Hindawi Publishing Corporation
New Journal of Science
Volume 2014, Article ID 271940, 14 pages
http://dx.doi.org/10.1155/2014/271940

Review Article

Macrophages, Neutrophils, and Cancer: A Double Edged Sword

Alberto Mantovani[1,2]

[1] *Humanitas Clinical and Research Center, 20089 Rozzano, Italy*
[2] *Department of Translational Medicine, University of Milan, 20089 Rozzano, Italy*

Correspondence should be addressed to Alberto Mantovani; alberto.mantovani@humanitasresearch.it

Received 6 March 2014; Accepted 20 May 2014; Published 13 August 2014

Academic Editor: Marco E. M. Peluso

The tumor microenvironment is a well-recognized framework, in which myeloid cells play important roles in cancer development from tumor initiation to metastasis. Immune cells present in the tumor microenvironment can promote or inhibit cancer formation and development. Diversity and plasticity are hallmarks of cells of the monocyte-macrophage lineage. In response to distinct signals the cells of the monocyte-macrophage lineage have the ability to display a wide spectrum of activation states; classical Ml or alternative M2 macrophages represent extremes of a continuum of this activation. Tumor-associated macrophages generally acquire an M2-like phenotype that is relevant for their participation in tumor growth and progression. There is now evidence that also neutrophils can be driven towards distinct phenotypes in response to microenvironmental signals. In fact they can interact with distinct cell populations and produce a wide number of cytokines and effector molecules. Therefore, macrophages and neutrophils are both integrated in the regulation of the innate and adaptive immune responses in various inflammatory situations, including cancer. These findings have triggered efforts to target tumor-associated macrophages and neutrophils. In particular, "reeducation" to activate their antitumor potential or elimination of tumor promoting cells is a new strategy undergoing preclinical and clinical evaluation.

1. Introduction

The tumor microenvironment plays important roles in cancer development and behaviour [1–4]. The tumor microenvironment consists of various host components such as stromal cells, growing blood vessels, and inflammatory infiltrate that cause a local chronic inflammation. Infiltrating leukocytes in the tumor microenvironment may exert a dual role on tumor development and progression. Tumor cells can be directly eliminated by the immune cells that had developed an antitumoral immune response but can also recruit and instruct immune cells to favour tumor growth and progression. The recruited immune cell consists of many players, including macrophages and neutrophils.

Tumor-associated macrophages (TAM) are components of the inflammatory infiltrate of several tumors and produce many mediators (e.g., chemokines) responsible for the activation and maintenance of the chronic inflammatory process [5]. In human peripheral blood neutrophils are the most abundant leukocyte subset and have a primary role in the defence against infective pathogens. They have long been considered to have a negligible role in cancer-related inflammation because of their short life span and their terminal differentiation. However it has been recently recognized, on the basis of evidence in animal models, that tumor-associated neutrophils (TAN) can also be polarized in different phenotypes in response to tumor-derived stimuli. TAN can thus exert pro- and antitumoral functions [6–9].

Cancer growth causes the expansion of a heterogeneous population of immunosuppressive cells, in addition to macrophages and neutrophils. These cells, called myeloid-derived suppressor cells (MDSC) [10, 11], have been classified in mice as monocytic $CD11b^+/Ly6C^+$ MDSC (Mo-MDSC) and granulocytic $CD11b^+/Ly6G^+$ MDSC (G-MDSC) on the basis of the expression of the Ly6C or Ly6G antigen, respectively, on their surface. Monocytic and granulocytic MDSC, however, are not fully separated cell subsets, since epigenetic silencing of the retinoblastoma gene [12] can induce in a large proportion of Mo-MDSC the phenotypic, morphological, and functional features of G-MDSC. Though MDSC share

functional and phenotypical similarities with TAM and TAN, this relationship needs further investigations and is beyond the scope of this review.

TAM and TAN exert their protumoral functions participating in the extracellular matrix (ECM) remodelling and enhancing cancer cell invasion and metastasis, angiogenesis, cancer cell proliferation, and lymphangiogenesis, while inhibiting the antitumoral immune surveillance [8, 13–17]. Hypoxia is a key determinant of the localization and phenotype of TAM [18]. On the other end, TAM and TAN exert antitumoral activities by direct killing of tumor cells and releasing a wide range of mediators (e.g., cytokines, chemokines, and growth factors) which are able to recruit and activate cells of both the innate and adaptive immune systems [4, 19–21]. In this review we will explore the immunobiology of TAM and TAN; particular attention will be given to the recruitment and polarization of these tumor-associated cells, to the roles they can play in tumor growth and progression, and to their significance in clinical settings.

2. Tumor-Associated Neutrophils

There is now evidence that the "small eaters" (microphages), neutrophils, in addition to their known antimicrobial functions, play an important role in tumor progression, beyond the "big eaters" macrophages. TANs have been proposed as key mediator of malignant transformation, tumor progression, and angiogenesis and in the modulation of the antitumor immunity [8].

