Review Article

A narrative review of tumor-associated macrophages in lung cancer: regulation of macrophage polarization and therapeutic implications

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Abstract: Lung cancer is the deadliest malignancy worldwide. An inflammatory microenvironment is a key factor contributing to lung tumor progression. Tumor-Associated Macrophages (TAMs) are prominent components of the cancer immune microenvironment with diverse supportive and inhibitory effects on growth, progression, and metastasis of lung tumors. Two main macrophage phenotypes with different functions have been identified. They include inflammatory or classically activated (M1) and anti-inflammatory or alternatively activated (M2) macrophages. The contrasting functions of TAMs in relation to lung neoplasm progression stem from the presence of TAMs with varying tumor-promoting or anti-tumor activities. This wide spectrum of functions is governed by a network of cytokines and chemokines, cell-cell interactions, and signaling pathways. TAMs are promising therapeutic targets for non-small cell lung cancer (NSCLC) treatment. There are several strategies for TAM targeting and utilizing them for therapeutic purposes including limiting monocyte recruitment and localization through various pathways such as CCL2-CCR2, CSF1-CSF1R, and CXCL12-CXCR4, targeting the activation of TAMs, genetic and epigenetic reprogramming of TAMs to antitumor phenotype, and utilizing TAMs as the carrier for anti-cancer drugs. In this review, we will outline the role of macrophages in the lung cancer initiation and progression, pathways regulating their function in lung cancer microenvironment as well as the role of these immune cells in the development of future therapeutic strategies.

Keywords: Macrophages; lung neoplasms; macrophage activation; immunotherapy

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Introduction

Lung cancer is the primary and the secondary cause of cancer-related death for men and women worldwide, respectively (1). It is categorized into two main types, including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), the latter type accounts for about 80–85% of all cases of lung cancer (2). More than 55% of all patients with NSCLC are diagnosed at advanced

stages of the disease (3). Available treatment options for NSCLC are surgical resection of the primary tumor or metastatic lesion, radiation therapy, and systemic therapies. Conventional chemotherapies, drugs targeting commonly mutated pathways in lung cancer, and immune checkpoint inhibitors are our current armamentarium of systemic therapies for NSCLC (4). Despite promising advances in the systemic management of lung cancer, 5-year survival rates for localized, regional, and metastatic lung cancer are 57.4%, 30.8%, and 5.2%, respectively (5). Cancer progression during or after treatment with systemic therapies are the major cause of death in NSCLC. Hence, resistance to systemic therapies is considered a serious obstacle in the therapy of NSCLC. Therefore, finding new therapeutic strategies can improve the prognosis of patients with NSCLC (6).

The functional association between inflammation and cancer is extensively investigated. The link between inflammation and cancer dates back to the 19th century when Rudolf Virchow for the first time proved the presence of leukocytes within tumors (7). Subsequently, it has been revealed that cancer-related inflammation can impact all stages of tumorigenesis, including initiation, promotion, angiogenesis, and metastasis of established tumors (8,9). Likewise, lung cancer is one of the cancers associated with inflammation. Immune responses mediate the effect of environmental exposures on lung cancer initiation, control the invasion and metastasis of lung tumor, and are therapeutic targets for lung cancer treatment (10).

The tumor microenvironment is composed of tumor cells, innate and adaptive immune cells, fibroblasts, vascular and lymphatic endothelial cells, extracellular matrix, growth factors, cytokines, chemokines, hormones, and proteases among others (6,11). Interplays between tumor cells and immune cells in the tumor microenvironment, e.g., through production and secretion of tumor-regulating cytokines by immune cells, control tumor cell survival, and metastasis (12). Innate immune cells, particularly natural killer cells (NK cells) and macrophages exert a great impact on cancer and play a pivotal pattern in the regulation of tumor progression and inhibition (13).

Macrophages within the tumor microenvironment are referred to as tumor-associated macrophages (TAMs). In the NSCLC microenvironment, TAMs constitute the predominant cellular component. They not only act as immunosuppressive cells enabling immune evasion of NSCLC but also directly contribute to cancer cell proliferation, survival, invasion, and metastasis (14). Two main macrophage phenotypes with different functions have been identified. They include inflammatory or classically activated (M1) and anti-inflammatory or alternatively activated (M2) macrophages (15). M1 macrophages usually arise in the setting of inflammatory surroundings that are usually induced by type 1 T helper (Th1) cytokines such as interferon gamma (IFN-γ), Toll-Like Receptor (TLR) agonists like lipopolysaccharide (LPS), and Granulocyte-Monocyte Colony-Stimulating Factor (GM-CSF) (16,17). This type of macrophage secretes higher levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1α/β, IL-6, IL-12, and IL-23, lower levels of IL-10 than M2 macrophages and produces inducible nitric oxide synthase (iNOS). The phosphorylated form of STAT1 as a transcription factor, CD80, CD86, and CD64 are some common M1 biomarkers (16,18-20). M1 macrophages regulate a potent immune response against pro-inflammatory situations and microbial activity. M2 macrophages are found in environments associated with type 2 T helper (Th2) cytokines. M2 phenotype consists of four well-defined subtypes including M2a induced by IL-4 and IL-13; M2b induced by immune complexes and IL-1 receptor agonists; M2c induced by IL-10, Transforming Growth Factor-Beta (TGF-β), and glucocorticoids; and M2d induced by IL-6, Leukemia Inhibitory Factor (LIF) and adenosine. Although M2 macrophage subtypes have anti-inflammatory and immunoregulatory roles in common, they also exhibit different functions. For example, M2a subtype is involved in tissue fibrosis, M2b subtype contributes to tumor progression, M2c subtype is responsible for tissue remodeling, and M2d subtype promotes angiogenesis (21). Some common markers for M2 macrophages are arginase, CD206, CD204, and CD163. M2 macrophages are well-adapted to inflammatory response inhibitors and tumor progression (17,21) (*Figure 1*).

Density, phenotype, and microanatomical localization of macrophages are considered efficient biological parameters for patient survival prediction (22). The increasing number of evidence indicates that M2 TAM density has a strong correlation with advanced tumor progression (23-25). Since M2 TAMs act as pro-tumoral macrophages and contribute to lung cancer progression (26), they are beginning to be assessed as promising therapeutic targets for lung cancer treatment. Strategies for TAM targeting and utilizing them for therapeutic purposes include limiting monocyte recruitment and localization, targeting the activation of TAMs, reprogramming TAMs to antitumor phenotype, and

