Novel insights in the regulation and function of macrophages in the tumor microenvironment

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Purpose of review
Tumors contain not only cancer cells but also nontransformed types of cells, the stromal cells. A bidirectional interplay exists between transformed and nontransformed cells leading to tumor progression and metastasis. Tumor-associated macrophages (TAMs) are the most abundant tumor-infiltrating leukocytes characterized by a high heterogeneity and plasticity. TAMs exhibit strong protumoral activities and are related to bad prognosis and worse overall survival in various cancer types.

Recent findings
Recent progress has delineated the existence of distinct TAM subsets in primary tumors and metastatic sites regulated by diverse mechanisms and triggering strong protumoral functions such as immunosuppression, angiogenesis, metastasis and resistance to current therapies.

Summary
Delineating the regulatory pathways governing TAM heterogeneity and activation could present a novel frontier in cancer therapy. TAM targeting/repolarization is considered as a promising novel therapeutic modality in combination with standard-of-care therapies or immuno checkpoint blockers.

Keywords
immune checkpoint blockade, metastatic niche, therapy resistance, tumor microenvironment, tumor-associated macrophage

INTRODUCTION
A meta-analysis of gene expression signatures from approximately 18,000 human tumors with overall survival outcomes across 39 distinct human cancer types revealed that prognostic gene clusters reflect cancer cell-intrinsic as well as cancer cell-extrinsic features of the tumor [1**]. The former include genes functionally linked to cell proliferation, cell cycle phase, cell adhesion and epithelial-mesenchymal transitions. Cancer cell characteristics have been studied since decades, which has led to the development of efficacious cancer cell-targeting medicines that have strongly improved life expectancy of patients. However, an equally important prognostic gene cluster relates to immunological processes and immune-response genes, illustrating the importance of tumor-infiltrating leukocytes [1**]. The regulation and molecular mechanisms employed by tumor-infiltrating leukocytes have only more recently attracted the attention of cancer researchers, providing a window of opportunity for the development of novel cancer therapies directed against immune cells. In this respect, tumor-associated macrophages (TAMs) are among the most abundant tumor-infiltrating leukocytes and are related to poor prognosis in 80% of the studies [2]. Importantly, signatures of tumor-associated M2 macrophages were found to predict worse outcomes than proinflammatory M1 macrophages [1**].

ONTOGENY OF TUMOR-ASSOCIATED MACROPHAGES
During steady state conditions, macrophages mainly derive from yolk sac progenitors that either directly (e.g. microglia in brain) or through a fetal liver
monocytic progenitor (e.g. Kupffer cells in liver and Langerhans cells in skin) differentiate into distinct tissue-resident macrophages during embryogenesis and self-maintain throughout life [3]. In inflammatory settings, however, peripheral blood monocytes may differentiate into macrophages and dendritic cells and replenish the tissue pool of these cells. In the tumor microenvironment, the relative contribution of tissue-resident versus monocyte-derived macrophages is to a large extent still an outstanding question, although a major influx of classical Ly6C\textsuperscript{hi} monocytes to the primary tumor has been reported [4,5]. During tumor evolution, an 'emergency' type of myelopoiesis provides a rapid and massive generation of granulocytes and monocytes. This excessive myelopoiesis is regulated by the retinoic-acid-related orphan receptor (RORC1/ROR\textsubscript{y}) at multiple levels, by suppressing negative and promoting positive regulators of myelopoiesis, by shielding these cells from apoptosis and by mediating TAM differentiation [6]. Consequently, important changes occur in the myeloid cell composition of bone marrow and spleen during tumor growth, raising the question of which organ provides the bulk of monocyte precursors for the generation of TAM. Using mice expressing the photoconvertible protein Kikume Green-Red, the origin of tumor-infiltrating monocytes, and hence TAM, was tracked to the bone marrow, although the spleen can also contribute to some extent [5].