That neutrophil infiltration within tumors which may be associated with poor clinical outcome has been suggested by several epidemiological evidences. This negative correlation between TAN infiltration and clinical outcome has been reported in metastatic and localized clear cell carcinoma [22, 23], in head and neck squamous cell carcinoma [24], in bronchoalveolar carcinoma [25], in hepatocellular carcinoma [26], and in esophageal squamous cell carcinoma [27]. In human gliomas [28] and in aggressive types of pancreatic tumors [29], infiltration of neutrophils correlates with tumor grade. However, a favourable prognosis associated with a high neutrophil count has also been reported in some tumors (e.g., gastric cancer) [30].

How neutrophils exert their role in the promotion and progression of cancer has been only partially elucidated. ROS-dependent and -independent mechanisms [31], including the production of granule proteins, cytokines, and angiogenic factors, have been reported as factors that link neutrophils and carcinogenesis. For instance, epithelial tumor cells adjacent to neutrophils can uptake the neutrophil elastase and hydrolyse the insulin receptor substrate-1 (IRS-1), causing the enhancement of PDGFR signalling and tumor cell proliferation [32]. In addition, in patients with bronchoalveolar carcinoma, the hepatocyte growth factor (HGF) is released by neutrophils upon stimulation with tumor derived cytokines (TNF-α, GM-CSF) and HGF promotes tumor cell migration [25]. In breast cancer, neutrophils release oncostatin M (OSM) when stimulated by the GM-CSF released by cancer cells. In turn, OSM induces VEGF in breast cancer cells and promotes their detachment and invasive behaviour [33]. Moreover a new mechanism by which TAN affect tumor growth has been very recently reported. In an experimental tumor model Mishalian and coworkers showed that TAN from established tumors produce CCL17, recruiting regulatory T cells (Tregs) into the tumor. Recruitment of Tregs was inhibited by anti-CCL17 monoclonal antibodies treatment and neutrophil depletion using anti-Ly6G monoclonal antibodies reduced the migration of Tregs into the tumors, highlighting a clear link between TAN and regulatory T cells (Tregs) in tumor immunosurveillance [34].

Neutrophil depletion in two murine models of transplantable tumors (melanoma and fibrosarcoma) revert the increased tumor growth, angiogenesis and metastasis observed in IFN-β deficient mice [35]. Neutrophil depletion decreased tumor growth also in normal C57Bl/6 mice transplanted with the T cell lymphoma RMA [36, 37].

TANs have been reported as key mediators in angiogenesis. In a genetic mouse model of pancreatic cancer, neutrophil-derived MMP9 was responsible of VEGF release from the ECM and neutrophil depletion inhibited VEGF-dependent angiogenic switch [38]. An increase in neutrophil infiltration into tumors is significantly correlated with glioma grade and in glioblastoma with acquired resistance to anti-VEGF therapy. In this model the effect was mediated by neutrophil-mediated overexpression of S100A4 in tumor cells, since downregulation of S100A4 inhibited tumor progression independently of the infiltration of neutrophils into the tumor [39]. It has been recently proposed that neutrophils exert their protumor activities, as progression and dissemination, through neutrophil extracellular traps (NETs), especially in the context of systemic infections that might occur in surgery oncology patients [40]. This study reports that circulating tumor cells become trapped within NETs in vitro under static and dynamic conditions. In a murine model of infection in tumor bearing mice, microvascular NET deposition and consequent trapping of circulating lung carcinoma cells have been demonstrated. NET trapping was associated with increased formation of hepatic metastases. NET inhibition with DNAse or a neutrophil elastase inhibitor abrogated these effects. Neutrophil elastase profoundly influences cancer growth and development directly inducing tumor cell proliferation [32]. Interestingly it can also modify the tumor suppressor role of some ECM components, as has been reported for EMILIN1 that is cleaved by neutrophil elastase in inactive fragments. The consequence of this proteolytic process was the impairment of its antiproliferative role [41].

As has been described for TAM, also neutrophils can display surprising plasticity [9]. In models as lung cancer and mesothelioma, infiltration of TANs, their tumor cytotoxicity, and their immunostimulatory profile were increased by TGF-β blockage. Interestingly, in these experimental animal models, $CD8^+$ T-cell activation increased after TAN depletion, whereas after TGF-β blockage, depletion of TANs decreased $CD8^+$ T-cell activation [6].

Therefore, as already established for macrophage polarization, it has been proposed that TAN can be polarized to N1 and N2 phenotypes. Thus TANs infiltrating the tumor are driven by TGF-β to acquire a N2 protumoral phenotype. In

contrast, TGF-β inhibition induces an antitumoral N1 phenotype [6]. TGF-β signaling in myeloid cells has been reported to be required for tumor metastasis [42]. This study identifies myeloid-specific TGF-β signaling as a critical mediator in tumor metastasis, distinct from the tumor-suppressive effect of TGF-β signaling on epithelial cells, fibroblasts, and T cells, suggesting a cell-type-specific cancer-targeting strategy.

Plasticity of TAN has been recently described, using two models of murine tumor cancer cell lines, the Lewis Lung carcinoma and the AB12 mesothelioma [43]. TAN from early tumors were more cytotoxic toward tumor cells and produce higher levels of TNF-α, NO, and H_2O_2, while in established tumors these functions were downregulated and TAN acquire a more protumorigenic phenotype, showing how the evolvement of tumor microenvironment influence TAN phenotype.

