DOI: 10.1002/JLB.MR0218-056RR

JLB JOURNAL OF LEUKOCYTE BIOLOGY

REVIEW

# The emerging roles of macrophages in cancer metastasis and response to chemotherapy

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## Abstract

Macrophages represent a heterogeneous group of cells, capable of carrying out distinct functions in a variety of organs and tissues. Even within individual tissues, their functions can vary with location. Tumor-associated macrophages (TAMs) specialize into three major subtypes that carry out multiple tasks simultaneously. This is especially true in the context of metastasis, where TAMs establish most of the cellular and molecular prerequisites for successful cancer cell dissemination and seeding to the secondary site. Perivascular TAMs operate in the perivascular niche, where they promote tumor angiogenesis and aid in the assembly of intravasation sites called tumor microenvironment of metastasis (TMEM). Streaming TAMs co-migrate with tumor cells (irrespective of the perivascular niche) and promote matrix remodeling, tumor cell invasiveness, and an immunosuppressive local microenvironment. Premetastatic TAMs are recruited to the premetastatic niche, where they can assist in tumor cell extravasation, seeding, and metastatic colonization. The dynamic interplay between TAMs and tumor cells can also modify the ability of the latter to resist cytotoxic chemotherapy (a phenotype known as environment-mediated drug resistance) and induce chemotherapy-mediated pro-metastatic microenvironmental changes. These observations suggest that future therapeutics should be designed to target TAMs with the aim of suppressing the metastatic potential of tumors and rendering chemotherapy more efficient.

KEYWORDS

cancer metastasis, tumor-associated macrophages, chemotherapy, environment-mediated drug resistance (EMDR)

## 1 | INTRODUCTION

In recent years, the role that the tumor microenvironment plays in cancer progression and metastasis has garnered much interest. New targeted therapies are now not only focused on targeting tumor cells themselves, but also on disrupting the interactions between tumor and stromal cells.[1] Traditionally, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), and various components of the cancer-associated endothelium have been considered the most impactful stromal cells in the tumor microenvironment.[2–13] Indeed, the traditional "hallmarks of cancer," described nearly 20 years ago,[14] focused on the acquisition of 6 critical disease hallmarks, either via cell autonomous mechanisms (e.g., driver mutations), or via heterotypic interactions between tumor and stromal cells, with an emphasis on CAFs, TAMs, and endothelial cells. More recently, however, the contribution of other stromal cells (or more distinctive subtypes of the aforementioned ones) begun to emerge. These include, but are not limited to, adipose cells, pericytes, neutrophils, bone marrow-derived progenitor cells, and mesenchymal stem cells.[1,15–24] An exhaustive description of these stromal cells in cancer progression would be complex and beyond the scope of this review. Here, we focus rather on the TAMs, perhaps the most influential stromal contributor in many solid carcinomas, and examine their role in regulating cancer

Abbreviations: ANG2, angiopoietin 2; CA4P, combretastatin-A4-phosphate; CAF, cancer-associated fibroblast; CD, cluster of differentiation; CSC, cancer stem cell; DCs, dendritic cells; ECM, extracellular matrix; EMDR, environment-mediated drug resistance; FGF, fibroblast growth factor; HDAC, histone deacetylase; IF, immunofluorescence; IHC, immunohistochemistry; ISH, in situ hybridization; KLK, kallikrein-related peptidase; LGR4, leucine-rich repeat-containing G-protein coupled receptor 4; MAFIA, macrophage-associated FAS-induced apoptosis; MENA, mammalian enabled; MMP, matrix metalloproteinase; MMTV, mouse mammary tumor virus; MRC1, mannose receptor; PA, plasminogen activation (-or); PDGF, platelet-derived growth factor; PDX, patient-derived xenograft; PSGL1, P-selectin glycoprotein ligand-1; PyMT, polyoma middle-T antigen; RSPO, R-spondin 1–4; SDF1, stromal derived factor 1; SNAIL, zinc finger protein SNAI1; STAT3, signal transducer and activator of transcription-3; TAM, tumor-associated macrophage; TEC, tumor endothelial cell; TMEM, tumor microenvironment of metastasis; VE-CAD, vascular-endothelial cadherin; VEGF, vascular endothelial growth factor; ZO1, zonula occludens-1

Received: 19 September 2018 | Revised: 7 January 2019 | Accepted: 8 January 2019

  

metastasis, as well as in regulating tumoral and immune responses to cytotoxic chemotherapy.

### 1.1 | Recruitment and maturation of TAMs in the tumor microenvironment

Among the plethora of stromal cell types in the tumor microenvironment, TAMs are among the best-studied ones. In general, macrophages play very important roles in tissue homeostasis, and they participate in a variety of pathophysiologic conditions, including cancer.[25–27] The extravasation of peripheral monocytes into the tumor microenvironment leads to their differentiation into tissue macrophages, which subsequently display a continuum of specialized phenotypes whose extremes are described as proinflammatory (M1) and anti-inflammatory (M2).[28–30] The recruitment of TAMs is a complicated process heavily dependent on the microenvironmental "context": cancer cell-mediated secretion of chemokines (e.g., CCL2); cytokines (e.g., IL-4, IL-13); and growth factors (e.g., vascular endothelial growth factor [VEGF], macrophage CSF [M-CSF or CSF1], granulocyte-macrophage CSF [GM-CSF or CSF2]). For example, the proinflammatory circulating monocytes expressing the $CCR2^{+}$ can readily respond to and subsequently infiltrate via chemotaxis, CCL2-producing tumors. Thus, the subsequent differentiation and maturation of these monocytes into functional TAMs depends on the specific cytokines and growth factors present in the local tumor microenvironment. It has been suggested that the CSF1/CSF1R axis is the most critical pathway that influence the monocyte fate.[27,31–38]

Although the pathways describing infiltration and maturation of bone marrow-derived monocytes are well established, tissue-resident macrophages of embryonic origin (i.e., those derived from the yolk sac and/or fetal liver) also contribute significantly to the TAM population. In pancreatic ductal adenocarcinoma (PDAC) for example, a proportion of TAMs, shown to be of embryonic origin, assumes functions independent of bone marrow-derived monocytes.[39] In transgenic K-$ras^{LSL\text{-}G12D/+}$/$p53^{R127H/+}$/PdxCre harboring PDAC tumors, these tissue-resident macrophages are capable of proliferation and self-expansion, and they express high levels of pro-fibrotic ECM-remodeling factors that facilitate tumor progression.[39] Importantly, the suppression of the CSF1/CSF1R axis in this tumor model does not significantly affect this TAM subpopulation (although it partially reduces tumor size), suggesting that PDAC progression is in part regulated by tissue-resident TAMs.[39]

### 1.2 | Functional diversity of TAMs in the tumor microenvironment

One critical question is: what roles do mature TAMs play in the primary tumor after they have been recruited? This is a difficult question to answer as these functions depend heavily on the context under which TAMs are recruited, as well as the interactions they experience with the local tumor microenvironment upon arrival.[28,29,40–44] Originally, researchers had divided TAMs into tumor-promoting and tumor-suppressive. However, as discussed earlier, these phenotypes are dynamic and interchangeable in a context-dependent manner.[8,30,40,41,43,45,46] This functional diversity leads to TAMs interacting not only with cancer cells, but also with a multitude of stromal cells, and participating in juxtacrine and/or paracrine signaling interactions all of which dictate the fate of tumor growth, metastasis, and other hallmarks of the disease.[1,25,38,41,43]

Early literature focused on TAMs as critical mediators of angiogenesis in various solid carcinomas, and TAMs have been associated with poor prognosis in breast cancer.[47] To date, TAMs are viewed as central mediators of most hallmarks of cancer, not only inflammation and angiogenesis. As will be thoroughly described in section 2 later, they can regulate the immunologic microenvironment, tumor growth, epithelial-to-mesenchymal transition (EMT), cancer stem cell (CSC) induction and maintenance, as well as dissemination and metastasis, including critical functions in secondary metastatic sites (e.g., preparation of a tumor-receptive premetastatic niche, colonization and re-dissemination of tumor cells to tertiary metastatic sites). These emerging concepts will be detailed in the current review.