PHENOTYPIC AND FUNCTIONAL HETEROGENEITY OF TUMOR-ASSOCIATED MACROPHAGES IN TUMOR PROGRESSION AND METASTASIS

Different TAM subsets exist, depending on the diverse stimuli present in their specific microenvironments. This phenotypic plasticity varies between cancer types, different stages of tumor growth and different intratumoral regions (Fig. 1).
Ly6C$^{\text{high}}$ inflammatory monocytes were shown to give rise to two distinct TAM subsets in several mouse tumor models: CD11b$^{\text{high}}$Ly6C$^{\text{low}}$MHC-1$^{\text{low}}$ TAMs that reside in hypoxic tumor regions exhibit an M2-like phenotype [high expression of macrophage mannose receptor (MR), scavenger receptor-A, interleukin (IL)-4Rα and arginase-1] and are strongly angiogenic and immunosuppressive and CD11b$^{\text{high}}$Ly6C$^{\text{low}}$MHC-1$^{\text{high}}$ TAMs that are found in less hypoxic regions, exhibit an M1-like phenotype and induce proinflammatory responses [7]. Recent evidence illustrates that tumor-derived macrophage-colony stimulating factor (M-CSF) (CSF-1) regulates monocyte extravasation, proliferation and differentiation toward MHC-II$^{\text{low}}$ TAM and shapes the MHC-II$^{\text{low}}$ TAM phenotype, whereas granulocyte macrophage-colony stimulating factor (GM-CSF) fine-tunes the MHC-II$^{\text{high}}$ phenotype [4]. Along the same line, orthotopic lung tumors were shown to harbor distinct macrophage populations with a clearly different transcriptome, suggesting that distinct TAMs play specific roles in tumor promotion [8].

MMR$^{\text{hi}}$ M2-like TAMs, which also express Tie2, have been found around blood vessels following chemotherapy [9]. These cells are attracted to that site via a CXCL12-CXCR4 axis and mediate tumor revascularization and relapse via the secretion of vascular endothelial growth factor-A (VEGF-A). Furthermore, Tie2 MMR$^{\text{hi}}$ VEGF-A$^{\text{A}}$ perivascular TAMs are crucial components of the tumor microenvironment of metastasis (TMEM). TMEM is a microanatomical site within primary tumors, which regulates metastasis by controlling tumor cell escape. In TMEM, macrophages interact directly with cancer cells through a paracrine epidermal growth factor/M-CSF signaling loop leading to higher cancer cell mobility and intravasation [10]. Moreover, macrophage-derived VEGF-A is responsible for blood vessel permeability and intravasation by causing disruption of vascular junction proteins [11]. Consequently, TMEM density is predictive of metastatic potential in human breast cancer [12]. Other mechanisms of TAM-mediated metastasis have been described. A positive feedback loop has been demonstrated between GM-CSF produced by breast cancer cells and CCL18 produced by TAMs, inducing epithelial-to-mesenchymal transition (EMT) of the cancer cells, metastasis and poor patient survival [13]. Similar results have been obtained in human pancreatic ductal adenocarcinoma (PDAC) in which CCL18 produced by M2-polarized TAMs promotes EMT, leading to metastasis [14].

A distinct subset of perivascular macrophages [CCR2$^{\text{V}}$ vascular endothelial growth factor receptor 1 (VEGFR1)$^{\text{Tie2}}$], known as metastasis-associated macrophages (MAMs), has been identified at metastatic sites. MAMs were proven to promote breast cancer cell extravasation to metastatic sites such as the lung [15]. Interestingly, angiopoietin-2 (ANGP2) was responsible for CCL2 expression by endothelial cells resulting in recruitment of the CCR2$^{\text{Tie2}}$ MAMs to the metastatic site [16]. Hence, ANGP2 blockade via an ANGP2-specific Ab leads to a significant decrease in metastatic growth and an increase in survival post mastectomy in mouse breast carcinoma models [16]. The importance of the CCL2-CCR2 axis to recruit classical monocytes and their differentiation into MAMs was further illustrated by the increased lung metastasis of intravenously inoculated melanoma cells in CCR2-deficient mice [17]. The CCL2-CCR2 axis prompts CCL3 production by MAMs, which in turn activates CCR1. This CCL3-CCR1 autocrine loop leads to early accumulation and retention of MAMs at the metastatic site by reinforcing MAM-cancer cell interaction through integrin α4, resulting in lung extravasation and metastasis [15]. MAMs of breast cancer pulmonary metastasis also express Fms-related tyrosine kinase 1 (FLT1) (VEGFR1). FLT1 signaling regulates a set of downstream inflammatory genes such as M-CSF. M-CSF is a crucial factor regulating macrophage differentiation/proliferation/survival which via an autocrine loop induces metastatic seeding and growth [18]. Notably, the metastasis-promoting activity of MAMs is counterbalanced by the presence of immunostimulatory CD103$^{\text{hi}}$ DC, which ingests tumor material and triggers antitumor immunity [17]. Moreover, CCR2-independent patrolling monocytes also perform an antitumoral role by reducing tumor metastasis to the lung through scavenging tumor material and recruiting natural killer cells to the lung vasculature [19]. These data further showcase the intricate involvement of distinct myeloid cell types in metastasis formation.

