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REVIEW

Tumor-associated macrophages: a short compendium

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Abstract
Macrophages play an important role in tissue development and homeostasis. They serve as a nexus between adaptive and innate immunity, and employ considerable plasticity. In cancer, they play a pivotal role in chronic inflammation and tumor growth either by directly stimulating the proliferation of cancer cells or by producing angiogenic and lymphangiogenic factors. Although numerous immune cells play an important role in the tumor microenvironment, tumor-associated macrophages (TAMs) are by far the most extensively studied. A better understanding of the role of TAMs in mediating chemo- and radiotherapy resistance and suppressing immunosurveillance has led to numerous strategies targeting TAMs as an anticancer therapy either by targeting them directly or by polarizing TAMs toward a tumoricidal phenotype.

Keywords Monocytes · TAM · Macrophage · Cancer · Innate and adaptive immunity

Introduction

Macrophages are phagocytic cells, critical for our innate and acquired immune response. They not only detect, engulf, and destroy cellular debris, foreign material, and pathogens but also cancer cells [1]. They are the first line of defense against anything that expresses signatures on their surface different from molecules on host cells, namely damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) [2]. Besides phagocytosis and its role in the innate immunity, they can also recruit the other immune cells such as lymphocytes and present antigens to T cells (adaptive immunity).

Macrophages have their origin in the bone marrow, where they pass a monoblast and promonocyte stage to the stage of becoming monocytes which enter the blood system. When inflammation occurs, monocytes undergo a series of changes to become a macrophage when they leave the bloodstream. A majority of macrophages, however, is of embryonic origin (tissue-resident macrophages) and are stationed in certain tissues (the mononuclear phagocytic system) where microbial invasion or accumulation of foreign material is common such as the liver, lymph nodes, and spleen [3, 4]. The main task of these strategically placed macrophages is ingesting foreign material and recruiting additional macrophages if needed. In contrast with circulating blood monocytes which have a half-life of about a day, the life span of tissue macrophages is several months or even years.

The main hallmark of macrophages is their versatile nature, whereby they can play multiple roles in the immune response. Besides the scavenger function, they can present antigens [Major Histocompatibility Complex (MHC) class II] along with dendritic cells and produce cytokines vital to the regulation of the immune response.

Monocytes can be classified as classical (in humans: $CD14^{high}$, $CD16^{-}$), intermediate ($CD14^{high}$, $CD16^{low}$), and non-classical ($CD14^{low}$, $CD16^{high}$) with all different phenotypes [5]. Classical monocytes are involved with phagocytosis and cytokine production, while non-classical can be pro-inflammatory depending on the context. Although considered as separate entities, the current evidence supports the

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existence of a monocyte continuum rather than incremental differences between the different subtypes [6]. Plasticity of monocytes is also proven by several clinical studies shown their diversity in pathological context. For example, we have shown that colon cancer cell-derived stimuli change their transcriptome [7], while others found a difference in the frequency of non-classical monocytes in breast cancer patients [8]. This plasticity makes them attractive cells for diagnosis and disease follow-up.

Similarly to the concept of a monocyte continuum, macrophages derived from all these monocyte subtypes are considered as well to be a wide spectrum of phenotypes. At its extremes, they can be divided into two main groups designated as M1 and M2, which can be identified by cell surface markers and their functional phenotype. "Killer" M1 macrophages are in vitro activated by interferon-gamma (IFN-γ), lipopolysaccharide, (LPS), and granulocyte–macrophage colony-stimulating factor (GM-CSF or also called colony-stimulating factor 2, CSF2) and secrete pro-inflammatory mediators such as interleukin (IL)-1, IL-12, IL-18, IL-23, and tumor necrosis factor (TNF). Phenotypically, they express high levels of MHC class II, CD68, and co-stimulatory molecules CD80 and CD86. Their main role is pathogen destruction and driving Type 1 T helper (Th1) responses [1]. "Repair" M2 macrophages, on the other hand, are activated in vitro by IL-4 or macrophage colony-stimulating factor (M-CSF or also called colony-stimulating factor 1, CSF1), and they are more involved in processes like wound healing and tissue repair and secrete an anti-inflammatory response by producing anti-inflammatory cytokines such as IL-10. Therefore, they are involved in angiogenesis, extracellular matrix remodeling, and resolution of inflammation [9, 10]. M2 macrophages are further divided into four major types based on their role (M2a, M2b, M2c, and M2d) [11, 12].

The dichotomy between M1 and M2 is believed now to be a more continuous spectrum where insufficient shifts between one of these types could be causative in the pathogenesis and complications of many diseases such as atherosclerosis, muscle regeneration, chronic infections, wound healing, and cancer.