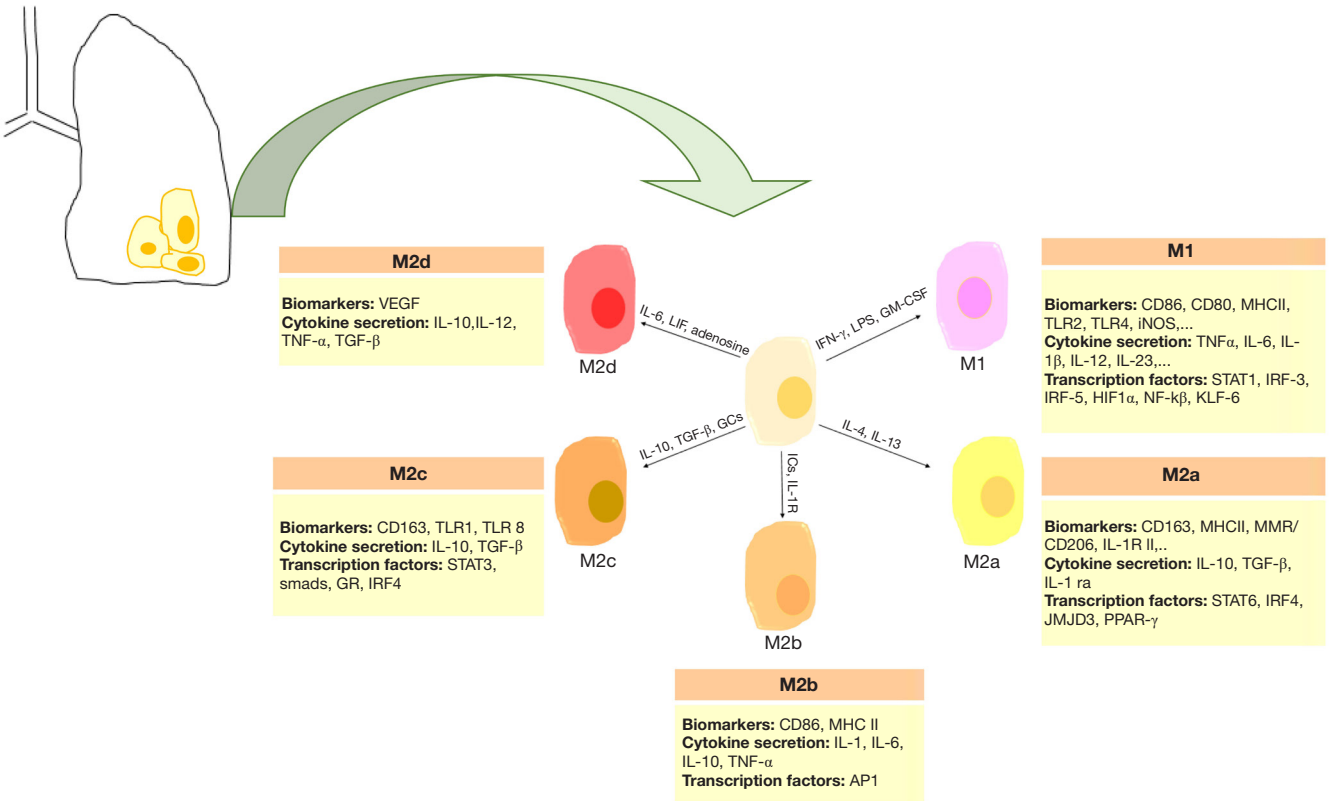

Figure 1 Pulmonary macrophage polarization. Various induction signals polarize pulmonary macrophages into M1 or M2 phenotype. AP1, activator protein 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; GR, glucocorticoid receptor; HIF1α, hypoxia-inducible factor alpha; ICs, immune complexes; IL, interleukin; IL-1R, interleukin-1 receptor; IL-1Ra, interleukin-1 receptor antagonist; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; JMJD3, Jumonji domain containing 3; KLF, kruppel-like factor; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MMR, macrophage mannose receptor; NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; STAT, signal transducer and activator of transcription; TGF, tissue growth factor; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

utilizing TAMs as the carrier for anti-cancer drugs (27). A detailed assessment of TAMs and pathways by which TAMs are involved in lung cancer progression can shed some light on designing novel drugs for lung cancer with a focus on TAMs.

In this review, we aim to describe pathways involved in macrophage polarization and discuss hopeful researches in TAM targeting strategies with a focus on lung cancer that leads to suppression of cancer progression and metastasis and improves patient prognosis. To obtain relevant literature, we searched Medline (via PubMed) using keywords "lung cancer", "macrophage", and "TAM" from the inception of the database to March 2020. Our search was restricted to publications in English language. We retrieved other eligible studies by manual searching of the reference lists of included studies.

We present the study in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-1241).

TAMs and lung tumor growth

Macrophages are involved in all stages of tumor progression. From the early pre-invasion stage, tumor cells attract macrophages and other inflammatory cells into the tumor stroma through the release of cytokines and exosomes (28), and in this place, macrophage directly evokes the tumor growth, migration, and metastasis (29).

Despite the fact that alveolar macrophages are increased and constitute the main cellular component in the bronchoalveolar lavage fluid of lung cancer patients (30,31), they may show diminished phagocytic abilities (32) and reduced expression of markers characteristic of M1 macrophages such as HLA-DR, CD83, and ICAM-1 (32,33). In addition, alveolar macrophages in lung cancer patients secrete lower levels of inflammatory cytokines such as IL-1 and TNF-α (32).

M2 TAMs induce invasion and progression of tumor cells, which lead to poor prognosis in NSCLC patients (34,35). TAMs release some molecules such as matrix metalloproteinases (MMPs), growth factors, cytokines, chemokines, and other inflammatory mediators to create an inflammatory environment and drive tumor growth (36-38). Many of these molecules, like vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and IL-10 are related to lung cancer progression and metastasis. VEGF-C not only affects tumor migration and angiogenesis but also promotes tumor progression and development through autocrine and/or paracrine pathways (39). Macrophages enhanced the expression of IL-8 in A549, CL1-0, CL1-5, and PC14 lung cancer cell lines. There was a certain correlation between macrophage infiltration density, and intra-tumor microvessels, and also a negative correlation with the survival of patients with NSCLC (40). Studies in lung cancer demonstrated that during tumor formation, TAMs expressed M1 markers (41), and macrophage phenotype switching from M1 into M2 occurred during lung cancer progression (41,42). Yuan and colleagues revealed that M2a and M2c raised invasion and xenograft tumor growth of A549 cells, whereas M1 macrophages significantly decreased the expression of fibrinogens and TGF-β, which support tumor progression in the cancer cells (43). Some studies have shown that upregulation of IL-10 in M2 TAMs in NSCLC and other malignancies are associated with poor prognosis, and overexpression of IL-10 in TAMs showed a positive correlation with the late stage of NSCLC (44-46). IL-10 provides tumor cells an immunosuppressive milieu by interacting with regulatory T lymphocytes (47). In addition, IL-10 confers resistance to apoptosis in Lewis lung carcinoma (LLC) cells (48).

Several reports of the association between macrophage density in lung tumor and patients' outcomes in terms of survival produce contradicting results (49). These conflicting results may stem from the different roles of macrophage phenotypic subtypes, the micro-distribution of TAMs in tumors of lung cancer patients, variability in the characterization methods of macrophage subtypes, insufficient statistical power, or using a particular tumor stage (50-52). A number of studies have shown that M2-polarized macrophage density is correlated with worse survival in lung cancer (53-56). Meanwhile, M1-polarized macrophage density is associated with better overall survival (49). The micro-distribution of TAMs in the tumor niche influences lung cancer progression. A few reports showed that the high density of $CD68^+$ cells and $CD68^+HLA\text{-}DR^+$ M1 TAMs in lung cancer tumor islets was associated with better overall survival, although $CD68^+CD163^+$ M2 density in tumor islets was not connected with overall survival. High stromal $CD68^+$ TAMs or $CD204^+$M2 TAMs were associated with poor overall survival and advanced lymph node stages, while the density of stromal $CD68^+HLA\text{-}DR^+$ M1 and $CD68^+CD163^+$ M2 TAM was irrelevant to overall survival. A low fraction of islet to stromal $CD68^+$ TAM displayed poor overall survival and showed that the localization of TAMs could be a prognostic predictor in NSCLC (49,51,57). The most common molecular marker used to recognize TAMs is the CD68 antibody. However, this surface molecule is not only expressed by TAMs but also by other components of tumor tissue such as malignant epithelial and stromal cells (58). In addition, labeling of macrophages just based on CD68 cannot discriminate between M1 and M2 subtypes (59).