Moreover, it is now clear that cancer is associated with the expansion of the myeloid compartment and with the appearance of a heterogeneous population of immunosuppressive cells functionally defined as myeloid-derived suppressor cells (MDSCs) [44]. MDSCs and TANs present with shared phenotypic (e.g., cell markers and morphology) and functional properties (e.g., recruitment, angiogenesis, and immunosuppressive activity) [45]. It is not yet completely elucidated whether TAN activity is at least in part due to MDSCs. In this regard, Fridlender and colleagues recently conducted transcriptomic analysis of TANs, naïve bone marrow neutrophils (NN), and granulocytic MDSCs (G-MDSC). Their data showed that TANs are a neutrophil population that markedly differs in their genetic profile from both NN and G-MDSC. They concluded that TAN are not "tissue-based G-MDSC" that undergo phenotypic modulation because of the tumor. In parallel, the NN and G-MDSC are more closely related to each other than TANs [46].

Finally, it can be remembered here that, as will be described in the following section regarding macrophage, cell polarization is a phenomenon that displays notable differences between mice and humans. Therefore, the existence and properties of N1-N2 in cancer-related inflammation in humans need to be carefully investigated.

3. Polarized Activation of Macrophages

Monocytes/macrophages undergo reprogramming when exposed to signals derived from microbes, damaged tissues, or activated lymphocytes. Functional reprogramming activates a spectrum of distinct functional phenotypes, in principle described as two different polarization states [47, 48]. IFNγ alone or in concert with microbial stimuli (e.g., LPS) or cytokines (e.g., TNF and GM-CSF) induces classically activated M1 macrophages. In contrast IL-4 and IL-13 induce the alternative M2 form of macrophage activation [49]. Moreover, IL-33 and IL-21 are associated with Th2 and M2 polarization [50, 51].

Classical M1 cells play a central role as inducer and effector cells in polarized Th1 responses; they are also effectors of resistance against intracellular parasites and tumors. They are high producers of IL-12 and IL-23 and low producers of IL-10. Moreover they are efficient producers of effector molecules (e.g., reactive oxygen and nitrogen intermediates) and inflammatory cytokines (e.g., IL-1β, TNF, and IL-6) [48, 52]. They can also be potent antitumor effectors, controlled by CD4+ Treg cells that prevent the recruitment and/or differentiation of Ly-6C^{high} monocytes favoring tumor progression [21].

In contrast, M2 macrophages generally produce low levels of IL-12 and IL-23 and high levels of IL-10 and release variable amounts of inflammatory cytokines. M2 cells are poor antigen presenting cells, suppress Th1 responses, actively scavenge debris, contribute to the dampening of inflammation, and promote wound healing, angiogenesis, tissue remodelling, and tumor progression [47]. M2 macrophages contribute to Th2 response and to elimination of parasites [53]. The IL-1 system, central in all inflammatory processes, appears differentially regulated in the two polarized population of macrophages; in fact M2 cells express and produce lower amounts of IL-1β and higher IL-1ra and decoy type II receptor in comparison with M1 cells [54].

M1 and M2 macrophages have a distinct ability to produce chemokines: M1 macrophages release Th1 lymphocyte attracting chemokines, such as CXCL9 and CXCL10, whereas M2 macrophages release CCL17, CCL22, and CCL24, chemokines that are involved in the recruitment of Tregs, Th2, eosinophils, and basophils [13, 55].

Metabolism of M1- and M2-polarized macrophages can also differ, as regards iron, folate, and glucose [56, 57]. Recently metabolism has been reported to be important in shaping the functional phenotype of macrophages in response to distinct polarizing stimuli in the tissue microenvironment, under normal as well as pathological setting. Indeed, recent data suggest that macrophage functional polarization is regulated by their distinct metabolic features [58].

It is now clear that M1 and M2 macrophages are an oversimplification: in the presence of various stimuli, such as antibody immune complexes together with LPS or IL-1, glucocorticoids, transforming growth factor-β (TGF-β), and IL-10, macrophages can acquire M2-like functional phenotypes with properties shared with IL-4- or IL-13-activated macrophages. In vivo modulation of M2 polarization has also been demonstrated (e.g., during helminthic and *Listeria* infection, in the placenta and embryo development, in obesity and cancer) [59]. Thus, M1 and M2 polarized macrophages are extremes in a continuum in a wide range of functional states [48]. Interestingly exposure of M2 macrophages to some immunological mediator molecules, as, for instance, IFN-γ, can induce "reeducation" into M1 cells and abrogate their immunosuppressive abilities [60].