Role of tumor-associated macrophages (TAMs) in cancer

Progression of cancer is not only based on the growth of malignant cells but also on behavior of the components of the tumor microenvironment (TME), which includes various immune cells as well as tumor-associated stromal fibroblasts [13]. However, just the presence of immune cells does not imply that they are activated to kill or stimulate cancer cell growth. The lymphoid component of the TME consists of tumor-infiltrating lymphocytes (TILs) as well as natural killer (NK) cells. Myeloid cells present in the TME include myeloid-derived suppressor cells (MDSCs), granulocytes, dendritic cells (DCs), and TAMs. TAMs include both M1-like cells harboring anti-tumor effector functions as well as M2-like macrophages which, similar to MDSCs, express immunosuppressive and tumor-promoting factors. Both M1- and M2-like TAMs show strong intrinsic plasticity and can cross-regulate each other's functions and do not represent fixed, frozen phenotypic conditions [14, 15]. TAMs can have, therefore, different effects on the tumor depending on their activation state [16] and, therefore, orchestrate the intratumoral inflammation. In general, higher M1-like infiltrates in the tumor correlate with a better prognosis (anti-tumoral effect), while higher M2-like TAM infiltrate correlates with poor prognosis (pro-tumoral), although M1-like macrophages can also promote malignant transformation by inducing chronic inflammation [17, 18]. In tumors, TAMs mainly originate from bone marrow-derived monocytes [19]. This infiltration is mainly regulated by chemokines such as CCL2, CCL5, CXCL12, and CSF1 (or M-CSF). Once in the tumor, TAMs can undergo a phenotypic switch based on microenvironmental factors such as hypoxia in conjunction with cytokine availability [20]. Moreover, some studies suggest that TAMs can also be activated by exosomes derived from cancer cells [21–23]. Exosomes are important signal mediators transferring cancer-associated signaling molecules to surrounding cells such as immune cells.

In an early phase, macrophages recognize the malignant cells and present their antigens to the effectors of the immune system. In a later phase, TAMs play a role in tumor progression by stimulating tumor growth, angiogenesis, metastasis, and immunosuppression (Fig. 1) [24]. Qian and Pollard classified TAMs into six functional subtypes: angiogenic, immunosuppressive, invasive, metastasis-associated, perivascular, and activated macrophages [25].

TAMs are abundant in all the stages of tumor progression [26]. By producing growth factors, they can stimulate carcinoma cell proliferation [27]. They can also produce proteolytic enzymes that digest the extracellular matrix to assist with tumor cell dissemination. Finally, they provide a supportive niche for metastatic tumor cells at distant sites [28].

Role of TAMs in tumor angiogenesis

Due to intensive proliferating and expanding tumor tissue, oxygen demand is surpassed by oxygen supply leading to tumor hypoxia. Cancer cells respond differently to hypoxia leading to cell death or survival depending on the time of exposure of hypoxia. Hypoxia induces the activation of a number of intracellular signaling pathways such as the major hypoxia-inducible factor (HIF) pathway, the PI3K/AKT/mTOR pathway and the NFkβ pathway [29–31]. In