### 1.3 | Polarization schemes of TAMs—oversimplification or not?

All the diverse functions of TAMs described earlier were mainly understood by categorizing them into specialized subtypes such as M1 and M2 macrophages. In this scheme, classically activated (M1-polarized) macrophages are activated by cytokines such as IFN-$\gamma$; they produce proinflammatory and immunostimulatory cytokines (IL-12 and -23), and they are involved in Th1 immune responses. Alternatively activated (M2-polarized) macrophages are activated by Th2 cytokines (IL-4, -10, and -13), and they promote proliferation, invasion and metastasis of tumor cells, angiogenesis, and immunosuppression.[6,41,48] Over the years, multiple sets of immunohistochemical and/or cell surface markers were proposed to distinguish between M1- and M2-polarized states in TAMs.[49] However, phenotypes that could not be explained by the traditional M1/M2 polarization paradigm were also found. To address this, Mantovani et al. proposed further subcategorization of M2 macrophages into M2a, M2b, and M2c, based on the specific mechanism of M2 phenotype induction.[50] However, even this subcategorization still may not fully describe the continuum of TAM phenotypes observed, and, although still widely accepted, due to convenience for understanding macrophage-related diseases, the M1/M2 dichotomy is increasingly viewed as too bipolar and oversimplified.[51,52] In one of the most comprehensive studies to-date, Gubin et al. (2018), performed RNAseq and CyTOF analyses of immune cell populations in the tumor microenvironment and defined 5 categories (based on gene-expression profiling), and 8 categories (based on protein-expression profiling) of monocytes/macrophages that could be distinguished by the markers CD206, CX3CR1, CD1d, and iNOS.[53] The study, however, concluded that the functional and structural diversity of macrophages within the tumor microenvironment reflects mostly to the activation and polarization of infiltrating monocyte subpopulations, rather than of preexisting, intratumoral macrophages.[53] In this review, we describe TAM functions, their involvement in cancer metastasis and response to cytotoxic chemotherapy, all from the viewpoint of their spatiotemporal localization within the tumor microenvironment, rather than their polarization status.

## 2 | TUMOR-ASSOCIATED MACROPHAGES IN CANCER METASTASIS

TAMs secrete a variety of ECM components, proteolytic enzymes, and other ECM-remodeling factors that act to modulate the tumor microenvironment, regulate angiogenesis, and facilitate the metastatic cascade in a context-dependent manner.[54–56] However, recent studies suggest that macrophages may not be simply "ECM-managers," but rather, active tumor cell partners involved in signaling networks which dictate cell fates during metastasis.[37,43,57] In this chapter, we describe studies that highlight these emerging roles of TAMs.

### 2.1 | TAMs as "ECM-managers" in the tumor microenvironment

The activated stromal cells in many solid carcinomas, primarily CAFs and TAMs, can readily secrete extracellular matrix (ECM)-remodeling factors, extracellular proteases, and/or protease inhibitors, which may directly or indirectly organize: collagen composition and structure (including collagen crosslinking); bioavailability of ECM-bound growth and chemotactic factors; extracellular receptor profiles; as well as the general tissue elasticity,[58–61] all of which may provide efficient conduits to metastasizing tumor cells. The effects of extracellular proteolysis, and enzyme-dependent remodeling of ECM in particular, have been long recognized as key factors of cancer cell invasion, migration, and metastasis.[54–56,62]

There are several extracellular proteolytic systems that are relevant in the context of cancer progression, but the plasminogen activation (PA) system, the matrix metalloproteinases (MMPs), and the recently described kallikrein-related peptidases (KLKs) have been the most thoroughly investigated families.[56,62–65] Individual members of these families have been thoroughly investigated in a significant number of reports (please refer to Almholt and Johnsen, 2003[66] and references therein), and the overall conclusion is that proteolytic systems tend to localize in activated stromal cells, including TAMs.

However, emerging evidence demonstrates that whereas the expression of proteolytic components is primarily mediated by fibroblasts, macrophages, and endothelial cells, tumor cell-mediated proteolysis of ECM and basement membranes also occurs and plays a significant role in metastasis. It has been shown that TAM-tumor cell interactions can lead to the formation of invadopodia in tumor cells.[67,68] Invadopodia are actin-forming, invasive cellular protrusions capable of degrading ECM through localized deposition of proteases, such as MT1-MMP, on their cell surface.[69] Indeed, in certain microanatomic contexts, such as during transendothelial migration of tumor cells, invadopodium-mediated ECM degradation by tumor cells (a phenotype elicited by TAMs), and not proteolysis by stromal cells, is critical for achieving this step of the dissemination pathway.[67,68]

### 2.2 | The emerging roles of TAMs in CSC induction and maintenance

TAMs, and their secreted products, are involved in the induction of EMT in many cancer settings.[57,70–72] EMT, originally described as a crucial cell-biologic program in embryonic development, is frequently phenocopied by metastasizing cancer cells, and involves a considerable re-allocation of the gene- and protein-expression profile from epithelial-like into a mesenchymal-like pattern, which facilitates the invasive and migratory capacity of tumor cells.[73–76] EMT is almost exclusively regulated by contextual signals and cues originating in the local microenvironment, including those derived by TAMs, CAFs, and other stromal cells.[73,75,76] Quite interestingly, it has been recently shown that distinct EMT programs, such as the one controlled by the EMT-transcriptional regulator SNAIL, may be associated with stem cell reprogramming. Moreover, EMT regulates CSC properties (e.g., tumor-initiating capability), which coincide with traditional stem cell-surface marker expression patterns (e.g., $CD44^{HIGH}/CD24^{LOW}$).[77–81]

The normal development of certain epithelia, such as the mammary gland, requires the presence of macrophages, which have been proposed to constitute part of the normal mammary stem cell niche.[82] More recent studies propose that TAMs may be involved in the induction and maintenance of the CSC niche, as well. For instance, it has been shown that if TAMs are co-injected with $CD90^{HIGH}$ CSCs, then the tumor-initiating activity and metastatic efficiency are significantly increased.[83] This suggests that macrophages can support or expand the CSC population, which was shown to result from a contact-dependent induction of the stem cell supportive cytokines IL-6 and IL-8 in tumor cells, following macrophage-induced activation of Eph4A signaling in tumor cells.[83]

In a different model of breast cancer, TAMs were shown to be important for CSC maintenance via a contact-independent mechanism involving a paracrine EGFR/STAT3/Sox2 signaling pathway.[84] However, these studies do not indicate whether the macrophages drive expansion of the CSC population by promoting expansive self-renewal and/or enhancing survival of existing stem cells, or whether they might be re-inducing a stem cell phenotype in their more differentiated offspring. Thus, more work is necessary to fully understand the relationship between TAMs and CSCs.

### 2.3 | The emerging roles of TAMs in cancer cell dissemination and intravasation

Monocyte infiltration in tumors is mediated by paracrine loops involving chemotactic receptors, such as CCR2 (see section 1.2). However, distinct chemotactic pathways (CXCL12/CXCR4 and CSF1/CSF1R) are involved in the translocation of specific TAM populations to various compartments within the tumor mass, such as for example, toward or away from blood vessels.[85] Tumor cells and tumor-associated stromal cells, including CAFs, often up-regulate and release systemically the corresponding ligands for these chemotactic pathways, resulting in increased myeloid cell and monocyte chemotaxis.[86–89] Once within the tumor microenvironment, TAMs can form heterotypic groupings with tumor cells and/or other stromal cells, and, through intricate juxtacrine and paracrine signaling networks/loops, can facilitate the metastatic process. Two examples of prominent heterotypic interactions among TAMs, tumor cells, and other stromal cells include: the assembly and function of a specialized cancer cell intravasation site called "tumor

microenvironment of metastasis" (TMEM) and cancer cell "streaming" migration toward TMEM sites as discussed next.