4. Mechanisms of Recruitment

Stromal and tumor cells produce a wide spectrum of chemokines and growth factors that recruit circulating monocytes to tumor sites and cause their differentiation into macrophages. For instance, CCL5/RANTES, CXCL12/SDF-1, and CXC3L1/fractalkine were found in neoplastic tissues and contribute to macrophage recruitment and tumor promotion [61–65]. In addition to chemokines and growth

factors monocyte recruitment and macrophage differentiation are induced by noncanonical chemotactic peptides, such as vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF), macrophage colony stimulating factor (M-CSF/CSF-1), and urokinase plasminogen activator (uPa), by the antimicrobial peptide β-defensin-3, by the lectin Reg3β [64, 66–72], and by tumor products, as oxysterols which recruit tumor-supporting neutrophils [37]. CXC chemokines (CXCL8, CXCL1, CXCL2, CXCL3, and CXCL5), known for their role in neutrophil recruitment in both physiological and pathological conditions and involved in cancer progression, are also produced in tumor-associated inflammation. This favors tumor angiogenesis and metastasis [8, 73, 74]. In a preclinical experimental model of tumor transplant CXCL17, the latest identified member of the CXC family, enhanced tumor growth increasing the recruitment of immature myeloid derived $CD11b^+Gr1^+F4/80^-$ cells in the tumor [75]. In animal studies, tumor-derived CXCL5 recruited intratumoral infiltrative neutrophils and these promoted tumor growth and metastasis. Immunohistochemical analysis of human ICC samples showed that overexpression of CXCL5 correlated strongly with intratumoral neutrophil infiltration, shorter overall survival, and high tumor recurrence [76]. In addition, in murine models of inflammation-induced skin tumors and intestinal colitis-associated or spontaneous cancer, myeloid cells infiltrated tumors, and CXCR2 deficiency or neutrophil depletion suppressed inflammation-related tumorigenesis and the onset of spontaneous tumors [77]. In a mouse model of colitis-associated cancer (CAC) infiltrated neutrophils have been shown to produce large amounts of interleukin-1 (IL-) 1β, that in CAC milieu promotes tumorigenesis by inducing IL-6 production by intestine-resident mononuclear phagocytes. It was also noted that commensal flora-derived lipopolysaccharide (LPS) was identified to trigger IL-1β expression in neutrophils [78]. Interestingly, epithelial barrier deterioration caused by colorectal-cancer initiating genetic lesions (i.e., loss of the adenomatous polyposis coli tumor suppressor) resulted in adenoma invasion by microbial products. These products are responsible for the production of IL-23 in myeloid cells and the development of a protumoral IL-17 response [79]. In a mouse model of lung adenocarcinoma driven by conditional activation of K-ras and of p53 inactivation, Cortez-Retamozo et al. reported that spleen was the site of localization of TAM and TAN precursors, from where they physically relocated to the tumor stroma [80]. Removal of the spleen caused reduced accumulation of TAM and TAN in the tumor and affected tumor growth, showing that the spleen is a reservoir for TAM and TAN precursors which play a central role in the control of tumor evolution [80]. Moreover overproduction of angiotensin II was also present in this model and it amplified self-renewing hematopoietic stem cells and macrophage progenitors [81]. In a K-ras mutant mouse model of lung cancer, depletion of neutrophils or inhibition of CXCR2 receptor or genetic deficiency of neutrophil elastase significantly inhibited lung cancer development [82].

In humans, CXC chemokines, notably CXCL8/IL-8, are produced by liver epithelial cells from hepatocellular carcinoma (HCC). These chemokines in turn promote neutrophil migration in peritumoral stroma [26]. Recently, Zhou and colleagues analyzed by immunohistochemistry samples from 919 HCC patients and found correlation of CXCL5 overexpression with neutrophil infiltration, shorter overall survival, and tumor recurrence [83]. Very recently the same group analyzed CXCL5 expression in tumor samples from patients with intrahepatic cholangiocarcinoma and reported that in multivariate analysis CXCL5 overexpression alone, or combined with the presence of intratumoral neutrophils, was an independent prognostic indicator for ICC patients and thus a potential therapeutic target [76]. In addition, neutrophil chemotaxis was induced by IL-8 present in the medium conditioned by head and neck squamous cells carcinoma (HNSCC) and macrophage migration inhibitory factor (MIF) produced by HNSCC induced neutrophil recruitment in a CXCR2-dependent manner [24, 84]. CCL4-STAT3 signaling induced in prostate cancer by androgens contributes to tumor promotion highlighting a connection between chemokines and hormones in prostate cancer promotion [85].

5. Functions of Tumor-Associated Macrophages

Plasticity is a well-known characteristic of macrophages; that is, they are able to integrate distinct signals from the microenvironment and to acquire distinct phenotypes [48]. From a simplistic point of view, as already mentioned in a previous paragraph, macrophages may result in two opposite polarization states. M1 macrophages are induced by the exposure to Th1 cytokines (e.g., IFN-γ) alone or in combination with microbial components (e.g., LPS); M1 participate in Th1 response and are mediators of resistance against microbes and tumor cells. Typically M1 polarized macrophages are efficient producers of effector molecules (e.g., reactive oxygen and nitrogen intermediates) and inflammatory cytokines (e.g., IL-1β, TNF, IL-6), produce high levels of IL-12, and display a low expression of IL-10 [48]. In agreement with their functions in Th1 responses, M1 macrophages produce Th1 attracting chemokines, such as CXCL9/Mig and CXCL10/IP-10. The cytokines IL-4 and IL-13 inhibit this classical activation and induce the alternative M2 form of macrophage polarization [49]. Unlike M1, M2 macrophages produce high amounts of IL-10 and low amounts of IL-12 and display a poor antigen presenting capacity [53]. Being involved in Treg cell, Th2, eosinophil, and basophil recruitment, M2 macrophages produce chemokines that recruit these cell populations, such as CCL17/TARC, CCL22/MDC, and CCL24/Eotaxin-2 [19, 55, 86]. Moreover, they suppress Th1 adaptive immunity, participate in the resolution of inflammation and in the protection against parasites, and promote wound healing, angiogenesis, and tissue remodelling [47]. A strong evidence of macrophage plasticity consists in their potential to be "reprogrammed" by some immunological stimuli, such as IFN-γ or IFN-α, from immunosuppressive M2 macrophages into immunostimulatory cells [60, 87]. Interestingly it has been reported that IFN-γ driven intratumoral microenvironment exhibits superior prognostic effect compared with