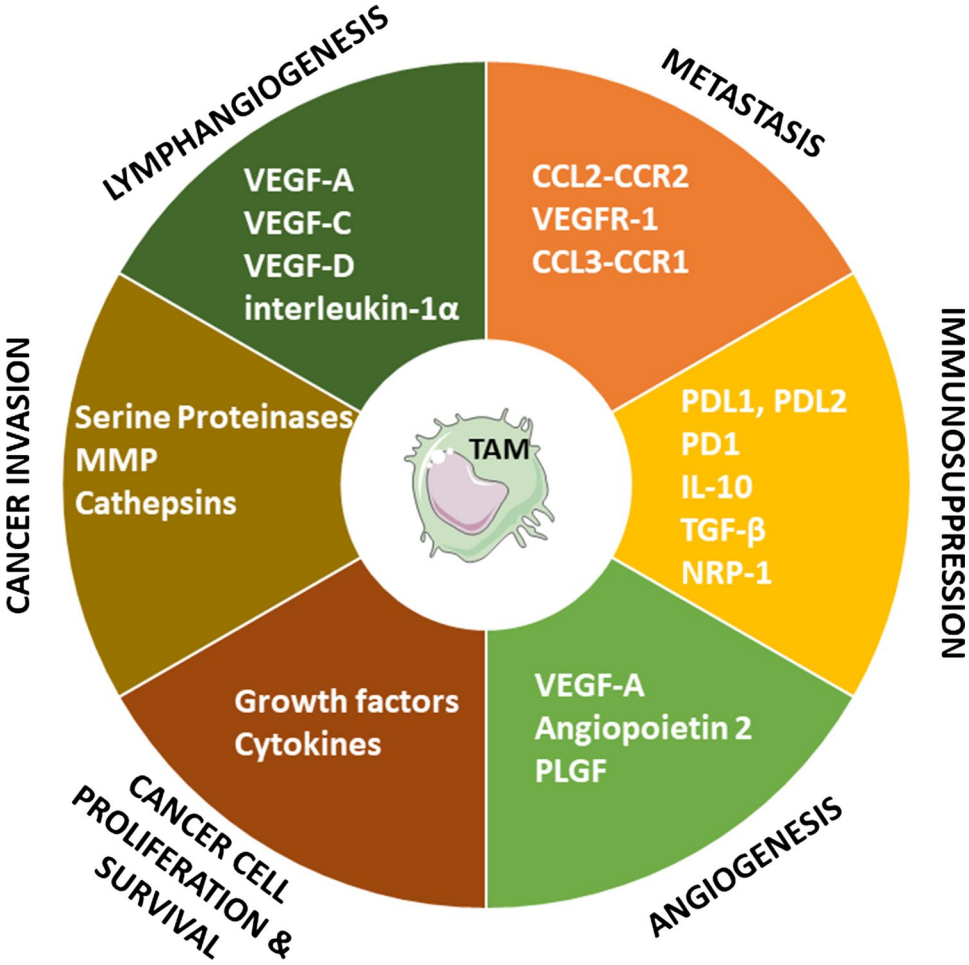

Fig. 1 TAMs importantly contributes to all the steps of cancer progression. Pro-tumoral effects of tumor-associated macrophages (TAMs) on cancer cells and the different molecules associated with the specific roles

cancer, these pathways can also be stimulated in a hypoxia-independent manner by growth factors, cytokines, and chemokines or by epigenetic changes and acquired mutations of the members of these pathways, such as mutations in the receptor tyrosine kinase leading to uncontrollable cell growth. However, the generation of tumor blood supply is often a rate-limiting step during tumor progression. Hypoxia induces an imbalance between pro- and anti-angiogenic factors leading to chaotic blood vessel formation. In contrast with normal blood vessels, tumor blood vessels are often abnormal, immature, and leaky. This onset of angiogenesis is called the "angiogenic switch" and can occur at different stages during tumorigenesis [32]. The most common mechanism is sprouting angiogenesis by which new vascular branches arise from pre-existing capillaries or postcapillary venules. Other mechanisms of tumor angiogenesis are vasculogenesis, vascular mimicry, intussusception, and vascular co-option [33].

Angiogenesis is initiated by activation of the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), expressed on blood endothelial cells, by binding to the ligand VEGF, and also requires the participation of other signaling molecules such as angiopoietin-2 and delta ligand-like 4 [34]. For the last decades, there was a lot of interest in the possibility of inhibiting cancer growth by blocking angiogenesis, for example, with monoclonal antibodies directed against either the VEGF ligand or the VEGF receptors. By contrast, inflammation-associated angiogenesis was rather a neglected field until recently. There is now accumulating evidence that immune cells can be involved in the modulation of angiogenesis [35].

In 1971, Judah Folkman, already suggested that immune cells, in particular macrophages and mast cells, could be a source of pro-angiogenic factors [36]. Later on, multiple studies confirmed that several cells of the innate and adaptive immune system can play a major role in tumor angiogenesis, such as monocytes, macrophages, mast cells, neutrophils, basophils, and eosinophils [37].

Although monocytes were assumed in the past to be only a transient precursor for tissue macrophages, they have now emerged as a highly plastic and dynamic cellular system. They can promote angiogenesis by producing VEGF after the stimulation of different molecules such as cysteinyl-leukotriene D4, M-CSF, and ATP [38–40]. On

the other hand, CD16$^-$ classical monocytes also express VEGFR1 and migrate more when activated by VEGF [41]. In 2005, De Palma et al. identified a unique subset of mouse and human monocytes expressing the tyrosine kinase receptor TIE-2 (TEMs) [42]. These monocytes are a functional distinct myeloid lineage that can induce angiogenesis and tumor growth. They have been identified in different human tumors such as kidney, colon, pancreas, liver, lung, and breast tumors, while they were excluded from surrounding healthy tissue [43–45]. In vitro, TIE-2 expressing monocytes migrate toward angiopoietin-2 (Ang-2) released by activated endothelial cells and angiogenic vessels [43]. Furthermore, angiopoietin-2 stimulated TIE-2 expressing monocytes also have an immunosuppressive and, therefore, pro-tumorigenic role by suppressing T-cell proliferation via IL-10 dependent mechanisms [46]. Targeting the Ang-2/TIE-2 axis might, therefore, be interesting approach to inhibit tumor growth [47].