Previously, multiphoton intravital imaging of breast cancer in live mice has demonstrated that intravasation does not occur throughout the entirety of the cancer-associated endothelium, but instead is localized to specific microanatomical doorways (TMEM) composed of a tumor cell (expressing the actin-regulatory protein mammalian enabled [Mena]), a perivascular macrophage, and an underlying endothelial cell—all in direct physical contact with one another.[90–92] Given that TMEM is the only known site where cancer cell intravasation has been directly observed, it is not surprising that TMEM density in patient tumors, as measured by standardized IHC assays, serves as a clinically validated, independent prognostic indicator of metastatic recurrence.[91,93,94] Kinetic, high-resolution vascular permeability studies have demonstrated that vascular permeability associated with tumor cell intravasation is always transient and strictly localized to TMEM sites.[92,95] Further IHC/IF analyses on TMEM sites have indicated that each functional TMEM site is composed of a perivascular macrophage expressing high levels of TIE2, VEGFA, and mannose receptor (MRC1),[92] suggesting they could represent a distinct subpopulation of M2 or M2-like TAMs. $TIE2^{HIGH}VEGFA^{HIGH}MRC1^{HIGH}$ macrophages have been intensely studied[8,96–115] as they can induce pro-angiogenic, pro-metastatic, immunosuppressive, and chemoresistant niches in a context-dependent manner. For example, using the well-described MMTV-PyMT mouse model of breast carcinoma, it was demonstrated that VEGFA secreted by the $TIE2^{HIGH}$ macrophage on TMEM sites disassembles the underlying vascular junction proteins, zonula occludens-1 (ZO1) and vascular-endothelial cadherin (VE-CAD), exposing a paracellular passage that metastasizing tumor cells use to escape into the circulation.[92] Thus, $TIE2^{HIGH}VEGFA^{HIGH}MRC1^{HIGH}$ macrophages are attractive pharmacologic targets for suppression of cancer progression.

The metastasizing tumor cells are highly migratory and highly invasive and are involved in paracrine/juxtacrine interactions with intratumoral TAMs, which are phenotypically divergent from the $TIE2^{HIGH}$ perivascular TAMs. Migratory tumor cells, along with their co-migrating TAMs, utilize one-dimensional highways composed of linearized collagen fibers that are directed toward the vasculature: a process known as multicellular "streaming" migration.[38,116] Typically, such streaming tumor cells have already undergone EMT and shifted their gene and protein-expression signature into that of a mesenchymal cell which facilitates their movement through the ECM.[117–119] Moreover, these tumor cells have an alternatively spliced Mena isoform pattern, which includes a prominent shift from the "noninvasive" isoform, Mena11a, to the more "invasive" isoform, $Mena^{INV}$: an expression pattern that has been described as $Mena^{Calc}$.[38,116–118,120–124] Mena is an actin-binding protein expressed by most cell types exerting migratory or protrusive functions, and is involved in cofilin-stimulated actin polymerization, a key activity that determines chemotactic migration and invasion.[38,116,122,125–127] The $Mena11a^{LOW}Mena^{INV\text{-}HIGH}$ isoform splicing pattern is particularly critical in streaming tumor cells, because $Mena^{INV}$ increases receptor sensitivity to chemotactic signals (e.g., EGF, HGF, and insulin growth factor-1 [IGF1]) secreted by stromal cells, including the partnering TAMs.[38,118,119,122,126,128–131] Moreover, $Mena^{INV}$ is critical for the formation and function of highly specialized, matrix-degrading cellular protrusions known as invadopodia that have been shown to orchestrate transendothelial migration and metastatic dissemination.[69,132,133] In this context, $Mena^{INV}$ plays a major role in promoting cortactin phosphorylation, and thus invadopodium maturation, by inhibiting a critical phosphatase, protein tyrosine phosphatase-1B (PTP1B).[132]

Co-migrating TAMs are critical to the process of cancer cell streaming, because these TAMs induce and maintain most (if not all) phenotypic characteristics of tumor cells leading up to migration, invasion, and interactions with the TMEM site. First, a juxtacrine pathway between TAMs and tumor cells is important for the induction and maintenance of $Mena^{INV}$ expression in the tumor cells. In particular, TAM-mediated Notch1 signaling results in a prominent up-regulation of $Mena^{INV}$ expression in the streaming tumor cells, both in vitro and in vivo, and the pharmacologic inhibition of the Notch pathway or suppression of direct cell-to-cell contact significantly reduces $Mena^{INV}$ expression in tumor cells.[133,134] Second, a paracrine pathway between TAMs and tumor cells assists in directional streaming toward the blood vessel. In particular, in vitro and in vivo evidence has demonstrated that streaming migration occurs in response to a well-described EGF/CSF1 paracrine loop. In this paracrine signaling loop, the tumor cells express EGFR and secrete CSF1, whereas TAMs express CSF1R and secrete EGF. Superimposed to the EGF/CSF1 relay chemotaxis is an endothelium-generated hepatocyte growth factor (HGF) gradient, which attracts cancer cell-macrophage streaming pairs toward blood vessels, where they intravasate at TMEM.[131,135,136]

From the earlier descriptions, it is evident that TAMs accompanying tumor cells during multicellular streaming migration are not identical to the $TIE2^{HIGH}VEGFA^{HIGH}MRC1^{HIGH}$ TAMs observed in perivascular areas or TMEM sites,[85] and that both types of TAMs respond to different sets of cytokines/chemokines, display different phenotypes, and individually serve distinct functions during cancer cell metastasis. However, experiments conducted in transgenic animal models in which macrophages were systemically depleted (e.g., FAS-induced apoptosis [MAFIA] mouse model), clearly indicate that TAMs are critical modulators of cancer cell dissemination and metastasis.[92] As such, the pharmacologic targeting of critical pathways involved in any of the steps described earlier, should result in suppressing metastasis. For example, a conditional VEGFA-KO mouse model of breast carcinoma in which VEGFA expression was specifically deleted in the monocyte/macrophage lineage, results in breast tumors with unaffected TMEM assembly, but impaired VEGF-dependent vascular wall disruption and cancer cell dissemination. These observations suggest that specific inhibitors targeting $TIE2^{HIGH}$ macrophages, such as rebastinib,[114] could be potentially used along with chemotherapy to suppress metastatic dissemination and growth, respectively.[110,114,137]

## 2.4 | The emerging roles of TAMs in local immunosuppression

It has been long known that tumor-promoting TAMs also promote an immunosuppressive tumor microenvironment. Certain notable

mechanisms include the secretion of immunosuppressive cytokines such as IL-10 and TGF-$\beta$ to suppress cytotoxic T-cell mediated antitumor immunity and dendritic cell (DC) maturation.[30,138–140] Interestingly, the production of IL-10 can also induce the expression of the co-stimulatory molecule PD-L1 in monocytes.[141] It has also been shown that TAMs found in hypoxic regions express PD-L1 in an HIF1a-dependent manner.[142] PD-L1, expressed by immunosuppressive macrophages under these circumstances, is a specific ligand for the inhibitory receptor programmed cell death protein 1 (PD1), which suppresses T-cell cytotoxic functions.[141] Other cytokines released by TAMs, such as CCL17, -18, and -22 may function as chemotactic factors, whereas additional mediators, such as $PGE_2$ and indolamine 2,3-dioxygenase, play important roles in the induction of T-regulatory cells (Tregs), which, in turn, suppress T-cell responses.[13,138,143]