Introduction to main pathways involved in macrophage polarization in cancers and take advantage of them in lung cancer researches

Since macrophages encounter various signals in the cellular microenvironment, the molecular mechanisms behind the macrophage polarization have not been fully characterized yet (60,61). However, in the most recent decade, there has been great progress in demystifying macrophage polarization under physiological and pathological conditions such as cancer. Macrophage polarization is a highly plastic process (62,63). The local cytokine microenvironment control macrophage polarization. Th1 cytokines, such as IFN-γ and TNF-α, drive macrophages to M1 phenotype, that produce pro-inflammatory cytokines including TNF-α, IL-6, IL-12, IL-1α, and IL-1β, through which remove pathogens during infection (64). Th2 cytokines, including IL-4 and IL-13, induces macrophage M2 polarization. This is mediated by STAT6 induction via IL-4 receptor alpha activation (64). So, cytokines are considered pivotal

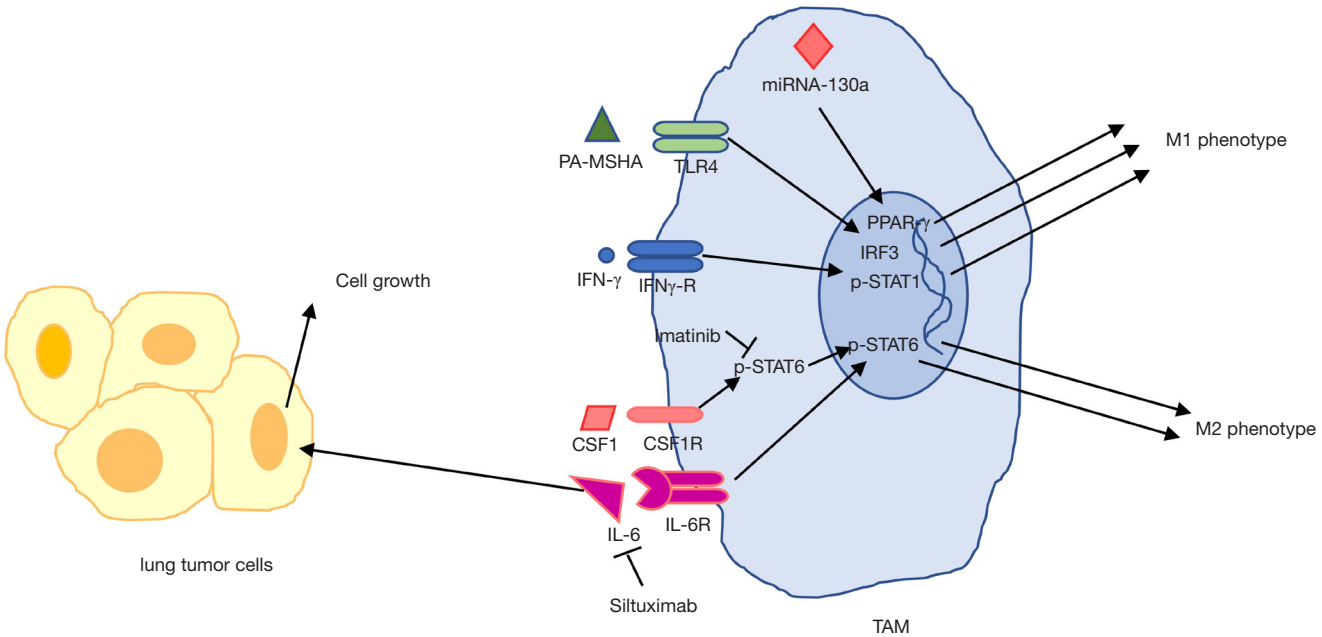

Figure 2 TAM targeting in lung cancer. Induction of TLR4 and IFN-γ receptor by their agonists skewed TAMs into anti-tumoral M1 phenotype. miRNA-130a by targeting PPAR-γ transcription factor regulates M1-related genes expression in TAMs in lung cancer. Targeting CSF1 and IL-6 by specific inhibitors prevents M2-polarized TAMs and suppresses lung cancer. Siltuximab by inhibition of STAT3 signaling prevents lung tumor growth. CSF, colony stimulating factor; IRF, interferon regulatory factor; PA-MSHA, pseudomonas aeruginosa-mannose-sensitive hemagglutinin; PPAR-γ, peroxisome proliferator-activated receptor gamma; STAT, signal transducer and activator of transcription.

contributors to macrophage polarization (*Figure 1*). Furthermore, macrophage switching is connected to the differential expression of diverse Toll-Like Receptors (TLRs) on macrophages (65).

IFN-γ

M1 macrophages augment tumor regression, while M2 macrophages promote tumor progression (66). IFN-γ, previously known as macrophage-activating factor (MAF), leads to polarization of TAMs from M2 toward M1 phenotype in the tumor niche. IFN-γ by upregulation of cytotoxicity-associated markers like NKG2D and granzyme A/B (67), is identified as a potent agent responsible for inducing macrophage tumoricidal activity (68). IFN-γ via its receptor activates STAT1, then phosphorylated STAT1 induces transcription of IFN-γ-related genes such as genes involving in M1 phenotype (69). Activated macrophages secrete IL-12 (70,71). IL-12 evokes NK and Th1 cells to produce IFN-γ, while IFN-γ itself induces the production of IL-12 (72). It is reported that IL-12 overexpression can reverse M2 macrophage to M1 (73). In lung cancer patients, plasma IFN-γ levels were considerably dropped (74). TAMs in IFN-$\gamma^{-/-}$ mice polarized into M2 phenotype, and those mice developed larger lung tumors than those in control mice (75). Several studies have shown the beneficial effects of IFN-γ in lung cancer inhibition. Low levels of this cytokine can induce tumor cell stemness through the ICAM1-PI3K-Akt-Notch1 pathway, whereas a high level of IFN-γ induced apoptosis in NSCLC via the JAK1-STAT1-caspase pathway (76). Recombinant IFN-γ enhanced the cytotoxic effects of TAMs through TNF-α and NO in lung cancer patients (77). A study by Ren and colleagues indicated that IFN-γ and/or celecoxib can modulate M2/M1 macrophage ratio in the tumor microenvironment that may prevent lung tumor growth (26). This study revealed that IFN-γ via p-STAT1 activates transcription of M1-related genes (26). In another study, it was concluded that IFN-γ, in combination with TLR agonists like LPS activates the M1 phenotype to inhibit LLC cell growth (78) (*Figure 2*). These data suggest that IFN-γ can reprogram TAMs and switch them toward

the M1 subtype, which improves tumor elimination. So, this protein can play a fundamental role in host antitumor immunity (69).

Interleukin-6

M2-polarized macrophages in lung cancer can secrete some interleukins such as IL-6 (79,80), IL-8 (80), and IL-10 (81) to promote tumorigenesis and metastasis. IL-6 is reported as a cytokine which primes macrophages for M2 polarization through stimulating IL-4 receptor expression (82). IL-4 receptor activates STAT6, even though other STATs may also be involved (83) (*Figure 2*). In another hand, TAM-derived IL-6 promoted mouse lung tumor through activation of STAT3 (84). Anti-IL-6 receptor monoclonal antibody abrogated the ability of triple-negative breast cancer cell (MCF-10A) to induce M2 phenotype in macrophages (85). Targeting IL-6 and IL-6 receptor using monoclonal antibodies Siltuximab and Tocilizumab in a K-ras mutant mouse model for lung cancer suppressed cancer progression via inhibition of STAT3 tyrosine phosphorylation (86,87) and polarized macrophages towards an anti-tumor phenotype (87). A study showed that IL-6 via STAT6 signaling polarized macrophages towards IL-4-dependent M2 phenotype by IL-4 receptor overexpression (82). Although the mechanism of Siltuximab action in TAM repolarization in lung cancer was not elucidated, it may work through blocking a similar mechanism. A phase I clinical trial showed acceptable safety profile of Tocilizumab in combination with chemotherapy and IFN-α2b in recurrent epithelial ovarian cancer (88). A small phase II randomized clinical trial on patients with high-risk smoldering multiple myeloma showed moderately increased progression-free survival with Siltuximab treatment (43 patients) in comparison with placebo (42 patients) (89). Several antibodies against IL-6 in lung cancer showed beneficial effects in lung cancer patients, although their roles in the re-education of TAMs have not been examined. ALD518, a humanized anti-IL-6 antibody, was examined in a randomized phase II clinical trial for NSCLC and showed preliminary evidence of efficacy for this population (90).