Not only circulating monocytes but also tissue-based TAMs can play a pro-angiogenic role. Preclinical mouse models have shown the functional importance of TAMs in tumor angiogenesis. Here, tumor-infiltrating, VEGF-producing macrophages were shown to facilitate the angiogenic switch and the progression to malignancy, because inactivation of TAMs by blocking the CSF1/CSF1R pathway, or broadly depleting TAMs, or genetic VEGF deletion in macrophages delayed the angiogenic switch, whereas genetic restoration of the macrophage population rescued the angiogenic phenotype [48–50]. TAMs also express proteases including matrix metalloproteinases (MMPs), plasmin, urokinase plasminogen activator (uPA), and serine or cysteine proteinases which can facilitate the infiltrative growth of tumor cells in the tumor microenvironment [51].

Another key molecule in angiogenesis is placental growth factor (PLGF), member of the VEGF subfamily. PLGF, produced by both tumor and stromal cells such as macrophages, was shown not only to be a chemoattractant for TAMs but also to play a role in their abnormal polarization [52, 53].

Besides monocytes and TAMs, also other myeloid cells such as neutrophils and MDSCs can play an important role in promoting tumor angiogenesis [54]. T cells, on the other hand, can negatively or positively regulate tumor angiogenesis based on the T-cell type. CD8$^+$ cytotoxic T lymphocytes and CD4$^+$ Th1 cells produce IFNγ that restrains endothelial cell proliferation and induces the production of angiostatic chemokines CXCL9, 10, and 11 in TAMs [55, 56]. In contrast, regulatory T cells (Tregs) suppress INFγ-expressing CD4$^+$ Th1 cells and secrete VEGF via hypoxia-induced CCL28, which both contribute to a pro-angiogenic tumor environment [57].

Role of TAMs in lymphangiogenesis

The lymphatic system primarily functions to regulate tissue fluid homeostasis, as well as collecting antigens and traffic immune cells from the periphery to the lymph nodes [58]. In contrast with blood vessels, they are not encircled by pericytes or smooth muscle cells, which make them highly permeable to interstitial fluids and immune cell migration. Lymphangiogenesis only takes place during embryogenesis and during pathological conditions such as tissue repair, inflammation, and tumor growth [59]. In cancer, the lymphatic system serves as a major route for tumor cell dissemination from the primary tumor site. The most important pro-lymphangiogenic factors are vascular endothelial growth factor (VEGF)-C and -D, which bind to the VEGFR3 expressed on lymphatic endothelium [60, 61]. The expression of VEGF-C in tumors correlates with poor prognosis in several tumor types, in part due to an increase in lymphangiogenesis [62]. Besides VEGFR3 also VEGFR2 and neuropilin receptor NRP-2 play a role in lymphangiogenesis [63, 64]. Several other factors with pro-lymphangiogenic activity have been identified such as hepatocyte growth factor, angiopoietin-1, fibroblast growth factor-1 and -2, platelet derived growth factor, insulin-like growth factor-1 and -2, adrenomedullin, and endothelin-1 [65].

Tumor-induced lymphangiogenesis is mediated by growth factors that are produced by either the tumors themselves or by stromal cells, activated platelets or TAMs. TAMs can promote lymphangiogenesis by expressing VEGF-C and -D [66]. This process is stimulated by cancer cells which activate macrophage-derived lymphangiogenesis by producing interleukin-1α in a highly specific manner [67]. Besides VEGF-C and -D, TAMs also secrete VEGF-A, more characterized for its role in angiogenesis, though this factor plays also an important function in lymph angiogenesis. First, VEGF-A recruits TAMs mostly via the activation of VEGFR1 on macrophages [68, 69], but also it directly induces the proliferation and migration of lymphoid endothelial cells (LECs) via VEGFR2 activation [63].

Macrophages have also been shown to contribute to chemotherapy resistance, for example, by production of cathepsins, leading to increased heparanase activity which in turn induces the expression of VEGF-C, ultimately leading to lymphangiogenesis and metastasis [70, 71].