Interestingly, it has been shown that macrophage elimination or repolarization strategies can also restore antitumor immunity, in particular $CD8^+$ T-cells, and improve cancer immunotherapy.[144] For instance, Tan et al. (2018) showed that leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4) and ligand R-spondin 1–4 (RSPO) interactions can induce a tumor-promoting phenotype in TAMs, characterized by suppression of $CD8^+$ T-cell activity, and resistance to immune checkpoint inhibitors in lung cancer and melanoma.[145] Indeed, specific inhibition of the LGR4/RSPO pathway resulted in TAM reprogramming, enhanced $CD8^+$ T-cell activity, and restored the sensitivity of the tumors to the immune checkpoint inhibitors.[145] In another approach, Guerriero et al. (2017) used a selective class IIa histone deacetylase (HDAC) inhibitor, TMP195, capable of modulating monocyte responses to CSF1-CSF2, and observed TAM repolarization in vivo, consistent with enhanced antitumor immunity and reduced tumor burden.[146] More importantly, the combination of this TAM repolarization strategy with immunotherapy produced an even more dramatic reduction of tumor burden and therapeutic efficacy.[146]

Because TAMs pair up with tumor cells while streaming to TMEM sites (as described in section 2.3), such TAM-dependent immunosuppressive mechanisms may provide localized immunosubversion along the metastatic pathway, allowing the metastasizing tumor cells to avoid immunologic destruction while disseminating. Interestingly, however, TAMs have also been shown to suppress $CD8^+$ T-cell activity via production of reactive oxygen species in metastatic sites.[147] This suggests that TAM-dependent immunosuppression is an essential program that accompanies tumor cells through the metastatic process, and dealing with it will be paramount for the efficacy of antitumor therapies and immunotherapies.

### 2.5 | The emerging roles of TAMs in the formation of the premetastatic niche

Accumulating evidence demonstrates that TAMs also play (through a complicated interplay with other immune cells) important roles in forming premetastatic niches in the organs to which tumor cells eventually metastasize. For instance, TAM-secreted TNF-$\alpha$, VEGF, and TGF-$\beta$ originating in the primary tumor, are believed to be transported through the bloodstream to distant organs where they induce naïve, tissue-resident macrophages to produce S100A8 and serum amyloid A3, which in turn recruit macrophages and tumor cells to the secondary sites and promote the formation of metastatic foci.[148] In yet another example, $CCR2^+$ TAMs are recruited in the premetastatic niche via CCL2, where they subsequently secrete CCL3 to increase their retention in the metastatic foci and to prolong tumor cell-TAM interactions, leading to metastatic colonization.[32] It was later demonstrated that circulating monocytes that migrate to the metastatic site first differentiate into $CD11b^{high}Ly6C^{high}$ metastasis-associated macrophage precursor cells (MAMPCs) (which confer an immunosuppressive microenvironment), and later differentiate into mature metastasis-associated macrophages (MAMs) capable of promoting the remaining hallmarks of metastasis, including colonization.[147] It is therefore clear that macrophages in the premetastatic niche can also undergo certain transitions, dynamically in time and space, to facilitate tumor metastasis.

In a final example showing the importance of TAM-mediated immunosuppression in the premetastatic niche, $CXCR2^+$ myeloid-derived suppressor cells (MDSCs) are recruited to the premetastatic niches as a result of TAM- and tumor cell-secreted CXCL1, -2, and -5 in the primary tumor site.[149–151] Once $CXCR2^+$ MDSCs are recruited, they can further attract monocytes/macrophages and other hematopoietic cells, and together form an immunosuppressive microenvironment susceptible to tumor seeding and growth.[152] Overall, TAMs play critical roles in the formation of a tumor-receptive, immunosuppressive microenvironment in metastatic sites through complex interactions with tissue-resident or newly recruited stromal cells.

## 3 | TUMOR-ASSOCIATED MACROPHAGES IN RESPONSE TO CHEMOTHERAPY

It has been long known that cytotoxic chemotherapies induce extensive tissue damage, accompanied by hypoxia, apoptosis, and necrosis, in the primary tumor microenvironment, and most likely in the microenvironment of the metastatic tumor sites. Chemotherapy-induced tissue damage results in a systemic release of cytokines and chemokines that triggers a wound healing response, characterized by mobilization of endothelial, monocyte, and other bone marrow progenitor cells into the primary tumor. $TIE2^{HIGH}$ monocyte and endothelial progenitors attracted in this manner can stimulate angiogenesis and a drug-resistant tumor microenvironment, refractory to subsequent treatment with chemotherapy, and as such, significantly facilitate local tumor relapse.[104,109] We[87,153] and others[154] have recently described previously unrecognized responses of $TIE2^{HIGH}$ TAMs to chemotherapy, resulting in the de novo induction of a metastasis-favorable tumor microenvironment. Because these newly reported mechanisms neither describe TAM-mediated tumor cell survival nor TAM-assisted evasion of apoptosis, they could not be classified within the traditional concepts of chemoresistance (environment-mediated drug resistance [EMDR]), and as such, were assigned the term "chemotherapy-induced metastasis" or "chemotherapy-exacerbated metastasis."[87,153,154] In this section, we focus on these two paradigms, "chemoresistance" and "chemotherapy-induced metas-

tasis," which are two diverse, and yet equally concerning side effects of chemotherapy.

### 3.1 | Emerging roles of TAMs in environment-mediated drug resistance (EMDR)

Over the past decades, it has been recognized that the mechanisms of resistance to therapies can be mediated not only by genetic events such as acquired mutations and selection of therapy-resistant tumor clones but also by the tumor microenvironment, which allows tumor cells to escape from the toxicity of chemotherapy, survive, and transiently become resistant:[155,156] a process known as EMDR.[157] As such, EMDR is a form of de novo drug resistance induced by complex interactions between tumor cells and a variety of cell types within the tumor microenvironment (e.g., CAFs, mesenchymal stem cells, adipocytes, endothelial cells, TAMs, DCs, etc.). An increasing body of work demonstrates that TAMs can induce EMDR in a context-dependent manner.[158–160] Foremost, TAM depletion by anti-CSF1 antibodies can enhance the antitumor activity of chemotherapeutic agents, such as taxol, etoposide, and doxorubicin in breast cancer xenografts.[161] In addition, CSF1 elimination can enhance the effectiveness of paclitaxel in MMTV-PyMT mammary tumors.[162] Along the same lines, live imaging has demonstrated that the activity of doxorubicin is improved in mice lacking $CCR2^+$ TAMs.[163]

In general, TAMs can limit the efficacy of chemotherapy either directly; by adhesion-dependent mechanisms that involve direct contact between macrophages and tumor cells (juxtacrine mechanisms) or adhesion-independent mechanisms through the secretion of soluble products (paracrine mechanism); or indirectly by modulating the immune system.