Finally, recent evidence also points to an effect of the primary tumor on more distal monocytes/macrophages. A 360-sample analysis of peripheral blood monocytes from patients suffering from colorectal cancer pointed to monocyte plasticity regulated specifically by tumor-derived factors [20]. Consequently, tumor-educated circulating monocytes are powerful candidate biomarkers for diagnosis and disease follow-up of colorectal cancer and it remains to be determined to what extent these primed monocytes further promote tumor growth. In the case of human renal cell carcinoma, IL-1R signaling was shown to instruct a protumoral phenotype in circulating monocytes [21]. Resident subcapsular sinus CD169$^{\text{hi}}$ macrophages in tumor-draining lymph nodes function as scavengers of tumor-derived extracellular vesicles (tEVs) in mice and humans. In the tumor-bearing state, this
subcapsular macrophage barrier is disrupted, allowing tEVs to interact with B cells in the lymph node cortex and foster tumor-promoting humoral immunity [22].

**NOVEL REGULATORS OF TUMOR-ASSOCIATED MACROPHAGE HETEROGENEITY**

Delineating important novel signaling pathways of TAM regulation could open up new horizons for TAM repolarization/targeting. Several novel mechanisms were described for the regulation of TAM infiltration in tumors. Pentraxin 3 (PTX3), a humoral pattern recognition molecule of innate immunity, was proven to act as an extrinsic onco-suppressor whose deficiency promoted protumoral inflammation by amplification of the complement system and CCL2 production, leading to increased TAM infiltration and tumor promotion. The PTX3 gene is epigenetically silenced in many human tumors such as colorectal carcinoma from an early tumor stage [23]. Another secreted factor, milk fat globule-epidermal growth factor 8, produced by tumors such as colorectal carcinoma from an early tumor stage [23]. Another secreted factor, milk fat globule-epidermal growth factor 8, produced by bone marrow-derived mesenchymal stromal cells, mediated acceleration of melanoma tumor progression and decreased survival in mice, at least partly because of enhanced M2-like CD206+ TAM infiltration [24]. M2 TAMs are also recruited to glioblastoma multiforme tumors (GBMs) via peristin (POSTN). Glioma stem cells produce POSTN which, in turn, recruits circulatory monocyte-derived protumoral TAMs through integrin α,β3, leading to enhanced tumor growth and decreased survival [25]. Recently, the metastasis suppressor Raf kinase inhibitory protein (Rkip) was shown to block high-mobility group AT-hook 2 (HMGA2), leading to a decreased production of the TAM chemotactic factor CCL5 in triple-negative breast cancer, which in turn inhibits TAM recruitment and the induction of a prometastatic TAM phenotype [26]. CCL5 overexpression in rKIP(+) tumors restores recruitment of prometastatic TAMs and intravasation, whereas treatment with the CCL5 receptor antagonist Maraviroc reduces TAM infiltration.