Myeloid colony-stimulating factors

Granulocyte-macrophage colony-stimulating factor (GM-CSF), also called CSF2 and Macrophage Colony-Stimulating Factor (M-CSF), also called CSF1 are known cytokines involved in the regulation of macrophage polarization. The major function of myeloid colony-stimulating factors is regulating the proliferation and differentiation of committed hematopoietic cells. GM-CSF is linked to M1 phenotype activation, and M-CSF is associated with M2 macrophage phenotype differentiation (91). Interaction of M-CSF with its cognate receptor CSF-1R leads to upregulation of PLC-γ2, STAT3, and Erk1/2 (92). Induction of GM-CSF receptor recruits JAK2 and leads to STAT5, ERK, AKT, NF-κB, and IRF5 activation (93). Many of these regulator molecules are also part of TLR and IFN-γ signaling pathways. GM-CSF induces IL-6, IL-8, M-CSF, G-CSF, TNF, and IL-1β in macrophages and monocytes (91). Although M-CSF and GM-CSF receptors share some common signaling pathways, they result in different transcriptional regulation and ultimate functional changes. For instance, both M-CSF and GM-CSF activate Ras/MAPK pathway in macrophages to upregulate the expression of scavenger receptor (SR)-A; However, M-CSF-mediated upregulation of SR-A is caused by binding of AP-1 to the enhancer of SR-A gene but GM-CSF increases the expression of SR-A through a different enhancer region (94). While IRF5 is implicated in GM-CSF-induced M1 macrophage phenotype, IRF4 orchestrates M2 macrophage polarization by M-CSF (95). A few studies reported high serum levels of M-CSF could be a biomarker in NSCLC patients (96,97). A study showed that TAMs with high expression of CSF1R were associated with a high death rate in lung cancer patients (98).

Both cytokines can be potent therapeutic targets for manipulation in human diseases such as cancer. A study demonstrated that Imatinib, by targeting CSF1R, prevented M2 polarization of TAMs in primary lung tumor or metastatic sites via inhibition of STAT6 phosphorylation (99) (*Figure 2*). Recombinant GM-CSF therapy promotes the anti-tumor activity of TAMs in lung cancer patients (100). CSF1R inhibitors may deplete M2 TAMs in a tumor microenvironment or modulate M1/M2 TAM ratio (101) (*Figure 2*). Several clinical trials are testing different classes of CSF-1R targeting agents in combination with cancer immunotherapy agents in various cancers such as NSCLC. Pexidartinib (NCT02452424) (102), classified as a small molecule kinase inhibitor, and ARRY-382 (NCT02880371) and Emactuzumab (NCT02323191), monoclonal antibodies (101), have been used for targeting CSF1R in NSCLC.

TLRs

TLRs are a class of pattern recognition receptors, which detect molecules of invading pathogens and are pivotal for innate immunity and inflammatory responses (103). Upon activation of TLRs, they transmit a signal through adaptor molecules and downstream mediators to regulate gene expression and induce pro-inflammatory responses (104). Alterations in the metabolic profile of macrophages alter their function and activation state. It is suggested that metabolic reprogramming can be induced via TLRs and influences activation, maturation, and immunogenicity of macrophages (105). Signal transduction through TLR1/2/4 in macrophages leads to mitochondrial Reactive Oxygen Species (ROS) production, which enhances the bactericidal function of macrophages (106).

It has been revealed that TLR4/5/7/8 and 9 are overexpressed in NSCLC than in normal lung tissue (107-110). TLR4 is activated by bacterial LPS and cationic polymers. TLR4 agonists through activation of Activator Protein-1 (AP-1) and interferon regulatory factor-3 (IRF-3) cascade, trigger activation of M1 phenotype-associated genes (111). It was shown that TLR4 signaling via NF-κB in TAMs is implicated in tumor promotion in experimental lung metastasis model (112). TLR2 is an important receptor for M2b phenotype polarization *in vitro* (113). Lewis lung carcinoma cell line-conditioned medium stimulates TNF-α production by macrophages through TLR2 activation, and in consequence, it promotes lung cancer progression (114). The overexpression of TLR7 is highly associated with tumor progression, poor clinical outcomes, and chemotherapy resistance in NSCLC (110). It has been suggested that TLR7 acts as a mediator of switching to M2 macrophage phenotype (65). Other TLR inducers like lipoteichoic acid (LTA) (115), R848 (116), and CpG (117) have potency for induction of antitumor M1 macrophages.

TLR signaling cascade regulates pro-inflammatory gene expression through activation of STAT1 and NF-κB (118). STAT1 has been proved to be a crucial regulator of biological responses of various TLRs. TLR/NF-κB axis is a vital signaling pathway in the regulation of macrophage polarization (119). In most cases, TLR/NF-κB activation promotes M1 macrophage phenotype. However, this depends on NF-κB subunit composition. For instance, NF-κB p65/p50 heterodimer boosts pro-inflammatory cytokines, and M1 macrophages are generated (120) while p50/p50 homodimer induces M2 macrophage phenotype (61).

Pseudomonas aeruginosa-mannose-sensitive hemagglutinin (PA-MSHA) through TLR4 activation re-educated M2 macrophage towards M1 in the malignant pleural effusion of lung cancer, and TLR4 blocking prevented this effect (121). Some studies confirmed the influence of PA-MSHA for advanced lung cancer treatment (111,121,122) (*Figure 2*). SPC-A1 NSCLC cell line in co-culture with TAMs overexpressed TLR1/6/7, and pretreatment with agonist ligands for these receptors led to induction of IL-1β, IL-8, and IL-6, which supported the inflammatory microenvironment and promoted the development and progression of lung cancer (116). The combination of Motolimod (a TLR8 agonist) with PEGylated liposomal Doxorubicin did not prolong overall survival or progression-free survival of recurrent ovarian, fallopian tube, or primary peritoneal carcinoma over placebo and PEGylated liposomal Doxorubicin in a phase II randomized clinical trial (123). EMD1201081 (a TLR9 agonist) and Cetuximab did not have superior efficacy than Cetuximab monotherapy in patients with recurrent or metastatic squamous cell carcinoma of head and neck revealed by a phase II randomized clinical trial (124). A phase I dose-finding trial enrolling previously-treated NSCLC patients showed acceptable safety and moderate overall response (15%) of IMO-2055 (a TLR9 agonist) with Erlotinib and Bevacizumab (125). Although a few researches have been performed in the manipulation of TLR pathways of macrophages in lung cancer for therapeutic aims, this pathway could open novel avenues for finding new approaches in the suppression of lung cancer.

PD-1/PD-L1 immune checkpoints

Programmed cell death protein 1 (PD-1)/PD-1 ligand (PDL) pathway plays an important part in establishing an immunosuppressive environment in the tumor and the evasion of cancer cells from anti-cancer immune responses. Of relevant clinical importance, PD-1/PD-L1 inhibition using blocking antibodies is being used for the treatment of many cancer types including melanoma, renal cell carcinoma, NSCLC, SCLC, squamous cell skin cancer, and triple-negative breast cancer (126). Tumor-infiltrating macrophages may express both PD-1 and PD-L1 (127). The anti-tumor effects of PD-1 and PD-L1 blocking antibodies were reversed following TAM depletion by anti-CSF1R antibody in a mouse model of colon cancer (CT26 cell line) (128); Hence, TAMs are significantly involved in the immunosuppressive potentials of PD-1/PD-L1

pathway in cancerous microenvironment. The majority of PD-1$^+$ tumor macrophages display M2 phenotype. PD-1$^+$ macrophages show defective phagocytic function in comparison with PD-1$^-$ macrophages (128). Interferon-sensitive responsive element (ISRE), STAT1, and STAT2 are pivotal for PD-1 expression in TAM (129).

TAMs are the most abundant PD-L1$^+$ immune cells in human NSCLC stroma (130). While PD-L1 is mostly recognized for its inhibitory effects on T cells through interacting with PD-1 (126), it seems that the main function of PD-L1 in TAM is skewing macrophage polarization towards an immunosuppressive state. There is evidence that PD-L1 affects TAM function through downregulating Akt/mTOR pathway (131). Additionally, TAMs that cross-present cancer antigen can evade immune destruction by T cells in a PD-L1 dependent manner (132). Pyruvate Kinase (PK) M2 isoform and Secreted Phosphoprotein 1 (SPP1) are the intracellular regulators of PD-L1 expression in TAM (133,134). To sum up, PD-1 and PD-L1 proteins are involved in regulating macrophage polarization.