an IFN-α driven microenvironment in patients with colon carcinoma, giving a successful proof-of-principle approach that complex cytokine interaction networks can be found and dissected in human tissues [88]. It can be remembered here that a Th1-dominated tumor micromilieu is strongly associated with a positive prognosis in CRC [89–91]. Tumor-produced molecules contribute to TAM polarization; thus modulation of the microenvironment can contribute to macrophage "reeducation." For instance, Wang et al. recently reported that Nodal, an embryonic morphogen member of the TGF-β superfamily, not detectable in healthy adult tissues, but that can reemerge in a number of human cancers and contribute to tumorigenesis, invasion, and metastasis, promotes to the in vitro generation of M2-like macrophages and downregulates the expression of IL-12. Its inhibition reversed TAMs to classically activated macrophage phenotype [92]. Drugs can also influence the TAM phenotype: using the HER2/neu transgenic (Tg-neu) mouse model lovastatin (Lov) was shown to significantly reduce the number of new oncogenic lesions in these mice, reducing the number of immunosuppressive and proangiogenic M2-like tumor-associated macrophages (TAM) and enhancing tumor infiltration by effector T cells [93]. Interestingly, microarray analysis identified a Lov-elicited genetic program in Tg-neu tumors that included downregulation of placental growth factor, which triggers aberrant angiogenesis and M2-like TAM polarization. Therapeutically "reeducated" macrophages to treat glioblastoma multiforme (GBM) have been described in a mouse model of proneuronal GBM. Treatment of mice with an inhibitor of the CSF-1 receptor (CSF-1R) to target TAMs significantly increased survival and regressed established tumors. [94]. Surprisingly, TAMs were not depleted in treated mice. Instead, glioma-secreted factors, including granulocyte-macrophage CSF (GM-CSF) and interferon-γ (IFN-γ), facilitated survival of TAM, whose expression of alternatively activated M2 markers decreased, consistent with impaired tumor-promoting functions. Moreover, multiple proneural GBM human xenografts responded similarly to CSF-1R inhibition, underscoring the therapeutic relevance of these findings [94]. Macrophages infiltrating the tumor may display dual functions, though often they participate to local inflammation, thus favoring tumor formation and progression. In facts several studies reported that TAMs have a M2-like phenotype [95]. The M2-like phenotype of TAM can be induced by the tumor cells. Katara et al. reported that vacuolar ATPase (V-ATPase) produced by tumor cells can promote tumor survival and growth. In particular cancer tissues and cells overexpress the a2 isoform of V-ATPase (a2V) and exposure of macrophages to the cleaved N-terminal domain of a2V induces expression and secretion of distinct TAM associated molecules, as elevated expression of mannose receptor-1, Arginase-1, interleukin-10, TGFβ, MMP9, and VEGF [96]. During tumor growth and progression macrophages can perform many functions, including extracellular matrix remodelling, promotion of tumor cell invasion and metastasis, angiogenesis, lymphangiogenesis, and immune suppression [19] (Table 1).

TAMs produce a number of proteolytic molecules, such as plasmin, urokinase-type plasminogen activator, cathepsin B, and matrix metalloproteases (MMP) which may directly remodel the ECM [98, 99, 105]. For instance, MMP participate to tumor progression through their capacity to degrade the basement membrane, activate growth factors, and enhance angiogenesis [103, 114, 115]. Moreover, the expression of nonproteolytic molecules by TAM can also have a role in the invasiveness of cancer cells. For instance, when macrophages were cultured in vitro in the presence of conditioned media from mammary epithelial cells containing FGF receptor 1-induced soluble factors, they increased the expression of CXCR2-binding chemokines. In turn, these CXCR2-binding chemokines induced migration of primary and tumoral mammary epithelial cells [116]. Expression of scavenger-receptor A in hematopoietic cells, as well as on macrophages, has been reported to be necessary for metastasis, in a mouse model of subcutaneously transplanted pancreatic cancer cells [100]. Moreover, expression of MMP-9 in glioma stem-like cells was induced by macrophages-derived TGF-β1, and MMP9 increased the invasiveness of tumor cells [101]. Finally, macrophages increased the expression of the antimicrobial peptide hCAP18/LL37 in the presence of tumor-derived versican V1 and the increased availability of hCAP18/LL37 contributed to ovarian tumor cell proliferation and invasion [102].