Role of TAMs in immunosuppression and regulation of adaptive immunity

One of the important roles of the immune system is to eliminate cancer cells [72]. More than 60 years ago, Prehn and Main showed, for the first time, that mice could generate immunity against carcinogen-induced tumors [73]. Since then, many developments in the field of immunology led to a better understanding of the role of immune cells in cancer. Escape of tumor cells from immunosurveillance is a critical event that regulates tumor growth and metastasis. In 2002, Dunn et al. proposed an improved version of the cancer immunosurveillance hypothesis called "tumor immunoediting" [74], resulting in one of three potential outcomes: tumor elimination, an equilibrium with the immune system, or escape from the immune system. The best understood phase of cancer immunosurveillance is the escape phase. For example, many studies have shown that patients with higher tumor-infiltrating T cells within the tumor have a better outcome [75, 76]. In addition, the more advanced tumors are, the less effector immune cells are not only present but also activated within the tumor microenvironment. For example, TAMs in hypoxic areas of the tumor respond to hypoxia with an altered gene expression profile leading to the development of a pro-tumoral phenotype that favors angiogenesis, metastasis, and suppresses anti-tumor immune response [77–79]. We have shown that TAMs' localization into hypoxic tumor areas is controlled by a Sema3A/Neuropilin-1 (NRP-1) signaling axis, leading to PlexinA1/PlexinA4-dependent VEGFR1 activation [68]. Blunting the Sema3A/NRP-1 pathway restored anti-tumor immunity and abated angiogenesis by confining TAMs inside normoxic regions and, thus, inhibiting tumor growth and metastasis [68]. Also other immune cells such as CD1d-restricted invariant natural killer (iNKT) cells contribute to cancer immune surveillance. In a mouse prostate cancer model, Cortesi et al. provide evidence that iNKT cells can remodel the TME by restricting pro-angiogenic TEMs and sustaining pro-inflammatory TAMs by cooperative CD1d, CD40, and Fas engagement [80]. Therefore, low iNKT cells and high TEMs can make cancers more aggressive.

Programmed cell death protein 1 (PD-1) is an immune checkpoint receptor, upregulated on activated T cells for the induction of immune tolerance. Tumor cells frequently overexpress the ligand PD-L1, facilitating their escape from the immune system. Besides its role on inhibition of T cells, a recent publication showed that TAMs also express PD-1 with an inverse correlation between the expression of PD-1 and the phagocytic potency of the TAMs [81]. This suggests that checkpoint inhibitors used in the treatment of many cancers such as melanoma, lung, head and neck, and bladder and renal cancer may also function through a direct effect on macrophages. Moreover, recent data showed that CSF1 expression by melanoma cells may limit the immune attack by activated $CD8^+$ T cells (adaptive resistance mechanism) and that simultaneous blocking of CSF1R with immune checkpoint targeting may be beneficial in cancers refractory to immune checkpoint blockade [82]. On the other hand, a population of PD1-negative TAMs has been involved in buffering anti-PD1 immunoglobulins by subtracting them to their target through the Fcγ receptors (FcγRs). Blockade of FcγRs before anti-PD1 administration enhances the effect of this treatment on cytotoxic T cells and induced the complete rejection of MC38 colorectal tumors in mice [83].

Although immunotherapy in cancer patients has been a clinical success, many patients experienced minimal effect or no clinical effect with the same treatment. We are far from understanding the complexity and diversity of the immune context of the TME and its influence on response to therapy [84]. Although numerous populations of immune cells have been reported to have suppressive functions in the tumor microenvironment, TAMs are the most extensively studied and well characterized.

Role of TAMs in chemoresistance and radioresistance

As TAMs can mediate chemotherapy and radiotherapy resistance by providing survival factors and activate anti-apoptotic programs, targeting TAMs for anticancer therapy has a clear rationale. For example, TAMs can limit the effect of cytoxic agents such as platinum-containing compounds, paclitaxel, gemcitabine, and doxorubicin [85–88]. De Nardo showed, in a mammary tumor-bearing mouse model, that response to chemotherapy is partly regulated by the tumor immune microenvironment and that cytotoxic drugs such as paclitaxel induce neoplastic cells to produce macrophage recruitment factors, which, in turn, enhance macrophage infiltration into mammary adenocarcinomas leading to tumor progression [85]. Another study in cervical and ovarian cancer cell lines suggests that a platinum chemotherapy-mediated increase in M2 macrophages may form an indirect mechanism for chemoresistance [86]. Finally, in a pancreatic cancer mouse model, Mitchem et al. showed that macrophages can directly induce tumor-initiating cell (TIC) properties by enhancing STAT3 activation and that $STAT3^+$ TICs enhance TAM-mediated immunosuppression. Thus, cross-talk between TAMs and TICs through STAT3 regulates the chemotherapeutic response by repressing anti-tumor cytotoxic T-cell activity [88]. Not only chemotherapy but also radiotherapy plays a major therapeutic role in the