#### 3.1.1 | Juxtacrine mechanisms of macrophage-mediated chemoresistance

TAMs can interact directly with tumor cells and induce chemoresistance. For instance, Zeng et al. described that the cell-cell contact between TAMs and human myeloma cells via P-selectin glycoprotein ligand-1 (PSGL1) and ICAM1 conferred chemoresistance and protected tumor cells from melphalan- and dexamethasone-induced apoptosis.[164,165] The interaction of these adhesion molecules induced the activation of pro-survival ERK1/2 and c-myc signaling pathways in tumor cells and suppressed the activation of apoptosis-related caspases that are typically induced by chemotherapy. Accordingly, pharmacologic blockade or genetic knockdown of PSGL1 or ICAM1 in myeloma cells could restore sensitivity to chemotherapy both in vitro and in vivo.[164,165]

#### 3.1.2 | Paracrine mechanisms of macrophage-mediated chemoresistance

There is abundant evidence that TAMs induce chemoresistance by releasing soluble products (i.e., through paracrine mechanisms). Yin et al. demonstrated that TAMs induce human and murine colorectal cancer cell resistance to several chemotherapeutic agents, such as 5-fluorouracil and oxaliplatin, and reduce drug-induced apoptosis by secreting IL-6 and activating signal transducer and activator of transcription 3 (STAT3)/miR204-5p pathway in tumor cells.[166] In addition, another study described that TAM-derived IL-10 protects human breast tumor cells from toxic effects of paclitaxel in a STAT3-dependent manner. In turn, the activation of STAT3 induces the up-regulation of Bcl2, a survival factor, mediating chemoresistance. This protective effect of IL-10 is abrogated in the presence of a neutralizing antibody, and consecutively restores the sensitivity of tumor cells to chemotherapy.[167]

A growing body of evidence demonstrates that STAT3 plays a central role in the crosstalk between TAMs and tumor cells,[168] and promotes the acquisition of chemoresistance.[169–171] For instance, coculture experiments demonstrated that TAMs enhance murine myeloma 5T33MM cell survival and chemoresistance to melphalan and bortezomib by activating STAT3 pathway in tumor cells and inhibiting caspase-3 cleavage. Indeed, a JAK2/STAT3 inhibitor, AZD1480, abrogates TAM-mediated chemoresistance in vitro and in vivo.[172] Interestingly, TAMs may induce chemoresistance via STAT3 to CSCs as well. For example, TAM depletion by either neutralizing CSF1R or inhibiting CCR2 improves chemotherapeutic response by decreasing the STAT3 activation in pancreatic CSCs.[173] In this regard, another study has shown that a TAM-derived factor, known as milk fat globule-epidermal growth factor VIII (MFG-E8), promotes chemoresistance to carboplatin via STAT3 and Hedgehog pathways activation in lung and colon CSCs.[174]

In addition, it has been reported that TAM-secreted cysteine cathepsins are major modulators of therapeutic response. Coculture experiments have shown that TAM-derived cathepsins B and S protect breast cancer cells from cytotoxic effects of chemotherapeutic drugs, including taxol, etoposide, and doxorubicin. This effect is reversed by a pan-cathepsin inhibitor and improves the response of MMTV-PyMT tumors to paclitaxel.[175]

Whereas much of the focus of the field has been on the secretion of soluble factors, there has been recent evidence that the secretion of exosomes could be another mechanism used by TAMs to induce therapeutic resistance in tumor cells. For instance, exosomal miR-21 secreted by TAMs confers cisplatin resistance in gastric cancer cells by enhancing the activation of PI3K/AKT signaling pathway.[176] Similarly, another study has shown that the exosomal miR-155 transferred by TAMs to neuroblastoma tumor cells induces resistance to cisplatin by directly down-regulating TERF1, a component of the shelterin complex and inhibitor of telomerase.[177]

#### 3.1.3 | Macrophage-mediated chemoresistance through the immune microenvironment

Consistent with the immunosuppressive roles of TAMs described earlier, an increasing amount of data suggests that TAMs could also mediate chemoresistance by suppressing the cytotoxic activity of T-cells in tumors. For instance, DeNardo et al. reported that TAM infiltration in breast tumors treated with paclitaxel limits the infiltration of $CD8^+$ cytotoxic T cells and reduces their antitumor activity. Depletion of TAMs by a CSF1R antagonist in combination with chemotherapy, improves survival of $CD8^+$ cytotoxic T-cells, and consequently the

response to chemotherapy.[162] Another study in MMTV-PyMT mice showed that TAM-derived IL-10 suppresses IL-12 secreted by DCs, thus reducing cytotoxic $CD8^+$ T cell activation in response to paclitaxel and carboplatin. Thus, specific neutralization of IL-10 improves tumor response to chemotherapy.[178]

## 3.2 | TAMs in chemotherapy-induced metastasis

The role of TAMs in chemotherapy-induced metastasis has become of special interest over the past several years, as emerging literature suggests that TAMs (and in general bone marrow-derived cells [BMDCs]) play a crucial role in the development of pro-metastatic features within the primary tumor microenvironment.[153] The increase of TAMs following chemotherapy is mostly the result of monocyte recruitment from peripheral circulation, and, to a lesser extent, proliferation of tissue-resident macrophages.[87,104,109,159,179] An increased expression of chemotactic agents known to recruit macrophages, including CSF1, CXCL12, and CCL2, are often up-regulated in tumor cells and tumor-associated stromal cells in response to cytotoxic chemotherapy.[109,162,180,181] It has also been established that hypoxia induces expression of several chemotactic factors, attracting a variety of BMDCs including monocytes, which differentiate into TAMs expressing the tyrosine kinase receptor TIE2.[179] $TIE2^+$ TAMs are closely associated with tumor vasculature and support angiogenesis in an angiopoietin-2- (ANG2)-dependent manner.[97–99,102,104,111] In this section, we focus on two microenvironmental modifications related to the increased metastatic potential of solid tumors following chemotherapy: neoangiogenesis and TMEM assembly, both of which are mediated by specialized TAM subpopulations.

### 3.2.1 | Chemotherapy-induced angiogenesis

Although stromal cells other than TAMs have also been implicated in the regulation of angiogenesis and neovascularization (refer to Bussard et al., 2016 and references therein[182]), TAMs are considered pivotal mediators of angiogenesis, and therefore targeted anti-angiogenic therapies are constantly proposed in this context.[104,108,183] Genetic analysis has unraveled that TAMs secrete critical pro-angiogenic molecules such as VEGF, TNF-$\alpha$, IL-1$\beta$, IL-8, PDGF, and bFGF, among others.[31] TAMs are known to secrete pro-angiogenic molecules under stressful microenvironments that are often seen following chemotherapy (i.e., hypoxia, low glucose levels, high lactate levels).[184] Increased TAM influxes, as observed during chemotherapy treatment, exert significant pro-angiogenic pressure on existing endothelia. Indeed, under the control of the CXCL12/CXCR4 signaling pathway, TAMs newly recruited into neoplastic tissues have been shown to transition into perivascular TAMs that express TIE2 and VEGFA.[85] The pharmacologic suppression of CXCR4 causes a reduction in the number of perivascular $TIE2^+$ TAMs, and therefore, a reduction in tumor revascularization and recurrence following treatment with chemotherapy.[109] Whether tumor angiogenesis is directly associated with increased metastatic risk is a subject of great debate, although it is generally accepted that tumor endothelial cells (TECs) contribute to critical steps of the metastatic cascade.[185,186] The association of angiogenesis with tumor metastasis seems to be due to interaction of TECs with $TIE2^+$ TAMs accumulated after chemotherapy at perivascular sites, which increases TMEM assembly and function.[137]

Although the current review focuses on chemotherapy, it should also be noted that a growing number of studies reported that TAMs limit the efficacy of anti-angiogenic therapies, mostly because TAMs shift the "angiogenic switch" toward the pro-angiogenic side.[187–189] For instance, Welford and colleagues have shown that hypoxia induced by combretastatin-A4-phosphate (CA4P), a vascular-disrupting agent, was associated with elevated levels of CXCL12 and increased $TIE2^+$ macrophage infiltration in mammary tumor models.[103] The blockade of $TIE2^+$ macrophage recruitment, either pharmacologically by a CXCR4 antagonist or genetically, enhances CA4P efficacy in subcutaneous mammary carcinomas.[103] Similarly, Sorafenib, a VEGFR2/Raf kinase inhibitor, increases CXCL12 levels and TAM infiltration in hepatocellular carcinoma xenografts, which in turn, triggers tumor angiogenesis.[190] Quite expectedly, the depletion of TAMs by clodronate, or with a specific CSF1R inhibitor, eliminates the tumor's resistance to Sorafenib and supports an anti-angiogenic microenvironment.[35,190]

Overall, these observations suggest that chemotherapy treatment leads to the rapid accumulation of proangiogenic TAMs in the tumor microenvironment, which, in turn, shifts the "angiogenic switch" toward a pro-angiogenic environment supporting cancer metastasis, and at the same time offsets the functions of anti-angiogenic drugs.