As mentioned above, macrophages have been described to be associated with the metastatic potential of several tumors [117, 118]. For instance, transfer of thioglycollate-elicited peritoneal macrophages in mice increased by up to 100-fold the number of artificially induced metastatic lung nodules induced by the intravenous injection of melanoma or Lewis lung carcinoma tumor cells [119]. In a mouse model of breast cancer, IL-4-treated macrophages upregulated the expression of cysteine protease cathepsin B, which promoted lung metastasis [120]. Moreover, macrophages exposed to M2 polarizing cytokines or tumor cells conditioned media express a truncated fibronectin isoform, namely, migration-stimulating factor (MSF), which exerts a potent chemotactic effect on tumor cells [121]. The metastatic potential of TAM is further supported by depletion studies demonstrating a reduced incidence of metastasis [122, 123]. Moreover, some TAM phenotypes can differ in primary tumor or metastasis. CX3CR1 expression can have a role in metastasis, as shown in an experimental colon cancer tumor model, where CX3CR1 deficiency significantly inhibited liver metastasis [124]. TAM density and their phenotype were evaluated in 246 samples from primary tumor and brain metastasis from renal cell cancer (RCC) patients. While the density of CD68(+) TAMs was similar in primary RCC and brain metastases, TAMs were more frequently CCR2-positive in brain metastases than in primary RCC ($P < 0.001$). Since CCL7 expression in cancer cells of brain metastases was more frequent compared with primary tumours, it was concluded that monocyte recruitment by CCR2 contributes to brain metastasis of RCC [125].

TAMs are associated with tumor angiogenesis and lymphangiogenesis: in fact factors that are directly or indirectly involved in new vessel formation and sprouting are expressed by TAM. These include TGF-β, VEGF-A, VEGF-C, PDGF, MMP-9, thymidine phosphorylase (TP), and chemokines

Table 1: Effector molecules involved in tumor promotion by TAM.

Molecules	Effect	Selected reference
EGF, bFGF,	Tumor Growth	Condeelis and Pollard, 2006 [4]
		Mantovani and Sica, 2010 [97]
Cathepsins, MMP-2, MMP-9	ECM Remodeling, Tumor Invasion, Metastasis	Gocheva et al., 2010 [98]
SR-A		Nagakawa et al., 2002 [99]
TGF-β		Neyen et al., 2013 [100]
LL37		Ye et al., 2012 [101]
		Li et al., 2013 [102]
MMP9	Angiogenesis and Lymphangiogenesis	Huang et al., 2002 [103]
VEGFs		Granata et al., 2010 [104]
PDGF		Wang et al., 2011 [105]
CXCL8		Hotchkiss et al., 2003 [106]
		Murdoch et al., 2008 [107]
IDO	Immunosuppression	Zhao, et al., 2012 [146]
IL-10		Sica et al., 2000 [108]
TGF-β		Hagemann et al., 2006 [109]
B7-H1 (PD-L1)		Kuang et al., 2009 [110]
B7-H3		Chen et al., 2013 [111]
B7-H4		Chen et al., 2012 [112]
Arginine Depletion		Chang et al., 2001 [113]

(e.g., CXCL8/IL-8) [104, 106, 107, 126–128]. For instance, TAMs induce the release of heparin-bound growth factors, such as VEGF-A, that have a crucial role in the angiogenesis switch, releasing MMP-9 [129]. On-site education of VEGF-recruited monocytes improves their performance as angiogenic cells [130]. The recruited monocytes derive from the abundant pool of circulating Ly6C^{hi} monocytes and upon arrival in the VEGF-rich environment they undergo multiple phenotypic and functional changes, endowing them with enhanced proangiogenic capabilities and, importantly, with a markedly increased capacity to remodel existing blood vessels.

In the tumor microenvironment, a proangiogenic program is triggered in macrophages by the increasing expression levels of HIF-1 and HIF-2 induced by low-oxygen tension. In this environment macrophages increase their expression levels of VEGF, bFGF, CXCL8/IL-8, and glycolytic enzymes [131]. Moreover, during local hypoxia the levels of adenosine markedly increase; this causes the release by human macrophages of angiogenic and lymphangiogenic factors [104]. Casazza and coworkers recently reported that TAMs' localization into hypoxic tumor areas is controlled by a Sema3A/Neuropilin-1 signaling axis. Confining TAMs inside normoxic regions by blunting the Sema3A/Neuropilin-1 pathway restores antitumor immunity and abates angiogenesis, overall inhibiting tumor growth and metastasis. Thus cancer cell-derived Sema3A, not VEGF, is responsible for TAM entry into hypoxic niches, through Nrp1 signaling, where TAM escape antitumor immunity and promote vascularization [132]. Modulating TAM localization and thus their phenotype can be a new approach to guide TAM activities against cancer. Moreover Laoui et al. reported that hypoxia is not a major driver of the TAM subset differentiation found in tumor infiltrate, namely, CD11b^{hi} F4/80^{hi} Ly6C^{lo}MHC-II^{lo} or MHC-II^{hi} TAM, both of which derived from tumor-infiltrating Ly6C^{hi} monocytes, but rather specifically fine-tunes the phenotype of M2-like MHC-II^{lo} TAM, that as a consequence contain higher mRNA levels for hypoxia-regulated genes than their MHCII^{hi} counterparts [18].

The angiogenic potential of TAM has been further proved by depletion studies demonstrating a reduced blood vessel density in tumor environment [133].