PD-1/PD-L1 pathway has clinical implications in NSCLC. PD-L1 expression, both in cancer cells and TAMs, was inversely associated with survival in early-stage NSCLC (135). The high density of PD-L1$^+$ TAM in tumor tissue predicted better survival in NSCLC patients who were treated with PD-1/PD-L1 inhibitors (130). PD-1/PD-L1 immune checkpoint inhibitors such as Pembrolizumab, Atezolizumab, and Nivolumab, either as single-agent or in combination with chemotherapy, have shown clinical efficacy in PD-L1$^+$ NSCLC (136-139). In conclusion, clinical application of PD-1/PD-L1 pathway has come of age for NSCLC.

Role of miRNAs in macrophage polarization:

Micro RNAs (miRNAs) are small non-coding RNAs that are able to regulate gene expression by binding to target messenger RNAs (mRNAs) that either lead to their decay or translation inhibition (140). miRNAs have long been known for their different roles in development, differentiation, and homeostasis (141). A great body of studies has suggested the involvement of miRNAs in the macrophage polarization in human and murine-derived macrophages (142,143). Functional miRNAs that are involved in polarizing macrophages have been identified (144). Namely, miRNA-125b decreases the level of IRF4 by targeting it. IRF4 is a key signaling molecule for the induction of M2 phenotype. In line, miRNA-125b overexpression in macrophages potentiates their antigen-presentation capabilities, priming of T cells, and cancer cell killing (145). The overexpression of miRNA-720 reprograms TAMs toward M1 phenotype by targeting GATA3 (146) (*Table 1*).

Neutrophils of high-risk heavy smokers secrets circulating miRNA-320a that through downregulation of STAT4 in macrophages promoted M2-like macrophage in lung cancer (180). It has been reported that miRNA-130a repolarized M2 macrophage towards M1 phenotype and had a prognostic role in NSCLC. Downregulation of miRNA-130a was correlated with poor overall survival, tumor progression, and metastasis in NSCLC patients (158) (*Figure 2*). Jingushi and colleagues reported that upregulation of miRNA-130b in NSCLC tissue specimens was associated with poor overall survival in patients suffering from NSCLC (181). miRNA-155 expression showed a prognosis role in patients with NSCLC and digestive system carcinomas (182). According to the link between miRNAs, immunosurveillance, and cancer progression, miRNA targeting could promote the development of new therapeutic strategies or diagnostic and prognostic tools in NSCLC.

Role of extracellular vesicles in macrophage polarization

Extracellular vesicles are membrane-bound structures that originate from cells and can be endocytosed by other cells. These vesicles contain macromolecules such as DNA, RNA, and proteins and the transfer of macromolecules between cells by the means of extracellular vesicles provides an intricate mode of intercellular communication and tissue and systems-level homeostasis. Intriguingly, neoplastic cells utilize extracellular vesicles for regulating the functions of other malignant cells, tumor microenvironmental cells, cells related to hematopoiesis, and distant organ niche. The main categories of animal extracellular vesicles are exosomes (30–120 nm), microvesicles (100–1,000 nm), and apoptotic bodies (800–5,000 nm) (183). Cancer cell-derived extracellular vesicles can promote M2 macrophage polarization. Non-coding RNAs including miRNA-103a, miRNA-25-3p, miRNA-130b-3p, miRNA-425-5p, and miRNA-301a-3p were present in lung cancer cell (CL1-5, NCI-42087, H1792, and H1437), colon cancer cell (HCT116), and pancreatic cancer cell (PANC1)-derived extracellular vesicles. These miRNAs targeted and decreased the level of PTEN which resulted in M2 macrophage polarization through STAT3 activation (184-186). Exosomes acquired from hypoxic epithelial

Table 1 Functional miRNAs regulate macrophage polarization

miRNAs	Promoted phenotype	Target(s)	Reference(s)
miRNA-155	M1	IL13Rα1, TLR4/IL-1R, C/EBP-β	(147-149)
miRNA-27a	M1	PPAR-γ	(150)
miRNA-27b	M1	PPAR-γ	(151)
miRNA-125a	M1	IRF4	(152)
miRNA-125b	M1	IRF4	(145)
miRNA-21	M1	STAT3	(153)
miRNA-127	M1	Bcl-6, Dusp1, JNK	(154)
miRNA-720	M1	GATA3	(146)
miRNA-223	M1	Pknox1	(155)
miRNA-26a	M1	KLF4	(156)
miRNA-9	M1	PPAR-δ	(157)
miRNA-125b	M1	IRF4	(145)
miRNA-130a	M1	PPAR-γ	(158)
miRNA-130b	M1	PPAR-γ	(159)
miRNA-125a	M2	KLF-13 (in mice)	(160)
miRNA-34a	M2	Notch1	(161)
miRNA-146a	M2	INHBA, TLR4/IRF3, IRAK1, TRAF6	(162-165)
miRNA-146b	M2		(166)
miRNA-223	M2	PBX/Knotted1 Homeobox 1 (Pknox1), STAT3	(167)
miRNA-210	M2	NF-κB, DR6	(168,169)
miRNA-33	M2	AMPK	(170)
miRNA-222	M2	STAT3	(171)
miRNA-127	M2	DUSP1	(172)
miRNA-132	M2	AchE	(173)
miRNA-124	M2	C/EBP-α, STAT3, TACE	(174,175)
miRNA-145	M2	IL10 gene silencer histone deacetylase 11	(176)
miRNA-93	M2	IRF9	(177)
miRNA-21	M2	SIRPb1	(178)
Let-7c	M2	C/EBP-δ, PAK1	(162,172)
miRNA-181a	M2	KLF6, C/EBP-α	(179)

ovarian cancer cells (SKOV3) harbored miRNA-21-3p, miRNA-125b-5p, miRNA-181d-5p, and miRNA-222-3p which degraded SOCS in macrophages and subsequently increased phosphorylated STAT3, a process which culminated in M2 macrophage polarization (171,187). In addition, STAT6 upregulation mediated by cGAS/STING pathway is reported to be involved in cancer cell-derived extracellular vesicle-induced M2 macrophage polarization (188). Exosomes derived from DLD-1 colon cancer cell line contained miRNA-145 which polarizes

TAMs towards M2 phenotypes by downregulating histone deacetylase 11 (189).

In a study by Chen *et al.*, extracellular vesicles derived from lung cancer cell lines (HCC827, LLC, A549, and H460) were taken up by macrophages and promoted M2-like phenotype in TAMs. These TAMs produced IL-1β which enhanced lung cancer cell stemness and survival. It was revealed that lung cancer cell-derived microparticles contained non-coding RNAs that could stimulate TLR3 activation which subsequently increased pro-IL-1β expression in a NF-κB and MAPK-dependent pathway. Simultaneously, extracellular vesicles uptake by macrophages increased cytosolic calcium concentration and mitochondrial ROS production. ROS activated NLRP3 inflammosome which is required for IL-1β activation (190). In another study, the E3 Ubiquitin ligase TRIM59 was delivered to macrophages by lung cancer cell line (H1299 and A549)-derived exosomes. TRIM59 promoted ubiquitination and proteasomal degradation of ABHD5, a hydrolase enzyme which is involved in lipid metabolism. Downregulation of ABHD5 activated NLRP3 inflammosome and resulted in enhanced IL-1β production (191). Collectively, these studies demonstrate the cardinal role of extracellular vesicles in regulating macrophage polarization.