### 3.2.2 | Chemotherapy-induced dissemination/intravasation

As already discussed in section 2.4, specialized subtypes of TAMs have been linked to critical signaling events in the individual steps of the metastatic cascade. For instance, the EGF-secreting, inflammatory TAMs can induce $Mena^{INV}$ expression in tumor cells during the process of streaming and make such cells highly capable of invasion, directed migration toward the perivascular areas,[133,134] and transendothelial migration.[133] Once the tumor cells reach these perivascular areas, a different TAM subtype, the pro-angiogenic $MRC1^+TIE2^+VEGFA^+$ macrophage, participates in a complex signaling cascade leading to both the assembly of new TMEM sites and TMEM-mediated vascular permeability, thus assisting in tumor cell intravasation.[92] In spontaneously developing tumors, hematogenous dissemination is continuous and the dynamic interactions of these TAM subtypes with tumor cells and the tumor microenvironment dictate the degree of dissemination.[90,122,153]

Interestingly, we, and others,[86,114,137] have reported that the total macrophage count in tumors remains unaltered in certain cancers treated by chemotherapy, although macrophage re-polarization and dynamic shifts between different TAM subpopulations are quite discernible in these contexts. Although the observed change between subpopulations varies in degree (most likely due to the differing technologies employed, or due to tumor heterogeneity in each animal model), all these reports agree and converge on the conclusion that pro-metastatic TAMs typically increase upon chemotherapy.[86,111,114,137] For instance, the infiltration of $TIE2^+$

monocyte and endothelial progenitors from the bone marrow following treatment with taxanes is extremely well documented.[191–193] These monocyte progenitors differentiate into TIE2$^+$ TAMs and mediate a well-described wound repair response against the cytotoxic stress/damage of chemotherapy, especially when given in the neoadjuvant setting.[137,154,192,193] In addition, it has been demonstrated that chemotherapy-induced hypoxia triggers proliferation of tissue-resident TIE2$^+$ TAMs, making this subpopulation a prominent component of TAMs in the primary tumor site.[108,179] Indeed, mice developing spontaneous MMTV-PyMT tumors, as well as breast cancer patient-derived xenografts (PDXs), respond with a dramatic increase of TIE2$^+$VEGFA$^+$ TAMs and TMEM assembly following treatment with paclitaxel, doxorubicin, and/or cyclophosphamide.[137] Moreover, multiphoton intravital imaging in live mice receiving paclitaxel demonstrated that such TMEM sites are functional, thus increasing the metastatic potential of tumors.[137] This de novo assembly of TMEM sites has been observed by a number of research groups studying pro-metastatic effects of neoadjuvant chemotherapy.[137,154]

In addition, treatment with chemotherapy may not only create a metastasis-favorable, perivascular tumor microenvironment, as described earlier, but could also directly affect the phenotypic characteristics and behavior of the metastasizing cancer cells. Indeed, in preclinical models of breast cancer, as well as in residual disease of breast cancer patients after completing neoadjuvant chemotherapy, it has been shown that the contact of tumor cells with TAMs, an event likely occurring near (or at) TMEM sites (as already described), can significantly increase Mena$^{INV}$ expression.[134,137] In addition, there is evidence that Mena$^{INV}$ confers to tumor cells taxane chemoresistance by altering the ratio of dynamic and stable microtubules in paclitaxel-treated cells.[194] Therefore, survival and selection, and de novo up-regulation, may all contribute to chemotherapy-related increases in expression of the highly invasive Mena$^{INV\text{-}HI}$ cancer cell subpopulation, capable of TMEM-dependent dissemination and metastasis.

Chemotherapy-induced metastasis is an emerging concept in the treatment of cancer, and a previously under-recognized effect of chemotherapy. It should be emphasized here that the molecular mechanisms behind the pro-metastatic phenotypes induced by chemotherapy represent an exacerbation of metastatic pathways already well established in the field of cancer biology, triggered as a stress response to the cytotoxic effects of chemotherapy.[87,153] Importantly, the dynamic shifts in, and the active recruitment of, specialized TAM subpopulations after chemotherapy, is paramount in the orchestration of these pro-metastatic phenotypes. As such, future therapies should focus on targeting TAMs (or an aspect of their biology), to suppress chemotherapy-induced metastasis. For example, rebastinib, a well characterized and selective TIE2 inhibitor, has been shown to efficiently suppress TMEM function and TMEM-dependent cancer cell dissemination in breast cancer.[114] Moreover, the co-administration of rebastinib, along with taxane-based chemotherapy, efficiently abrogates the pro-metastatic potential of chemotherapy[137] and increases metastasis-free survival, when compared to chemotherapy-treated alone, in preclinical mouse models of breast cancer.[114]

## 4 | CONCLUSIONS

The complex and diverse roles of the immune system, and especially macrophages, in promoting angiogenesis, intravasation, dissemination, and survival at primary and metastatic tumor sites has only recently begun to emerge. TAMs are now recognized as not only simply matrix-remodeling cells involved in cancer-related inflammation, but also multifaceted interlocutors, capable of creating complex signaling networks and loops that regulate the fate of almost all hallmarks of the metastatic cascade at the microanatomic level. Foremost, this review has discussed that TAMs represent a type of innate immune cell with remarkable phenotypic plasticity within the tumor microenvironment. In particular, TAMs can be polarized in elaborate ways into different subpopulations that specialize in resolving specific barriers and obstacles that tumor cells meet while in the process of the metastatic dissemination.

From this perspective, we may envision TAMs as "multitasking" tumor cell partners, facilitating key steps of the metastatic cascade (Fig. 1). One subpopulation of TAMs, for example, operates away from vessels, in the primary tumor microenvironment, and can simultaneously: (i) induce the overexpression of Mena$^{INV}$ in tumor cells through Notch signaling, making these tumor cells highly migratory, highly invasive and direction sensing; (ii) guide Mena$^{INV}$-expressing tumor cells toward the underlying blood vessels through a paracrine signaling loop involving chemotactic cytokines; (iii) remodel (through the secretion of proteolytic enzymes) the ECM, while simultaneously leading Mena$^{INV}$-expressing tumor cells toward vessels; and (iv) create an immunosuppressive local microenvironment that constantly shields the disseminating cancer cells from immunologic destruction. In the meantime, a second subpopulation of TAMs (TIE2$^+$) operates in the perivascular niche, to: (i) provide appropriate signals that promote tumor angiogenesis; (ii) orchestrate the assembly of intravasation sites called TMEM; and (iii) regulate TMEM function to disrupt the endothelial cell barrier for subsequent transendothelial migration of Mena$^{INV}$-expressing tumor cells. Finally, a third TAM subpopulation acts on the distant metastatic site independently to: (i) prepare a tumor-receptive premetastatic niche and (ii) facilitate the survival and colonization of the newly arrived tumor cells. Therefore, it is not surprising that tumor cells have opted for a strategic alliance with TAMs to overcome obstacles that would otherwise make metastasis an "impossible" rather than an "inefficient" process, as currently thought.[195,196]