M-CSF is known since several years to regulate TAM differentiation, as well as to determine their M2 orientation [27,28]. More recently, granulocyte macrophage-colony stimulating factor receptor (GM-CSFR) and macrophage-colony stimulating factor receptor (M-CSFR) signaling were found to fine-tune the phenotype of distinct M1-like and M2-like TAM subsets, respectively [4], suggesting the predominance of these cytokines in separate tumor regions. Along the same line, IL-13 was reported to stimulate TAM’s M2 orientation, but this was counterbalanced by intratumoral tumor necrosis factor secretion, again illustrating the simultaneous presence of opposing cytokines in the tumor microenvironment [29]. In fact, TAMs developed mechanisms that limit the production of inflammatory mediators. For example, the p38α-tristetraprolin (TTP) axis is an important regulator of inflammatory cytokine/chemokine production, whereby TTP is an adenosine uracil-rich RNA-binding protein that downregulates the expression of inflammatory chemokines/chemokines by inducing adenosine uracil-rich mRNA decay. In late stage TAMs, TTP expression is constitutive without effect on adenosine uracil-rich mRNA stability but with a suppressive effect on translation of adenosine uracil-rich mRNAs. Interestingly, elimination of TTP caused excessive inflammatory mediator output by TAMs and tumor cell death [30]. Similarly, absence of Dicer-1, a micro-RNA (miRNA)-processing enzyme, in TAMs skew TAM polarization into a proinflammatory, M1-activation state, resulting in retarded tumor growth. The Dicer-1 effect is mediated, in part, through the Let-7-5p miRNA family which silences an interferon-gamma-induced proinflammatory TAM state [31]. Of interest, several other miRNAs are also important regulators, either as activators or suppressors, of TAM activation/infiltration [32,33]. Notably, in pancreatic ductal adenocarcinoma, the immunosuppressive properties of TAM are regulated by necroptosis-induced SAP130 that binds to the macrophage receptor Mincle [34]. Blockade of necroptosis or Mincle initiates T-cell-mediated antitumor immunity.

Apart from secreted factors, hypoxia is an important feature of the tumor milieu in many cancer types, which is known to attract many macrophages. Indeed, hypoxia promotes the upregulation of Semaphorin 3A, which, in turn, attracts TAMs via interacting with Neuropilin-1 (Nrp1) and the induction of a PlexinA1/A4-dependent VEGFR1 transactivation [35]. In the absence of Nrp1, TAMs accumulate outside of the hypoxic areas and adopt a more M1-like and immunostimulatory phenotype, resulting in reduced tumor growth and metastasis. Hence, hypoxia instructs a protumoral activity on macrophages. Accordingly, a better oxygenation of tumors does not influence TAM differentiation, but rather affects the M2-like phenotype of TAMs by downregulating hypoxia-sensitive and angiogenesis-related genes [7]. In addition, hypoxia promotes the upregulation of CD45 tyrosine phosphatase activity in monocyctoid myeloid-derived suppressor cells (MO-MDSCs), which leads to a downregulation of signal transducer and activator of transcription 3 (STAT3) activity resulting in a trans-differentiation of these
MDSCs into TAMs [36]. An intriguing question is how hypoxia instructs the TAM phenotype. One possibility is the enhanced production of lactate through anaerobic glycolysis in hypoxic tumor regions. Indeed, tumor-derived lactate induces hypoxia-inducible factor 1 alpha-mediated TAM polarization toward an angiogenic M2 state [37].

**NOVEL REGULATORS OF TUMOR-ASSOCIATED MACROPHAGE-MEDIATED THERAPY RESISTANCE**

Of great importance is the TAM-dependent tumor resistance to current therapies such as radiation therapy [2], chemotherapy [9**] and antiangiogenic therapy [38*], as well as resistance to CSFR-1R inhibition [39*]. Indeed, therapeutic failure and subsequent metastasis are the main causes of cancer-related lethality, so a better understanding of the molecules that regulate TAM-mediated therapy resistance is warranted.