TAM express also immunosuppressive potential, through a wide range of molecules, such as TGF-β, iNOS, arginase-1, IDO, and IL-10, known for their immunosuppressive role [97, 108, 109, 134]. In murine models of breast cancer, T-cell suppression is dependent, at least in part, on TAM metabolic activities via arginase-1 or iNOS expression [113, 135–137]. Moreover, TAM have been shown to express the immunosuppressive molecule B7-H1 in hepatocellular carcinoma, B7-H4 in ovarian and lung cancer, and B7-H3 in lung cancer [110–112, 138]. In addition, TAM have the capacity to induce the expression of these molecules on cancer cell surface, thus providing a novel mechanism by which cancer cells escape the immune surveillance [111]. The immunosuppressive molecules of the B7 family can be expressed also by tumor cells. For instance, B7-H3 was highly expressed in non-small-cell lung cancer and its expression, evaluated by immunohistochemistry, significantly correlated with the patients' survival time; higher levels of B7-H3 expression were associated with a shorter survival time. Interestingly, in vitro soluble B7-H3 was capable of inducing

macrophages with an anti-inflammatory/reparatory (alternative/M2) phenotype; these M2 macrophages express high levels of macrophage mannose receptor (MMR) and IL-10 and low levels of HLA-DR and IL-1β [139]. The interaction between tumor-infiltrating hematopoietic cells and epithelial cancer cells can result in their fusion and the expression by tumor cells of hematopoietic markers, including CD45 and CXCR4. In this case tumor infiltrating leukocytes promote tumor growth equipping tumor cells with molecules that enhance cancer cells dissemination and escape from immune mechanism destruction [140]. This observation may have implications on the bone marrow contribution to the cancer stem cell population. It was hypothesized that the superior migratory potential gained by the cancer cells due to the fusion helps in its dissemination to various secondary organs upon activation of the CXCR4/CXCL12 axis. This finding has repercussions on CXCR4-based therapeutics and opens new avenues in discovering novel molecular targets against fusion and metastasis.

Due to their overall protumoral effects, targeting TAM is a useful and promising tool for new antitumoral therapies. Accordingly, several therapeutic strategies were proposed to inhibit their recruitment, interfere with their survival, or reprogram them into a M1 antitumoral phenotype [141–145].

6. Prognostic Significance

Prognostic significance of TAM in humans has been recently critically evaluated [146]. Though there is epidemiological evidence that in many human cancers, such as breast, cervix, bladder, and gastric cancer, high numbers of TAM are significantly associated with poor patient prognosis, [14, 147]; other studies have reported that the prognostic significance of TAM can be controversial [146]. For instance, in high-grade osteosarcoma patients $CD68^+$ TAM associated with reduced metastasis and improved survival [148], whereas in patients with classical Hodgkin's lymphoma and with large B-cell lymphoma no such correlation was found [149, 150]. However a recent study in patients with diffuse large B-cell lymphomas treated with Rituximab-CHOP reported that an increase of CD163(+) cells predicts poor prognosis, while an increase in CD68(+) cells was related to improved overall survival [151]. No correlation was also found for CD68+ cells in epithelial and stromal tumor compartments and cancer progression in patients with endometrial carcinomas [152].

In gastric cancer, number of TAM has been positively correlated with tumor cell apoptosis and the presence of CD8-positive cells [153]. Moreover, the number of TAM was found to be independent predictor of patient better survival [153]. The prognostic significance of TAM in patient with colorectal cancer (CRC) is controversial and could depend on distinct phenotypes acquired on distinct localization within the tumor [154]. In CRC it has been recently reported that an IFN-γ driven tumor microenvironment exerts a superior effect on patients' survival and outcome compared with microenvironments driven by IFN-α or without an IFN-associated immune reaction. IFN-γ was found expressed mainly in the desmoplastic stroma and only rarely in the tumor cells, while most of the IFN-α-positive cells were found to be of tumor epithelial origin. These results suggest that complex cytokine interaction networks can have greater relevance for the determination of the prognosis of patients than the mere presence of tumor infiltrating leukocytes [88]. Moreover the positive role of macrophages in CRC has been found to be related to factors secreted by CRC cells that induced TAMs of a mixed M1/M2 phenotype, which in turn could contribute to a "good" inflammatory response [155]. This suggests that reeducation of macrophages might allow for important therapeutic advances in the treatment of human cancer.

These controversies can also be the consequence of the consistent heterogeneity of the studies performed (i.e., experimental procedures and techniques used to identify tissue macrophages) could lead to these controversies [146].

The relationship between TAN infiltration and prognosis in human cancer has been recently reviewed [22]. In many cases neutrophil infiltration was associated with a worse clinical outcome, as in patients with metastatic and localized clear cell carcinomas, bronchioloalveolar carcinoma, hepatocellular carcinoma, colorectal carcinoma, head and neck squamous cell carcinoma, and resectable esophageal squamous cell carcinoma [23–27, 156]. Correlation between neutrophils infiltration with the tumor grade in human gliomas and with more aggressive types of pancreatic tumors has been reported [28, 29]. In contrast, TAN have been associated with better prognosis in gastric carcinoma [30]. In analogy to TAM, also the prognostic significance of TAN largely depends on the type of tumor and on the methodology used to detect and evaluate the neutrophils within the tumors (e.g., hematoxylin-eosin stain versus immunohistochemistry) [30, 134, 157].