Macrophage immunotherapy in cancer

As a matter of macrophage contribution to tumor growth, angiogenesis, and metastasis, there has been remarkable attention in macrophage therapy. Macrophage immunotherapy can be divided into those that reduce the number of TAMs in the tumor microenvironment by targeting TAM recruitment or survival and reduce circulating monocytes that are the progenitor of TAMs, those that reprogram activities of TAMs and using macrophages as carriers of anti-cancer drugs. Novel therapeutic approaches target survival, recruitment, polarization, and other properties of TAMs in cancer progression. Combinatorial approaches have emerged and shown efficacy in preclinical studies (58). These strategies are currently in assessment either to increase tumor immunity during standard radio- or chemotherapy or in combination with T cell-mediated immunotherapy (192). We will discuss some potential pathways that can be translated into therapeutic strategies to inhibit TAM-mediated immune suppression. *Table 2* summarizes the main molecular pathways that have been employed for TAM targeting in clinical trials so far.

Targeting TAM recruitment and survival

One method to diminish the number of TAMs in the tumor microenvironment is preventing their replenishment by circulating inflammatory monocytes. Monocytes are mainly dependent on CCL2-CCR2 signaling for migration from the bone marrow and recruitment towards inflammatory centers. Therefore, CCL2-CCR2 inhibition limits them in the bone marrow and reduces the number of TAMs in primary and metastatic sites (230). In preclinical models, inhibition of these targets improves the efficacy of chemotherapy, radiotherapy, and immunotherapy (231). A study indicated that PF-04136309, a CCR2 antagonist, prevents the movement of CCR2+ monocytes from bone marrow to the tumor, which causes TAM depletion (232). CCL2 blockade using specific antibodies showed effective results in combination with chemotherapy in various cancer models such as lung, prostate, and liver neoplasms (233-235). Cross-talk between TAMs and lung cancer cells through CCR2 could play a critical role in lung tumor proliferation and metastasis. CCR2 antagonist (RS504393) treatment LLC murine model showed diminished primary tumor growth and metastasis and inhibited TAM accumulation (236). Anti-CCL2 monoclonal antibody reduced primary tumor growth in syngeneic flank and orthotopic animal models of NSCLC and suppressed lung metastases in spontaneous lung metastases of NSCLC. Although the CCL2 blockade did not influence the number of TAMs recruited into the tumor in this study, there was a significant decrease in the M2 macrophage phenotype (233).

The CSF1-CSF1R axis is another target pathway of interest for inhibition of TAM recruitment to tumor microenvironment in preclinical models. This axis is important for differentiation, survival, and recruitment of TAM (237). In numerous tumors, blockage of CSF1-CSF1R signaling leads to the elimination of a remarkable portion of TAMs or repolarization of them (101,238,239). In animal models, CSF1R blockage improves T cell responses in combination with chemo- and radiotherapeutic agents (240-242). The combination of CSF1 and/or CSF1R blockage with immune checkpoint inhibitors such as CD40 agonists, PD-1 or CTLA4 antagonists, and T cell therapy is a promising spot in TAM therapy (243,244). Small molecule CSF1R inhibitor BLZ945 in phase I/II clinical trial (NCT02829723) is being tested for advanced solid

Table 2 Clinical trials of potential drugs for TAM targeting

Strategy	Target	Drug	Drug type	Tumor type	Clinical trial number	Ref.
TAM recruitment and survival	CCL2-CCR2 axis	CNTO 888 (Carlumab)	CCL2 inhibitor (mAb)	Solid tumors Prostate cancer	NCT00537368 NCT00992186	(193,194)
		Trabectedin	CCL2 inhibitor (small molecule)	Ovarian cancer Liposarcoma Leiomyosarcoma	NCT02163720 NCT01343277	(195,196)
		PF-04136309	CCR2 inhibitor (small molecule)	Pancreatic ductal adenocarcinoma	NCT02732938 NCT01413022	(197,198)
		MLN1202	CCR2 inhibitor (mAb)	Bone metastasis	NCT01015560	(199)
	CSF1-CSF1R axis	LY3022855	CSF1R inhibitor (mAb)	Solid tumors Breast or prostate cancer	NCT01346358 NCT02265536	(200,201)
		AMG 820	CSF1R inhibitor (mAb)	Solid tumors	NCT01444404 NCT02713529	(202,203)
		PLX3397 (Pexidartinib)	CSF1R inhibitor (small molecule)	Solid tumors Glioblastoma Multiforme Pancreatic ductal adenocarcinoma and colorectal cancer	NCT02452424 NCT01349036 NCT02777710	(204-206)
		RO5509554/ RG7155 (Emactuzumab)	CSF1R inhibitor (mAb)	Solid tumors	NCT01494688	(207)
		Cabiralizumab	CSF1R inhibitor (mAb)	Solid tumors Pancreatic ductal adenocarcinoma	NCT03158272 NCT03599362	(208,209)
		BLZ945	CSF1R inhibitor (small molecule)	Solid tumors	NCT02829723	(210)
	CXCL12-CXCR4 axis	LY2510924	CXCR4 inhibitor (small molecule)	Solid tumors	NCT02737072	(211)
		X4P-001 (Mavorixafor)	CXCR4 inhibitor (small molecule)	Melanoma	NCT02823405	(212)
TAM activation	CD40	ChiLob 7/4	CD40 agonist (mAb)	Non-Hodgkin lymphoma and solid tumors	NCT01561911	(213)
		CP-870,893	CD40 agonist (mAb)	Solid tumors Melanoma	NCT00607048 NCT01103635	(214,215)
		GM.CD40L	GM-CSF/CD40 Ligand (vaccine)	Lung adenocarcinoma	NCT01433172	(216)
		APX005M	CD40 agonist (mAb)	Melanoma and NSCLC	NCT03123783	(217)
	TLR7	Imiquimod	TLR7 agonist (small molecule)	Breast cancer Basal cell carcinoma	NCT01421017 NCT00899574 NCT00803907	(218,219)
	TLR7, 8, and 9	IMO-8400	TLR7,8, and 9 inhibitor (anti-sense oligonucleotide)	Diffuse large B cell lymphoma	NCT02252146	(220)

Table 2 (*continued*)

Table 2 (*continued*)

Strategy	Target	Drug	Drug type	Tumor type	Clinical trial number	Ref.
	TLR7/8	Resiquimod	TLR 7/8 agonist (small molecule)	Melanoma Cutaneous T cell lymphoma	NCT00821652 NCT01676831	(221,222)
		NKTR-262	TLR 7/8 agonist (small molecule)	solid tumors	NCT03435640	(223)
	TLR8	Motolimod (VTX-2337)	TLR8 agonist (small molecule)	Ovarian cancer ovarian, fallopian tube or primary peritoneal cancer Squamous cell carcinoma of the head and neck	NCT02431559 NCT01666444 NCT01334177	(123,224,225)
	TLR9	EMD 1201081	TLR9 agonist (small molecule)	Squamous Cell Carcinoma of the Head and Neck Cancer	NCT01040832	(124)
		IMO-2055	TLR9 agonist (small molecule)	Colorectal Cancer NSCLC	NCT00719199 NCT00633529	(125,226)
		SD-101	TLR9 agonist (synthetic CpG oligonucleotide)	Low-grade B-cell lymphoma Melanoma	NCT02254772 NCT02521870	(227,228)
	IL6-IL6R axis	Tocilizumab	IL6R inhibitor (mAb)	Ovarian cancer	NCT01637532	(88)
		Siltuximab	IL6 inhibitor (mAb)	Smoldering multiple myeloma	NCT01484275	(89)
	SIRP-α/CD47 axis	TTI-622	SIRP-α inhibitor (Fc-fusion protein)	Non-Hodgkin lymphoma	NCT03530683	(229)

CCL C-C, motif chemokine ligand; CCR C-C, chemokine receptor; CD, cluster of differentiation; CSF, colony stimulating factor; CXCR, C-X-C chemokine receptor; GM, granulocyte-macrophage; IL Interleukin; mAb, monoclonal antibody; NSCLC, non-small cell lung carcinoma; TLR, toll-like receptor; SIRP, signal regulatory protein.

tumors such as lung cancer (245). This inhibitor limited the experimental LLC-induced malignant pleural effusion model (246). Pass and colleagues showed that CSF-1R inhibitor JNJ-40346527 reduced expression of genes related to epithelial-to-mesenchymal transition (EMT), stem cell markers, and cisplatin resistance genes in multiple lung cancer CSF-1R positive cell lines (A549, NCI-H1299, NCI-H157, CALU-1, NCI-H1975, NCI-H358, and NCI-H4660) (247).

