recruitment, reprograms TAMs and stimulates T-cell infiltration in the sarcoma microenvironment.¹⁸⁴ PLX3397, a CSF1R antagonist, was shown to reduce the number of TAMs in a melanoma model, and its combination with CD8⁺ T-mediated immunotherapy significantly delayed the activity of T cells that promote tumor proliferation.¹⁸⁵ Vascular endothelial growth factor receptor 1 (VEGFR-1), which is classified as a tyrosine kinase receptor, is another promoter of macrophage recruitment that facilitates aggregation of TAMs in the TME and enhances tumor proliferation as a result of upregulation of vascular endothelial growth factor A/B (VEGF-A and VEGF-B). VEGFR-1 inhibitors can not only able to reduce macrophage infiltration in neoplasms, but also enhance tumor suppression when co-administered with an immune checkpoint inhibitor (ICI).¹⁸⁶

Macrophage reprogramming

The conversion of M2 macrophages into M1 macrophages is a promising strategy for macrophage engineering in the context of cancer therapy. TLR7, a critical element of Toll-like receptors (TLRs), plays a crucial role in macrophage reprogramming and influences inflammatory pathways via its associated connexin. By administering LET-7-equivalent TLR7 agonists, TLR7 can reduce IL-10 production and reprogram macrophages to alter the TME.¹⁸⁷ In contrast to TLR agonists, STING agonists cannot only modulate FcγR expression in vitro but also reverse the suppressive FcγR spectrum in vivo, which enhances FcγR activation on TAMs and significantly promotes macrophage reprogramming.¹⁸⁸ Inhibition of metabolic pathways associated with M2 macrophages may facilitate macrophage reprogramming given the different metabolic phenotypes of M1 and M2 macrophages. For instance, RIPK3 (Receptor Interacting Serine/Threonine Kinase 3) upregulation in hepatocellular carcinoma tissues may lead to increased production of reactive oxygen species (ROS) and enhanced PPAR (peroxisome proliferator-activated receptor) lysis, effectively inhibiting fatty acid metabolism, reducing the polarization of macrophages toward the M2 phenotype and slowing tumor growth.¹⁸⁹ In addition, the polarization of macrophages can be altered by therapies that affect macrophage RNA. Deletion of DICER1 (Dicer 1, ribonuclease III) in mice has been shown to

increase the proportion of M1 macrophages, thereby improving tumor suppression. A CpG oligodeoxynucleotide (CpG ODN), known as an agonist of Toll-like receptor 9 (TLR9), has the potential to facilitate macrophage repolarization. However, it is susceptible to inflammatory responses in vivo as it cannot effectively penetrate cell membranes. Intravenous administration of CpG ODN encapsulated by the highly acidic nanocellular carrier ferritin in combination with a peptide targeting M2 macrophages significantly prevented tumor progression in mice with tumors and transformed macrophages from the M2 phenotype to the M1 type. Remarkably, this approach has been shown to have the potential to alter the phenotype of human macrophages. These findings support its potential clinical application.¹⁹⁰

Challenges, and Clinical Translation

Despite the promising medical potential of macrophage-based platforms, certain safety issues must be considered before further translation into clinical practice. Unlike small molecules traditionally used in drugs, macrophage-based therapeutics and engineered macrophages are complex biological entities that interact with the immune system. Therefore, thorough evaluation of immunogenicity, biodistribution, manufacturing, and overall safety is crucial for further translation into clinical practice.