Phosphatidyl inositol 3-kinase (PI3K) signaling in myeloid cells is responsible for resistance to antiangiogenic therapy that targets the VEGF-VEGFR pathway [38*]. Cancer cells directly induce PI3K signaling in myeloid cells, after which activated PI3K initiates an immunosuppressive and angiogenic state in these cells. PI3Kγ signaling is activated, among others, by hypoxia-related molecules such as stromal cell-derived factor-1 alpha (SDF-1α) and IL-6, leading to the speculation that TAMs in hypoxic regions may be responsible for resistance to antiangiogenic therapy. Along the same line, it was demonstrated that inhibitory targeting of PI3Kγ stimulates antitumor immune responses, leading to improved survival and responsiveness to standard-of-care chemotherapy in animal models of PDAC [40]. PI3Kγ regulates an immunosuppressive transcriptional program in macrophages, so blockade of PI3Kγ in PDAC-bearing mice was able to reprogram TAMs to stimulate CD8(+) T-cell-mediated tumor immunity and to inhibit tumor cell invasion, metastasis and desmoplasia. In several mouse tumor models, tumors relapse after chemotherapy. As mentioned earlier, this phenomenon is mediated by perivascular TAMs that are recruited by CXCL12 [9**]. It is unclear which environmental signals instruct the protumoral/angiogenic phenotype of these perivascular macrophages, although the authors suggest a high level of oxidative stress in these postchemotherapy regions. Upon antibody-mediated colony stimulating factor-1 receptor (CSF-1R) blockade, about 50% of GBMs in mice eventually recur. In this scenario, TAMs are stimulated by IL-4 to activate STAT6 and nuclear factor of activated T cells (NFAT), leading to the expression and secretion of insulin-like growth factor-1 (IGF-1). IGF-1R ligation on cancer cells and subsequent PI3K activation in these cells leads to their survival and invasiveness, boosting tumor recurrence [39*].

**CONCLUSION**

Considering the implication of TAMs in stimulating primary tumor growth, metastasis and relapse, these cells are now considered as promising novel therapeutic targets. In this respect, strategies to shift the balance to a more antitumoral, immunostimulatory type of TAM activation are elegant and could be combined with current immune checkpoint blockade therapies. For example, in PDAC, M-CSFR blockade was shown to reprogram TAMs, leading to a T-cell-mediated antitumor response as well as enhancing the efficacy of immune checkpoint therapy with programmed death 1 (PD1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antagonists [41*]. Many molecules have by now been targeted to trigger TAM repolarization to an antitumoral M1 state. Very recent examples are PI3Kγ and/or Bruton’s tyrosine kinase (BTK) inhibition in PDAC [40,42], anti-Ang-2/VEGF bispecific antibody-mediated inhibition which could also overcome resistance to anti-VEGF therapy in GBM [43,44] and anti-macrophage receptor with collagenous structure (MARCO) antibodies targeting suppressive MARCO+ TAMs which could also ameliorate the effect of anti-CTLA4 therapy in melanoma and colon cancer [45]. Most likely, more molecular targets on TAMs will be identified and the challenge will be to identify the most promising targets that are amenable to therapeutic intervention in combination with standard-of-care therapies or the novel generation of immune checkpoint blockers.

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**Conflicts of interest**

_There are no conflicts of interest._
REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest


This article integrates data from 18000 human tumors across 39 malignancies to show correlations between the immune cell infiltrate and disease outcome.


The first demonstration that M-CSF and GM-CSF differentially steer monocyte development within tumors.


Making use of photoconversion to show that the majority of tumor-infiltrating monocytes are derived from the bone marrow.


The first proof that hypoxia affects the phenotype of one TAM population without influencing monocyte differentiation in tumors.


This article establishes a clear link between perivascular CD206-high macrophages and tumor release after chemotherapy.


One of the first articles to clearly describe a role for macrophages at the metastatic site and the mechanisms via which these cells are regulated.


State-of-the-art imaging providing a detailed insight in the early events of cancer cell dissemination to the lung.


The first demonstration that patrolling monocytes contribute to the progression of cancer by inhibiting lung metastasis.


Together with reference [28], this article reveals blocking anti-CSF-1R antibodies to be a valuable therapeutic option.


One of the rare studies investigating the protumoral mechanisms of monocytes/macrophages in cancer patients. IL-1R signaling was shown as tumor promoting pathway.


This is one of the first articles to demonstrate an important role for macrophages outside of the tumor microenvironment during tumor promotion.


Clear demonstration that the effectiveness of antiangiogenic therapy is hampered by TAM.


The first article to analyze in detail the mechanisms behind tumor resistance against anti-CSF-1R therapy.


Clear demonstration that the effectiveness of immune checkpoint blockade is hampered by TAM.
Novel insights in the regulation of tumor macrophages Bolli et al.