Another signaling pathway that is involved in macrophage recruitment is CXCL12-CXCR4. CXCL12-CXCR4 inhibition in combination with radiotherapy and immunotherapy has displayed anti-tumor efficacy, and this axis has emerged as a target to intervene with the immune system in clinical trials (248). Selective CXCR4 antagonists have been developed by some pharma companies that can be divided into four major classes: (I) small peptide CXCR4 antagonists such as BL-8040 and T140, (II) non-peptide CXCR4 antagonists such as AMD3100 and AMD070, (III) antibodies against CXCR4 such as LY2624587, and (IV) modified agonists and antagonists for CXCL12 such as CTCE-0214 and CTCE-9908, respectively (249,250). The expression of CXCR4 is high in lung cancer, specifically in SCLC. It has been shown that administration of AMD3100, in monotherapy or in combination with standard chemotherapy composed of etoposide and cisplatin, diminished the proliferation of SCLC primary tumor and inhibited metastasis in a xenograft mouse model (251).

Despite the beneficial aspects of these kinds of studies, tumor microenvironment can lead to the development of resistance to targeted pathways. Long-term administration of CSF-1R inhibitor BLZ945 in the animal model of Glioblastoma Multiforme (GBM) developed acquired resistance to CSF-1R inhibition by

elevated phosphoinositide 3-kinase (PI3K) signaling that led to disease recurrence without tumor cell intrinsic modifications (252). Compensatory resistance pathways and loss of tissue-resident macrophage populations, which are crucial for maintaining homeostasis, limit the application of these methods in clinical trials (192).

Targeting TAM activation

Another strategy in macrophage immunotherapy is the inactivation of macrophage immune-stimulatory activities as the primary phagocyte and professional antigen-presenting cell inside tumors. Reprogramming or macrophage repolarization towards an anti-tumor phenotype can be an effective approach to enhancing the efficacy of other types of immunotherapy.

Targeting the main molecular pathways which drive the immunosuppressive effects of M2-like phenotype TAMs, such as IL-4, IL-10, and IL-13, can be utilized as effective therapies against the pro-tumorigenic subtype of TAMs in cancers (58,253). IL-10 showed a close association with M2 TAMs, and it is reported that upregulation of IL-10 in TAMs correlates with late stage of lung cancer (46). IL-10 in cultured human macrophages induces chemoattractants, pattern recognition receptors such as TLRs, macrophage receptor with collagenous domain (MARCO), and cytokine receptors (254). MARCO drives macrophages towards immunosuppressive phenotype M2. In human melanoma samples and B16 melanoma murine tumors, MARCO is broadly expressed by TAM, and anti-MARCO antibody treatment significantly reduces the rate of a distinct TAM population that expresses *arginase 1* (ARG1) and inhibits *in vitro* T cell proliferation (255).

TAM receptors, including Tyro3, Axl, and MERTK, are a group of tyrosine kinase receptors with common ligands Gas6 and Protein S. They stimulate macrophage polarization toward M2-like phenotype (256). Some studies have reported overexpression of Axl and MERTK in some cancers such as NSCLC (257-259). Small molecule inhibitor of MERTK, UNC2025, decreased tumor xenograft growth in the murine model of NSCLC (260).

TAMs express a membrane glycoprotein called Signal Regulatory Protein-alpha (SIRP-α). Interaction of SIRP-α with CD47 on cancer cells inhibits cancer cell phagocytosis by TAMs (261). Inhibition of SIRP-α by blocking antibody prevented the development of resistance to anti-angiogenic therapy in the mouse model of NSCLC (262). Mechanistic studies revealed that antibodies against SIRP-α increased the phagocytic function of TAMs and macrophage-mediated cytotoxicity against cancer cells (262,263). CD40 signaling is implicated in monocyte maturation and differentiation into M1 macrophage and dendritic cells and can re-educate M2 phenotype macrophages into M1. Targeting CD40/CD40L is a common strategy in cancer immunotherapy (264). CD40L gene transfer into 3LLSA murine lung cancer cells increased the anti-tumoral activity of TAMs and stimulated the production of NO, TNF-α, and IL-12 (265). Pilot clinical trials of CD40 agonist antibodies in combination with chemotherapy or immune checkpoint inhibitors have shown acceptable safety profile and preliminary response in a variety of solid tumors including NSCLC (213-215,217).

Epigenetic reprogramming of macrophages by inhibition of histone deacetylases (HDACs) can elicit a T cell supportive role. M2 macrophages express HDAC2, and its inhibition leads to macrophage repolarization to M1 phenotype (266). Inhibition of this enzyme using genetic approaches (siRNA) or pharmacological inhibitors (ISAHA, VPA) in M2 macrophages and TAMs from lung tumors skewed them to M1-like phenotypes and controlled tumor cell functions (266). A selective class IIa HDAC inhibitor evokes anti-tumor macrophage phenotypes that assist T cell responses and augments responses to chemotherapy and immune checkpoint inhibition, particularly in mammary tumor models (267).

It has been demonstrated that activation of $PI3K_{\gamma}$ signaling leads to TAM immunosuppressive activities in models of melanoma, lung, and pancreatic cancer (268). In animal models, $PI3K_{\gamma}$ blockage causes macrophage reprogramming and enhancement of T cell responses as a single agent or in combination with T cell checkpoint inhibition (269,270). Imatinib, through inhibition of STAT6 phosphorylation and nuclear translocation, suppressed M2 macrophage polarization and reduced migration of LLC cells both *in vitro* and *in vivo*, although it did not influence lung tumor growth (99).

In conclusion, blocking the activating molecules of TAM is a promising strategy to enhance the efficiency of other therapies such as checkpoint inhibitor therapy.

Using macrophages as a carrier for anti-cancer drugs delivery

TAMs can be employed as a carrier for anti-cancer drug delivery systems (*Figure 3*). Some key characteristics of TAMs make them suitable for drug delivery in cancer settings. Separating circulating monocytes from peripheral

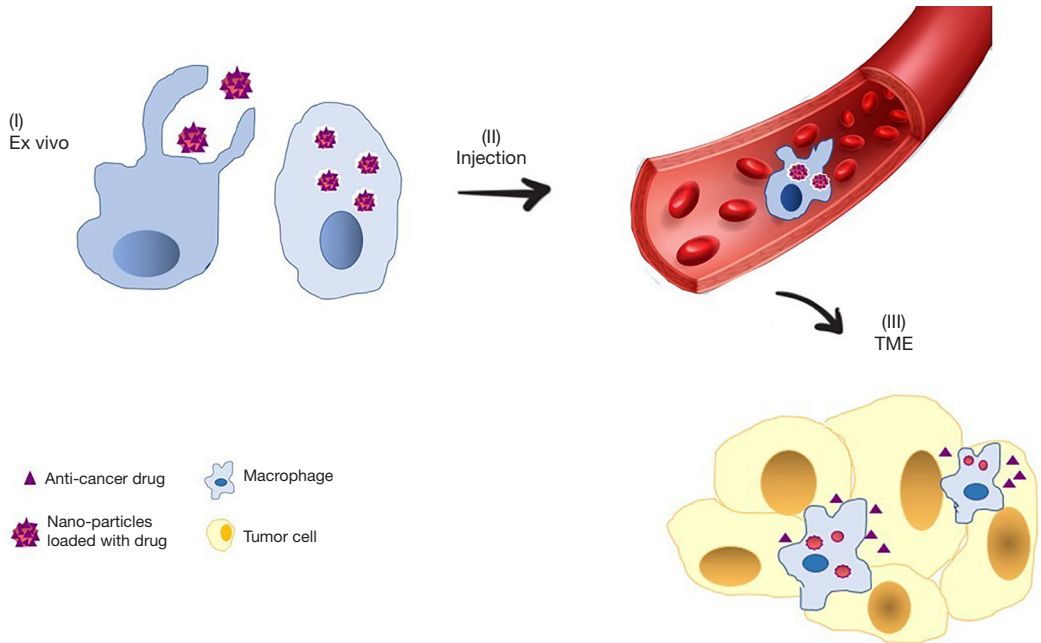

Figure 3 Macrophage-based drug delivery to tumors. (I) Nanoparticles that are loaded with anti-cancer drugs are engulfed by macrophages *ex vivo*. (II) Macrophages that contain nanoparticles are injected intravenously and migrate to tumors. (III) Anti-cancer drugs are released from macrophages into TME to kill cancer cells. TME, tumor microenvironment.