Immunogenicity is one of the main safety concerns associated with the use of macrophage-based strategies for disease treatment. Although macrophages are native immune cells with good biocompatibility, donor macrophages or their derivatives can activate the immune response because of differences in HLA expression, surface proteins, or other cellular components. In particular, synthetic receptor expression by engineered macrophages or modifications of their extracellular vesicles may provoke unwanted immune activation and, consequently, reduce therapeutic persistence and promote inflammation.^{191,192}

Thrombogenicity may be an additional risk factor, especially for intravenous cellular therapies and EV-based therapies. Inflammation, coagulation, and platelet activation are regulated by surface proteins on macrophages and EVs. Altered expression of TF/CD142, phosphatidylserine exposure, or inflammation markers may contribute to coagulation activation in specific cases. Thus, comprehensive evaluation of coagulation, platelet activation,

complement activation, and thromboinflammatory effects should be part of the preclinical and clinical testing of therapies.^{193,194}

Accumulation at off-target sites and unintended tissue interactions continue to limit macrophage therapies. Although macrophages have inherent tumor-homing properties, their migration is not exclusive to malignant sites. Engineered macrophages and macrophage-based nanoparticles may accumulate in inflammatory sites, the liver, spleen, or other organs of the reticuloendothelial system, leading to unintended effects. The same applies to EV-based therapies, which may exhibit broad biodistribution profiles based on size, markers, and mode of delivery. Improved targeting through surface modifications, receptor engineering, and the addition of tumor-specific ligands could be employed to enhance selectivity.^{195,196}

Scalability and standardization of manufacturing are major hurdles to translational research. The production of macrophage cells involves complex processes such as isolation, differentiation, genetic engineering, expansion, quality testing, and functional validation. Compared with regular drug development, cell therapy and biological vesicle manufacturing are highly complex and must comply with GMP standards. Limited proliferation capacity of macrophages, donor dependence, and functional instability during manufacturing are major challenges that affect production.¹⁷²

Another significant challenge to translational research is batch-to-batch variation. The biological properties of macrophages and macrophage derivatives vary according to factors such as donors, cell source, differentiation, activation, culture conditions, and the isolation process. For EV therapies, parental cell phenotypes, cargo composition, concentration, and surface markers can affect therapeutic properties. Standardized release criteria such as particle number, particle size profile, molecular markers, potency tests, and functional assays must be defined.^{197,198}

In general, although macrophages-based therapy offers some distinct features such as tumor tropism, immunomodulation, and biological compatibility, addressing the mentioned concerns becomes essential for the efficient application of this approach. Further research should focus on developing effective manufacturing protocols and safety testing methods.

CONCLUSIONS

The clinical use of macrophages as a therapeutic platform will require addressing several manufacturing and regulatory challenges. Standardized processes for isolating, engineering, expanding, and ensuring the quality of macrophages and their products must be developed to guarantee consistency and reproducibility across batches.² The heterogeneity of macrophage populations, donor-to-donor variability, and the absence of standardized potency assays present significant obstacles to large-scale clinical production. Developing GMP-compliant procedures for generating engineered macrophages, nanoparticle-coated macrophage membranes, and macrophage-derived extracellular vesicles will be essential in this context.^{50,199}

First, we highlighted the growing evidence supporting the development of macrophages for drug delivery and targeted cancer therapies. Based on current research, two approaches for macrophage-based cancer treatment were proposed and comprehensively evaluated: 1) using macrophages and their cellular derivatives or mimetics as therapeutic agents and drug delivery systems and 2) counteracting the pro-tumor effects while utilizing the anti-tumor capabilities of macrophages. A deeper understanding of the functions of macrophages in cancer and the development of more efficient macrophage-centred therapeutic strategies would improve the clinical treatment of cancer patients in the future.

Although there is a promising future for the targeting and engineering of macrophages for cancer treatment, numerous concerns regarding cost, safety and reliability remain. Not only is the cost of targeted therapy significantly higher than that of conventional chemotherapy, but the results are also uncertain. In addition, safety, which is foremost among all potential issues, is considered essential, as many patients fear that this targeting and engineering of macrophages could cause other serious adverse effects. Engineering macrophages is primarily concerned with ensuring the sustained efficacy and safety of administering significant quantities of drug-loaded macrophages, which requires rigorous and reliable clinical studies prior to widespread use in the treatment of human tumors. Macrophage membrane mimetics preserve the structural features and biological functions of macrophage cells, allowing them to evade immune defenses, prolong their circulation time and reach target