In this context, we discussed the literature demonstrating that monocytes which infiltrate tumors can potentially become streaming macrophages, and eventually perivascular macrophages, following a unidirectional transition driven by blood vessel-derived chemotactic gradients.[85] Although this is direct evidence of phenotypic plasticity in these TAMs, one could argue it is indirect evidence of lineage plasticity, as well. For example, gene expression analyses of TAM polarization markers have suggested that perivascular macrophages are mostly shifted toward an M2 phenotype, expressing the tyrosine kinase receptor TIE2 and MRC1,[92,109] which are prominent hallmarks of M2 polarization.[28,30,41,51,52] Streaming macrophages on the other side do not express these markers, and yet, they can potentially

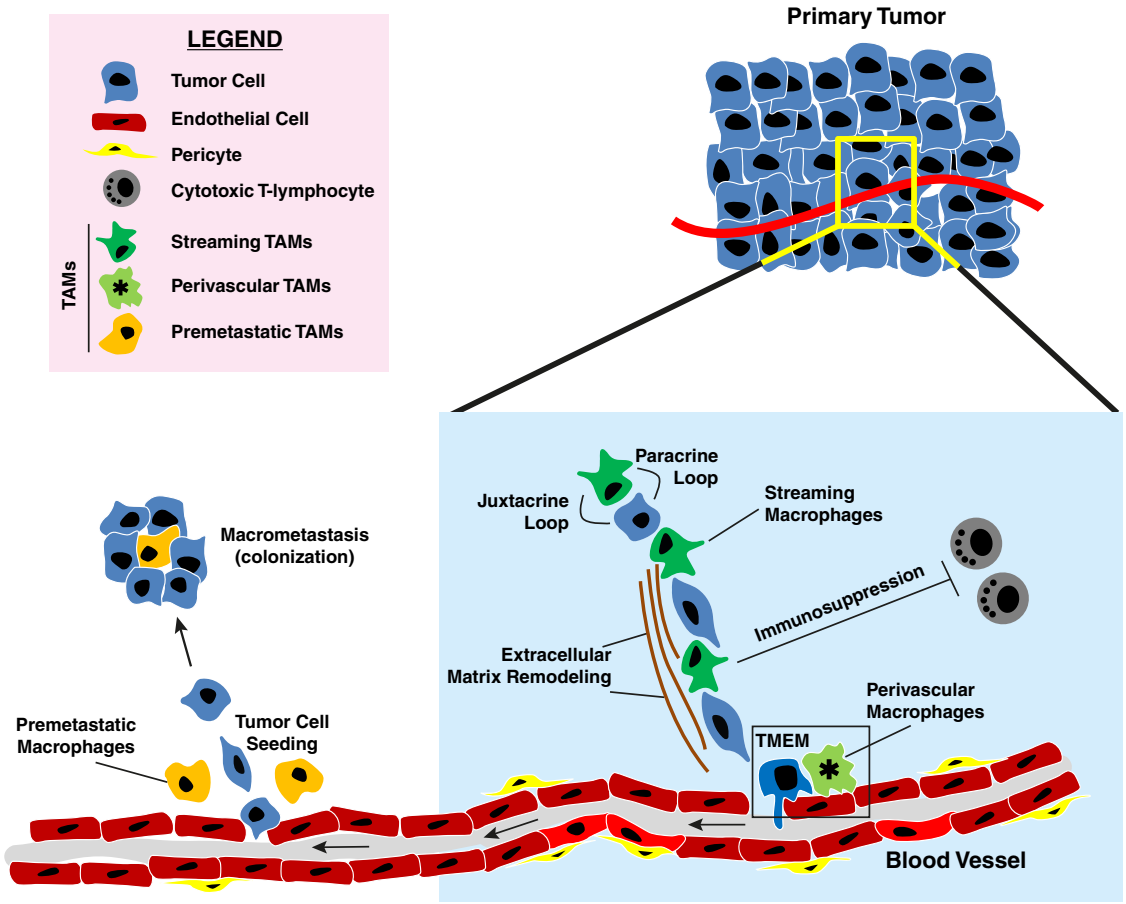


**FIGURE 1** **The contribution of tumor-associated macrophages (TAMs) in metastasis.** Conceptual model on how specific TAM subtypes can "multitask" in the primary and secondary tumor microenvironments to assist tumor cells to overcome obstacles in the metastatic cascade and to achieve all the hallmarks of metastasis. An intratumoral area in close proximity to a vessel is shown as a magnified inset (yellow box). Streaming TAMs (dark green color) operate in the primary tumor site, irrespective of proximity to the vasculature and co-migrate with tumor cells toward TMEM through a paracrine and juxtacrine signaling loop. Streaming TAMs are capable of modifying the ECM appropriately to facilitate invasion, and provide protection of tumor cells from immunologic destruction. Perivascular TAMs (light green color, with asterisk-shaped nuclei) operate in the perivascular niche, where they can provide pro-angiogenic signals and form intravasation sites. Finally, premetastatic TAMs (orange color) operate in the distant site to create a premetastatic niche. These TAMs are recruited, even before tumor cells arrive, through a chemokine network orchestrated by the primary tumor and the associated stromal cells. These premetastatic TAMs facilitate tumor cell extravasation, seeding, survival, and subsequent colonization on the secondary site

turn into perivascular macrophages expressing TIE2. Indeed, despite the original thought that TIE2$^+$ macrophages may either arise as tissue-resident macrophages, or from committed TIE2$^+$ monocyte progenitors, there is now strong evidence that hypoxia stimulates TIE2 expression.[111,179,192] Furthermore, recent studies have collectively shown that macrophage repolarization in the tumor microenvironment can be achieved by inhibition of the CSF1/CSF1R signaling pathway, and is associated with phenotypic modifications such as activation/enhancement of CD8$^+$ T-cell mediated immunity and suppression of the angiogenic potential.[197–199] This evidence may indicate that the TAMs described in this review are not terminally polarized, but subjected to repolarization dependent on contextual cues from the tumor microenvironment. Also, therapeutic intervention seems to also be a viable possibility. However, more studies, including lineage tracing studies, are needed to address these questions in the future.

Emerging literature suggests that tissue-resident TAMs originating from the yolk sac have distinct functions compared to macrophages originating from bone-marrow derived monocytes.[39] At this point it would be premature to discuss, or even speculate, whether the described phenotypic TAM subpopulations (i.e., streaming, perivascular, premetastatic) are associated with committed monocyte progenitors originating from the bone marrow, or whether they represent denizens traceable back to their yolk sac predecessors. Answering this question, however, is critical to our understanding of TAM involvement in metastasis, especially, because macrophages of different embryonic origins assume different functions, and each tumor type appears to be characterized and regulated by a unique macrophage ontogeny.[200]

Moreover, when faced with different drug treatments, especially cytotoxic chemotherapies, the phenotypic plasticity of macrophages makes them perhaps the most adaptive cells of the tumor stroma. In this review, we distinguished two types of response to cytotoxic chemotherapy, involving TAMs (Fig. 2). The first falls into the category of EMDR,[157] and describes how heterotypic interactions between TAMs and tumor cells offer advantageous survival signals