blood is more feasible than other cellular carriers such as mesenchymal stem cells; macrophage phagocytic property can be used for drug loading into macrophage; and they inherently home to tumor microenvironment in the body (271). M1 phenotype is usually considered as the carrier for nano-particle delivery. M1 macrophages have more phagocytic potency for loading anti-cancer drugs nano-particles than other types of macrophages and can naturally home into tumor tissues. Furthermore, the tumor microenvironment cannot influence M1 cells, and their own activity can inhibit tumor progression (272,273). In a pivotal study by Choi *et al.*, TAMs containing Gold Nano-shells moved along the central hypoxic region of breast tumor and destroyed surrounding cancer cells after irradiation with near-infrared laser waves (274). Wang and colleagues reported that monocytes internalized Fe/Fe_3O_4 magnetic Nano-particles combined with topoisomerase I inhibitor SN38 by the carboxylesterase-cleavable linker and delivered them to tumors (275). Anchoring lipopolysaccharide (LPS) on macrophages mediates production of microtubule networks with A549 cells and leads to selective Doxorubicin delivery to cancer cells via intercellular microtubule conduits in orthotopic lung cancer model (276). Some limitations, including the low-load capacity of carrier, relatively low drug release, the possibility of drug cytotoxicity, and susceptibility of drugs to lysosomal degradation within macrophage have restricted the applicability of macrophages in this context (277). Further studies are needed to improve this approach and overcome its defects.

Therapeutic limitations of macrophage regulation

TAM targeting for cancer treatment is challenged by some obstacles including predisposition to infection, organ dysfunction, the need for multiple dosing, and the presence of redundant pathways. Macrophages are cardinal in protecting body from invading pathogens. TAM targeting strategies that are based on eradicating macrophages may increase susceptibility to infections. On the other hand, tissue-resident macrophages such as liver Kupffer cells and brain microglial cells are ubiquitous throughout the body and are significantly involved in maintaining organ homeostasis and their depletion may give rise to serious organ dysfunction. One possible solution for overcoming these problems is to find novel targets that are specifically upregulated by macrophages in cancerous

microenvironment (25). Potential candidate molecules and pathways for TAM targeting may be present in diverse cell populations beyond TAM. For instance, CCR2 and CXCR4 are also expressed by lymphocytes (278,279). Alterations of immune and non-immune cellular functions caused by unintended influence on cells that share the target with TAM may result in complications. This issue also necessitates finding of targets that are specific to TAM (25).

The optimal dosing and frequency of TAM targeting therapeutics have not been determined. One special difficulty related to TAM repolarization is that TAM phenotype and function may return to its primary immunosuppressive state after the drug is withdrawn from tumor microenvironment (280).

The ultimate functional status of TAM is controlled by the complex interactions of several microenvironmental and intracellular regulators. The presence of redundant pathways of macrophage polarization and other immunosuppressive cells in tumor microenvironment may explain the observation that not all the tumors respond to TAM targeting strategies. Simultaneous interference with multiple contributors of immunosuppression and cancer progression either by combinatorial approaches or using agents with multiple relevant targets may enhance TAM targeting efficacy (192).

To conclude regarding the possible roles of TAM targeting in NSCLC, as TAMs are abundant in the tumor microenvironment of NSCLC and plays important parts in its progression, targeting TAM, either by eradicating them or re-educating them towards anti-cancer phenotype, will be a rationale strategy that can be translated to clinical application.

Macrophage and drug resistance

Drug resistance prevalently occurs against traditional chemotherapy drugs and targeted therapies in NSCLC patients (281,282). Neoplastic cell-extrinsic factors are involved in cytotoxic therapy resistance (283). *In vivo* studies have indicated that macrophages not only support tumor growth and progression but also mediate chemotherapy resistance by providing survival factors and upregulating genes responsible for anti-apoptotic programs in malignant cells. Soluble factors secreted by macrophages, such as IL-6, as well as extracellular deposition and cell-cell interactions, are involved in chemotherapy resistance (283-285). TAMs via CSF-1 signaling pathway diminishes the effectiveness of a combination therapy using Cyclophosphamide, Methotrexate, and 5-FU in a MCF-7 breast cancer xenograft model (286). TAMs defend MMTV-PyMT tumor cells against paclitaxel-induced cell death by secretion of lysosomal enzymes, Cathepsin B and S (287). In the animal model of colorectal cancer, TAMs mediate chemoresistance to 5-FU through secretion of IL-6 and the activation of IL-6R/STAT3 signaling (288). TAMs mediate Gemcitabine resistance in pancreatic ductal adenocarcinoma cells by upregulating cytidine deaminase, the metabolizing enzyme of Gemcitabine (289). TAMs have a supportive role in cancer stem cell functions (290). The interplay between TAMs and cancer stem cells by macrophage-derived factors, including IL-6 and Milk-Fat Globule-epidermal growth factor-VIII (MFG-E8) through activation of Hedgehog signals and STAT3 mediates drug resistance and induces distinct important maintenance signaling for cancer stem cells (291-293).

TNF-α is one of the crucial factors mediating chemoprotection, either directly by NF-κB activation (294) or indirectly through stimulated IL-6 expression and further STAT3 activation (58). Macrophages can be considered as the main source of TNF-α *in vivo* (295). In this regard, it has been documented that macrophage-derived TNF-α promotes resistance to MAPK inhibitors in melanoma. The protective effect of TNF-α on melanoma cell lines was mediated by the upregulation of the microphthalmia transcription factor (MITF) in melanoma cells (296). In a recent study, it has been demonstrated that TAMs can evoke autophagy in hepatocellular carcinoma (HCC) cell lines, which are involved in resistance to Oxaliplatin (297).

Conclusions

NSCLC is one of the most common malignancies in the world, and researchers are making considerable efforts for finding effective therapies. Unfortunately, all strategies do not work for all tumors, and all patients do not respond in the same manner to those therapies. It is partly because of tumor immunogenic properties and tumor niche composition. Increasing evidence demonstrates that TAM is one of the main components of the immune-suppressive tumor microenvironment. Since high macrophage infiltration causes poor survival in most cancers, these cells have emerged as promising aims for anticancer therapies. As briefed in this review, various studies have demonstrated that TAM targeting can synergistically improve the response to other conventional cancer treatments. However, most of these therapies are still at the preclinical stage, blocking

antibodies or antagonists have been examined in clinical trials for malignant solid tumors (101). Studies at the preclinical level have shown that the combination of TAM targeting strategies with checkpoint inhibitors can improve the therapeutic response in melanoma, colon, lung, and breast tumors (127). One main obstacle of TAM targeting is the occurrence of negative side effects in the patients. Due to the countless roles of macrophages, it seems that systemic depletion of these cells leads to increased infections or disabled activities of tissue-resident cells to do their normal function. To overcome this obstacle, one reasonable approach is to identify TAM-specific markers in order to precisely targeting this tumor immunosuppressive cell population. Targeting TAMs in lung cancer showed promising effects in the suppression of primary tumor growth or preventing lung metastasis.

Totally, preclinical and early clinical studies show that targeting lung TAMs could remarkably enhance the efficacy of conventional therapies and immunotherapies, but there is a long way between understanding the roles and mechanisms of TAMs in lung cancer progression and using TAM-based immunotherapy to cure cancer.

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