treatment of most solid tumors. Next to inducing lethal DNA damages in the tumor, radiotherapy also impacts the tumor microenvironment with its associated immune system. For example, radiotherapy can promote tumor immune response by eliciting immunogenic cell death, and activate tumor antigen release and subsequently immune cell activation. Therefore, radiotherapy can program macrophages leading to either radiosensitization or radioresistance according to the tumor type and the radiotherapy regimen. Many studies have shown that irradiation induces macrophage infiltration in tumors that in turn limits the efficacy of radiotherapy. Moreover, macrophages are also one of the most radioresistant cells, due to a high production of anti-oxidative molecules [89]. In addition to the intrinsic radioresistance of macrophages, radiation also leads to a high recruitment of myeloid cells at the tumor site, possibly leading to tumor regrowth which was shown in an intracranial xenograft model of glioblastoma [90] as well in a pancreatic cancer mouse model [91]. Another study showed that depletion of macrophages by liposomal clodronate before radiation promoted the anti-tumor effects of radiotherapy [92]. After radiotherapy, TAMS accumulate in hypoxic regions of the tumor by induction of the expression of SDF1α, which, upon interaction with its receptor CXCR4, induce the recruitment of macrophages that restored tumor vasculature and promote tumor regrowth [93]. Likewise, the inhibition of CXCR4 was shown to significantly delayed xenograft lung tumor regrowth after radiotherapy [94]. Altogether, it is clear that strategies aiming to target TAMs or TAM functions carry the potential to synergize with standard chemo- and radiotherapeutic treatments.

Metastasis-associated macrophages

Although most research has been focused on TAMs, less is known about the distinct role of the so-called metastasis-associated macrophages (MAMs) which include both tissue-resident macrophages as bone marrow-derived macrophages. They are located at the metastatic site promoting tumor cell extravasation, seeding, and persistent growth. Several studies demonstrated that myeloid cells and, in particular, MAMs are important for the 'preparation' of the metastatic niche via the release of matrix proteins at the metastatic sites, and for this reason, these cells are also entrained by the primary tumor into the premetastatic niche before the lodging of cancer cells [95–97]. MAMs are derived from circulating monocytes recruited by CCL2/CCR2 chemokine signaling [80]. In a metastatic breast cancer mouse model, Kitamura et al. showed that circulating monocytes differentiate into a distinct myeloid cell population characterized as $CD11b^{high}Ly6C^{high}$ in the metastatic lung where they further differentiate into MAMs [98]. These authors also found that accumulation of the $CD11b^{high}Ly6C^{high}$ cells was increased when micrometastases started to outgrow. MAMs are enriched for the expression of VEFGR1 and activation of this receptor has been shown to be important for metastatic growth but not for cancer cell extravasation [99, 100]. We have recently shown that VEGFR1 exposure on the cell surface can be restrained by caveolin-1 (likely through the formation of caveolae). CSF2 (or GM-CSF) keeps high the levels of caveolin-1 in the interstitial macrophages of the lungs. It follows that macrophage-associated caveolin-1 is critical for hindering metastasis and represents an intrinsic antimetastatic surveillance mechanism in the pulmonary microenvironment whereby its upregulation prevents excessive exposure of VEGFR1 and thereby limits downstream MMP9 and CSF1 expression, angiogenesis, and finally metastatic growth [99]. Since the physiology of the lung is to counter dangerous signals that are coming from the outside (i.e., airways), it is not surprising that the block of metastasis by this axis in macrophages was seen in lungs but not in livers [99]. On the contrary, MAMs can aid metastatic growth by inhibiting tissue destructive immune response, promoting angiogenesis and cellular growth. Several mechanisms through which these macrophages contribute to metastatic progression have already been explored such as stimulation of the CCL2–CCR2 and the CCL3–CCR1 axis [101–104]. Further effort will be required to understand how the pro-metastatic axis represented by the CCL2/CCR2–CCL3/CCR1 axis in MAMs is specific for breast cancer and lung metastasis or whether this pathway is also observed in the other tumors and/or metastatic sites. Further understanding of the similarities and differences in TAM and MAM function is critical for developing new therapeutic macrophage targeting agents.