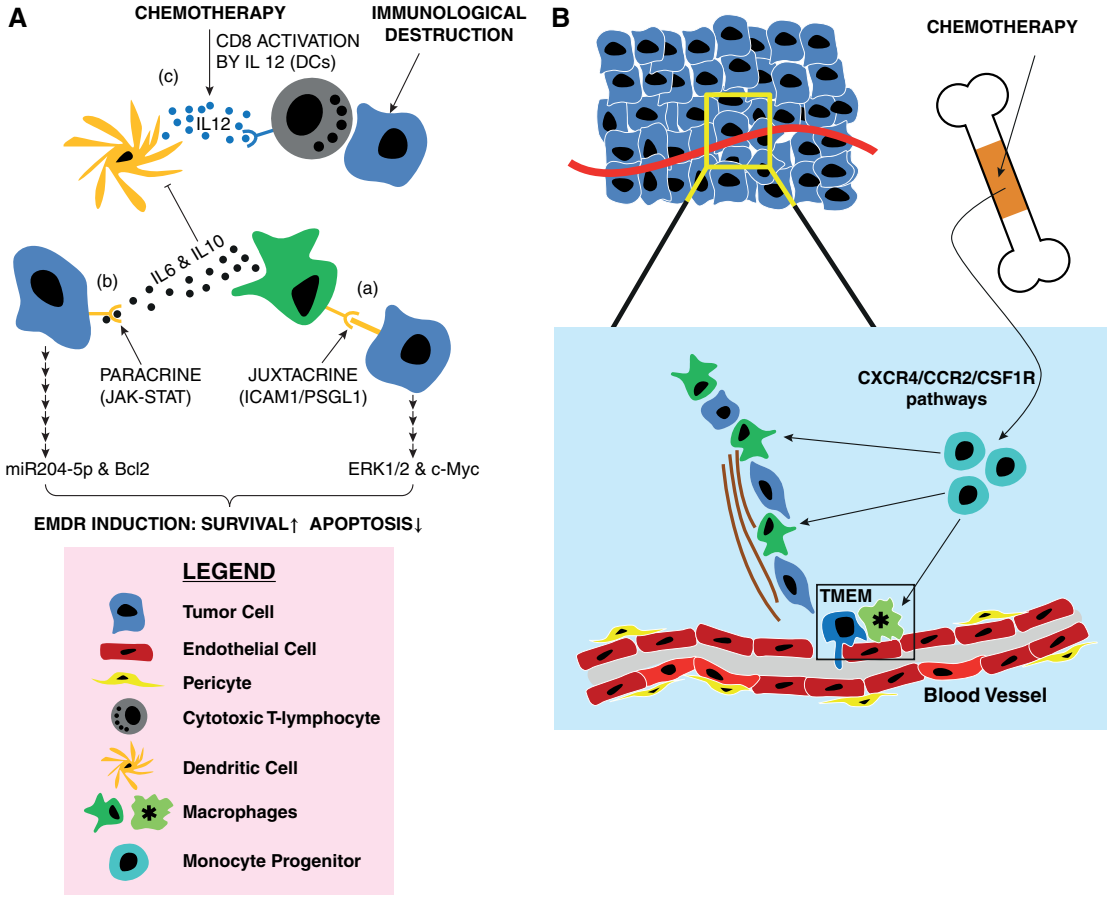


**FIGURE 2 The contribution of tumor-associated macrophages (TAMs) in: (A) Environment-mediated drug resistance (EMDR), and (B) chemotherapy-induced metastasis.** (A) Examples of EMDR phenotype induction in tumor cells by TAMs. Conceptual model showing three examples of how TAMs may induce an EMDR phenotype in tumor cells: (a) the juxtacrine ICAM1/PSGL1 pathway activates ERK1/2 and c-myc in tumor cells, thus supporting pro-survival function in the latter; (b) the paracrine pathway: IL-6 and IL-10 secreted by TAMs activate JAK/STAT signaling pathway in tumor cells, which in turn, activates Bcl2 and miR204-5p pro-survival and anti-apoptotic pathways in the latter; (c) the modulation of the immunologic microenvironment: in the absence of TAMs, chemotherapy induces the secretion of IL-12 by DCs, facilitating $CD8^+$ T-cell activation and immunologic destruction of tumor cells. However, TAM-secreted IL-10 suppresses IL-12 secretion by DCs, offering protection of tumor cells through inactivation of $CD8^+$ T-cells. (B) Chemotherapy-induced metastasis. Cytotoxic chemotherapy attracts bone marrow-derived monocyte progenitors (light blue color) to the primary tumor site, as a result of a wound-response mechanism. Chemotactic pathways, including CXCR4/CXCL12, CCR2/CCL2 and CSF1R/CSF1, mediate these responses. The monocyte progenitors differentiate and eventually give rise to different TAM subpopulations, which in turn mediate the hallmarks of the metastatic cascade as discussed in more detail in Figure 1. Specifically, an increase in the numbers of streaming and perivascular TAMs results in increased TMEM assembly and function, as well as increased $MENA^{INV}$ expression in the metastasizing cancer cell subpopulations, all leading to an increased metastatic potential

to the latter, as well as resistance to apoptosis upon treatment with cytotoxic chemotherapy. The second falls into the category of "chemotherapy-induced metastasis," and has been recently defined by our group[87,137,153] as a mechanism of de novo generation of a pro-metastatic tumor microenvironment. We anticipate that this review lays solid groundwork for other researchers to distinguish between the two, because different pathways are involved in each type of response, and as such, different therapeutic strategies and interventions should be considered to reverse these unwanted side effects of cytotoxic chemotherapy.

Randomized prospective trials have shown that addition of taxanes into the preoperative chemotherapeutic regimen of breast cancer patients increases pathologic complete response (pCR), but does not improve overall survival.[201] The preclinical studies described in this review indicate that TAMs are essential for both EMDR and chemotherapy-induced metastasis, thus the lack of improvement in overall survival may be partially due to chemotherapy's effect on TAMs. This implies that new therapies must be developed to supplement current chemotherapy regimens, particularly with a focus on targeting TAMs or TAM-related signaling pathways. To this end, our group has already initiated a phase 1b trail of the TIE2 inhibitor rebastinib, in combination of antitubulin therapy of either paclitaxel or eribulin for treatment of metastatic breast cancer (clinicaltrails.gov NCT02824575). Thus, acknowledgment of the newly recognized effects of chemotherapy on the tumor microenvironment will lead to more effective therapeutic approaches for treatment of metastatic disease.

In conclusion, we attempt in this review to provide an overview of the emerging roles played by different TAM subpopulations from a spatiotemporal and contextual perspective, rather than the well-accepted

M1/M2 polarization spectrum. This new classification scheme, which involves streaming, perivascular, and premetastatic TAMs, has been proposed with an aim of providing a fresh perspective on how molecular and cellular cues from the tumor microenvironment can dictate TAM plasticity and functional diversity. Interestingly, the traditional polarization schemes and the spatiotemporal paradigm described here are intertwined. For example, TIE2$^+$ macrophages previously described as M2 or M2-like, protumoral, and highly angiogenic, are viewed in our scheme as perivascular macrophages capable of additionally assembling TMEM intravasation doorways and facilitating cancer metastasis. These observations suggest that the evolving scheme proposed here should be viewed in conjunction with the existing schemes, rather than as a brand new paradigm. They also underscore the necessity of broadening communication between, and collaboration among, research groups that focus on specific classification schemes. Such combined approaches will offer the potential of novel translational and clinical applications with which we will be able to target the contextual prerequisites for the metastasis-promoting functions of macrophages.

## AUTHORSHIP

G.S.K., M.H.O., and J.S.C. conceived the idea of the manuscript; L.R.S., L.B., D.E., J.S.C., M.H.O., and G.S.K. wrote the manuscript; G.S.K. and L.R.S. designed the illustrations; all authors approved the final form of the manuscript.

## ACKNOWLEDGMENTS

This work was supported by grants from the NCI (CA100324, CA150344, and CA216248), the Gruss-Lipper Biophotonics Center and its Integrated Imaging Program, and Montefiore's Ruth L. Kirschstein T32 Training Grant of Surgeons for the Study of the Tumor Microenvironment (CA200561).

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**How to cite this article:** Sanchez LR, Borriello L, Entenberg D, Condeelis JS, Oktay MH, Karagiannis GS. The emerging roles of macrophages in cancer metastasis and response to chemotherapy. *J Leukoc Biol*. 2019;1–16. https://doi.org/10.1002/JLB.MR0218-056RR