tissues in a controlled manner through membrane surface proteins. However, these membranes are mainly derived from peripheral blood and macrophage cell lines, the former of which faces challenges such as limited lifespan and variability, while the latter consist of cancer cell lines. Therefore, it is important to establish a consistent and reproducible source of macrophages to improve their further use in cancer treatment. Previous research has shown that macrophage membranes can be produced on a large scale by sequential centrifugation and extrusion processes. To date, there is no standardized method for the production of macrophage membranes, which may hinder their application in the clinic. The yield, integrity and variability of the macrophage membranes produced must be considered. Macrophage-derived EVs could potentially be of greater importance than their membranes, as they can be produced in large quantities for clinical applications according to established protocols. The use of EVs as carriers of drugs or therapeutic agents for cancer treatment has been explored in various clinical trials with promising results. Further research on the efficacy and safety of macrophage-derived EVs in cancer treatment could provide new insights. The integration of light, sound, electricity, magnetism and other responsive nanomaterials also represents a promising approach for the development of a multimodal drug delivery and treatment system.

The complex role of macrophages in cancer underscores the promising prospects of targeting these cells for therapeutic interventions in oncology. Further exploration of the underlying mechanisms controlling both anti-tumor and pro-tumor functions of macrophages is essential for the identification of novel therapeutic targets in tumor treatment. The integration of existing therapeutic modalities with macrophage-targeted therapies could significantly improve the efficacy of tumor treatment strategies. Targeted inhibition of specific macrophage subsets has been proposed as a viable approach for cellular therapy. While previous research indicates that depletion of macrophages or disruption of their chemotactic processes leads to significant attenuation of tumor progression in mouse models, these methods have not yet proven viable or successful in the treatment of human malignancies. Given the abundance of macrophages and their capacity to amplify their own activation and recruitment, strategies aimed at

leveraging their potential to act as anti-tumor effector cells warrant considerable attention. Although there are promising results, therapies targeting macrophages may face challenges due to the considerable heterogeneity of these complex subpopulations. Therefore, it is critical to thoroughly dissect the diversity of macrophage differentiation in different tumor environments at the single cell level while identifying the distinct markers and functions associated with different macrophage subsets to enable macrophage repolarization targeting and improve the precision of macrophage-targeted therapies. Further efforts are needed to improve macrophage-centered therapeutic strategies to facilitate their transition into clinical use. Once the previously described obstacles are overcome, macrophage engineering and targeting could offer innovative approaches for cancer treatment.

Naïve monocytes/macrophages can differentiate into either pro-inflammatory M1-like macrophages with anti-tumor potential or immunosuppressive M2-like tumor associated macrophages (TAMs), depending on specific environmental stimuli and cytokines. M1-like macrophages show an increased capacity for antigen presentation, production of inflammatory cytokines, generation of reactive oxygen/nitrogen species and cytotoxicity toward tumor cells, while M2-like TAMs stimulate tumor progression through their immunosuppressive, angiogenic, extracellular matrix remodeling, invasive and metastatic activities.

In TME, the M2-like TAM polarization and functional activity depend on sophisticated crosstalk between TAMs and tumor cells, cancer associated fibroblasts (CAFs), regulatory T-cells (Tregs), myeloid derived suppressive cells (MDSCs), ECM molecules, hypoxia, changes in cellular metabolism, as well as soluble factors such as IL-10, TGF- β , CCL2 and CSF1. The major therapeutic intervention points targeting macrophages, including inhibition of macrophage recruitment (CCR2/CCL2 and CSF1R blockade), depletion of tumor-promoting macrophages, blockade of pro-tumor signaling pathways, macrophage repolarization toward an anti-tumor phenotype using TLR, STING, or CD40 agonists, metabolic reprogramming, and enhancement of macrophage-mediated anti-tumor responses through cytokine, nucleic acid, or cellular engineering approaches.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY

No new datasets were generated or analyzed for this study. The findings and information discussed in this manuscript are based on previously published literature, which is cited appropriately throughout the manuscript.

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The authors used an artificial intelligence (AI) tool solely for language editing and improving the clarity and readability of the manuscript.

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