Macrophages as a therapeutic target in cancer treatment

Numerous strategies are being explored to target either macrophages directly or polarizing TAMs toward a tumoricidal phenotype (Fig. 2) [105, 106]. Ways to target directly the biology of macrophages can be achieved by either blocking the myeloid growth factor receptor CSF1R or the monocyte chemoattractant protein CCL2. Zhu et al. demonstrated in a mouse model of pancreatic ductal adenocarcinoma that inhibiting CSF1R can reprogram macrophage responses enhancing antigen presentation leading to anti-tumor T-cell responses [107]. CSF1/CSF1R signaling drives the recruitment and differentiation of TAMs toward an M2 phenotype [108]. Numerous clinical studies are exploring the effect of monoclonal antibodies (ex. emactuzumab and cabiralizumab) or small molecules (ex. pexidartinib) targeting CSF1 or CSF1R, either in monotherapy as in combination with

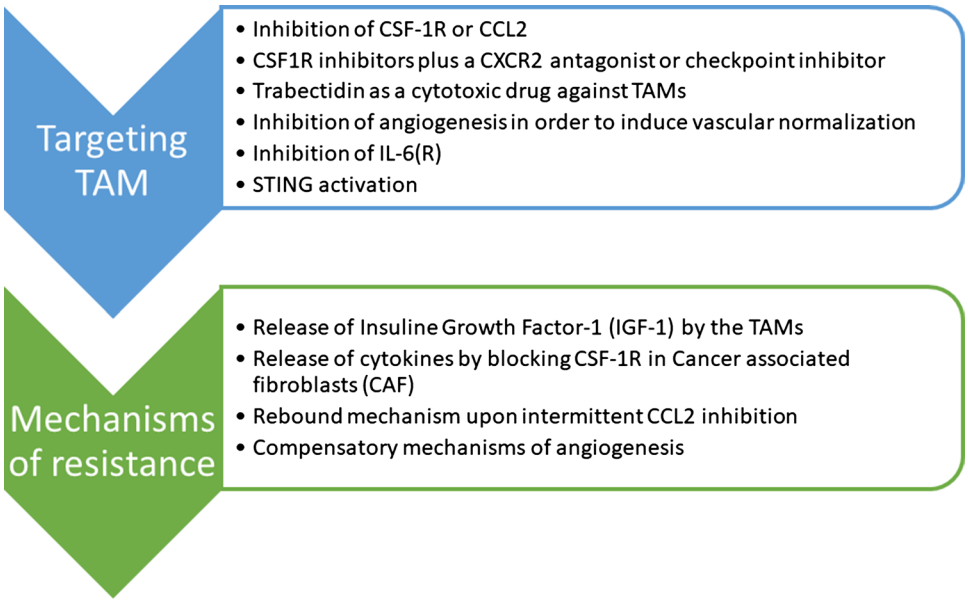

Fig. 2 Different ways to target tumor-associated macrophages (TAMs) and potential mechanisms of resistance

checkpoint inhibitors. The rationale for this combination treatment has been highlighted only recently. In mouse and human melanomas, CSF1 expression was found to correlate with the abundance of $CD8^+$ cytotoxic T cells (CTLs) and $CD163^+$ TAMs. Mechanistically, it was found that, partly because of CTL-release IFN-γ, melanoma cells increase their production of CSF1 as a kind of "defensive, immunosuppressive mechanism" to tone down the CTL response. Combination of anti-PD1 and anti-CSF1 receptor (CSF1R) antibodies induced the regression of $BRAF^{V600E}$-driven, transplant mouse melanomas, implying CSF1 and TAMs as $CD8^+$ T-cell-dependent adaptive resistance mechanism. This approach has also limitations that probably can partly explain the limited success of this approach in the clinic [109]. Quail et al. have proven that prolonged treatment with CSF1-targeted therapies causes the release of IGF-1 by the TAMs which in turn activates an IGFR-PI3 K cascade that drives resistance and tumor growth [110]. Another mechanism of resistance to anti-CSF1(R) treatment is completely engaged by the stroma. An unprecedentedly described activation of CSFR1 in cancer-associated fibroblasts (CAFs) was found recently responsible for silencing the expression of granulocytic cytokines. It follows that a CSF1 block causes the release of this cytokines with consequent recruitment of granulocytes and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC). This resistance mode is, at least in mice, overcome by the combination of CSF1R inhibitors with a CXCR2 antagonist [111]. Also targeting CCL2 led to tumor regression in a preclinical prostate cancer tumor model [112]. By blocking CCL2, not only recruitment of monocytes at the tumor site is inhibited but also the retention of metastasis-associated macrophages. Carlumab (CNT088), a human monoclonal antibody against CCL2, was evaluated in solid tumors in which it was administered either in monotherapy as well as in combination with the other chemotherapy agents [113]. The study did not show prolonged inhibition of CCL2, probably because the drug bound-free CLL2 with lesser affinity than reported from in vitro studies, making it less efficient in inhibiting CCL2 in humans [114]. Genetic and pharmacologic evidence has shown that CCL2 inhibition of MAMs in breast cancer metastasis is efficient till the treatment is endorsed [102, 115]. However, upon treatment withdrawal, a strong increase in CCL2 and other factors such as IL6 and VEGF was boosting macrophage release from the bone marrow to the metastatic site with poor disease outcome. This indicates that macrophage phenotype manipulation rather than a block in macrophage recruitment can be a safer therapeutic option [53]. Trabectedin, a marine-derived antineoplastic drug, currently used for the treatment of sarcomas, has not only direct effects against cancer cells, but also host-modulating properties such as depletion of TAMs as well as inhibition of monocyte recruitment and angiogenesis [116]. The mechanism of action is very complex, as it binds to DNA, blocks transcription, and also interferes with the DNA repair efficiency. Trabectedin is very cytotoxic for TAMs by engaging monocyte-specific TRAIL receptors 1 and 2 and mediating a caspase 8 dependent apoptosis [117].

Another key molecule involved in innate and adaptive immunity is CD40, a member of the TNF receptor superfamily, expressed on antigen presenting cells such as monocytes, macrophages, and dendritic cells. Many studies have shown that administration of an agonistic antibody directed against CD40 produced protective T-cell immunity in murine cancer models [118, 119]. Also CD47, known as integrin association protein, belonging to the immunoglobin superfamily is

an interesting therapeutic target. CD47 acts as a "don't eat me" signal to macrophages. CD47 is found to be overexpressed on cancer cells and interaction with signal-regulatory protein alpha (SIRPα) inhibits macrophage phagocytosis, allowing cancer cells to escape immune surveillance [120, 121]. Current CD47 antagonists undergoing clinical trials include, for example, Hu5F9 (an anti-CD47 antibody that directly inhibits the CD47-SIRPα interaction) and TTI-621, (a fusion protein composed of CD47-binding domain of human SIRPα and linked to the Fc region of IgG1).

Next to strategies that deplete (anti-CSF-1 antibodies and CSF-1R inhibition) or stimulate (agonistic anti-CD40 or inhibitory anti-CD47 antibodies) TAMs also pharmacologic modulation of macrophage phenotype could produce an anti-tumour effect which has been shown in a recent publication by Guerriero et al. [122]. By utilizing a macrophage-dependent autochthonous mouse model of breast cancer, they demonstrated that treatment with a class IIa histone deacetylase (HDAC) inhibitor altered the tumor microenvironment and reduced tumor burden and metastases by modulating macrophage phenotypes. Moreover, combination with chemotherapy regimens or checkpoint inhibitors significantly enhanced the durability of tumor reduction.

Interleukin-6 (IL-6) is a cytokine relevant in many inflammatory diseases and cancer. Several agents targeting either IL-6 itself (siltuximab, olokizumab, and sirukumab) or its receptor IL6-R (tocilizumab) are currently in clinical trials for inflammatory diseases such as rheumatoid arthritis but also cancer [123]. Remarkably, tocilizumab is also used in the clinic for the treatment of severe or life-threatening chimeric antigen receptor (CAR) T-cell induced cytokine release syndrome [124].

A protein called stimulator of interferon genes (STING) is part of the innate immune system as the first line of defense against pathogens. When activated, STING enforces the production of interferons and cytokines. One of the first STING analogs DMXAA (vadimezan) showed promising results in many preclinical models, but failed in a phase III clinical trial in non-small cell lung cancer [125, 126]. The lack of efficiency might be explained by DMXAA not binding to human STING. Many companies are, therefore, currently developing STING agonists or agents targeting the STING pathway, to be tested in clinical trials either in monotherapy or in combination with checkpoint inhibitors.

Finally, as hypoxia in the tumor microenvironment is a major factor that polarizes TAMs to a pro-tumoral phenotype [68], reduction of hypoxia can be an alternative approach to induce anti-tumor TAMs. This could be achieved by inducing vascular normalization through the inhibition of VEGF and angiopoietin-2. For example, a preclinical study published by Peterson et al., showed that dual inhibition of VEGFR and Ang-2 prolonged survival in glioblastoma models by reducing tumor burden, improving vessel normalization, and altering TAMs [127]. Overall, from the original aim of depleting TAMs from the tumor, the current strategy is to re-educate macrophages towards their more ancestral function, i.e., to protect the body against harmful stimuli.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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