7. Therapeutic Targeting

Preclinical cancer models in the mouse indicate that it may be possible to "reeducate" tumor-promoting TAMs to reject neoplastic cells [141, 158]. In this regard, Beatty and coworkers investigated in both humans and mice whether the systemic CD40 activation with an agonist CD40 monoclonal antibody can circumvent tumor-induced immune suppression and favour antitumor immunity. In human studies they conducted a phase II clinical trial of the fully human CD40 agonist antibody CP-870,893 in combination with gemcitabine chemotherapy, in twenty-one patients with advanced pancreatic cancer and observed tumor regression in some patients. Investigating the mechanisms responsible for the observed partial clinical effect in a murine model of pancreatic cancer, they found a modified macrophage phenotype with upregulated MHC class II and CD86 expression [141]. In relation to these data it is interesting that the Th1 cytokine IFNγ promoted the antitumor effector function of CD40 ligand-activated macrophages [159].

Recruitment is a key determinant sustaining macrophage infiltration at sites of inflammation. To interfere with infiltration sustained by the CCL2/CCR2 axis, antibodies against CCL2 or its cognate receptor CCR2 have been tested in experimental models. In some tumors as prostate cancer [160] and breast metastasis [161] anti-CCL2 therapy reduced

tumor growth. Combination of anti-CCL2 with chemotherapy improved survival in preclinical settings [144]. On these bases, anti-CCL2 antibodies are being evaluated in humans in prostate and ovarian cancer [162]. Targeting CSF-1 receptor can be another way to modulate recruitment. To this aims, CSF-1 receptor (CSFR1) kinase inhibitors have been generated and have been shown to display antiangiogenic and antimetastatic activity in acute myeloid leukemia and melanoma models [163]. Anti-CSF-1 antibodies and antisense oligonucleotides suppressed macrophage infiltration and xenograft mammary tumor growth in mice [164, 165]. As described above, TAMs have a role in promoting invasion of breast carcinoma cells via a CSF-1/EGF paracrine loop [166]. Thus, new therapeutic strategies to inhibit macrophage and tumor cell migration and invasion can include blockade of EGF receptor or interference with CSFR1 signalling. TAMs may also influence the tumor's response to chemotherapy. In a genetic mouse model of breast cancer, chemotherapy with paclitaxel caused upregulation of the macrophage chemotactic factors CSF1, CCL8, and IL-34 and increased in CSFR1 expression in TAM. Inhibitors of CSFR1 that block macrophage recruitment combined with chemotherapy enhanced therapeutic activity, inhibited metastases, and increased T cells in the tumors and mRNA for various cytotoxic effector molecules such as granzyme A and B and perforin-1. If $CD8^+$ cytotoxic effector T cells (CTL) were depleted, the positive effects of the block of macrophage infiltration disappeared, suggesting that macrophages depletion enhances chemotherapeutic response in a $CD8^+$ CTL-dependent manner [167].

Yondelis (Trabectedin) is an EMEA-approved natural product derived from the marine organism *Ecteinascidia turbinata*, with potent antitumor activity; it is specifically cytotoxic for human and murine macrophages and TAMs and inhibits of the production of CCL2 and IL-6 both by TAMs and tumor cells [168]. Evidence now indicates that targeting TAM represents a major determinant of the antitumor action of this agent [143].

A therapeutic strategy alternative to the reduction of macrophage recruitment can be the inhibition of TAM effector functions. The bisphosphonate zoledronic acid is a prototypical MMP inhibitor. In cervical cancer this compound suppressed MMP-9 expression by infiltrating macrophages and inhibited metalloprotease activity, reducing angiogenesis and cervical carcinogenesis [169]. The halogenated bisphosphonate derivative clodronate is a macrophage toxin, which depletes selected macrophage populations. In particular, clodronate encapsulated in liposomes has been shown to efficiently deplete TAMs in tumor models, as murine teratocarcinoma and human rhabdomyosarcoma xenografts. TAM depletion significantly inhibited tumor growth [133].

Recently, Daurkin and coworkers suggested that enhanced 15-Lipoxygenase-2 (15-LOX2) activity in renal cell carcinoma (RCC) microenvironment could be implicated in monocytes recruitment through the CCL2-CCR2 axis. In turn, TAMs could exert immunosuppressive actions on T cells by inducing FOXP3 and CTLA4 in LOX-independent fashion. Therapeutic approaches directed toward the manipulation of 15-LOX2-mediated arachidonic metabolism could represent a novel strategy to counteract cancer-related inflammation and subvert the immunosuppression in patients with RCC [170]. Liver X receptor (LXR) ligands, also named oxysterols, are released by cancer cells and inhibit CCR7 expression on maturing DCs, therefore dampening DC migration to draining lymph nodes and antitumor immune responses [171]. At the experimental level it has been demonstrated that interfering with the oxysterol-CXCR2 axis delays tumor growth and prolongs the overall survival of tumor-bearing mice. Moreover some freshly isolated human tumor cells release oxysterols able to bind both LXR and CXCR2 [36] and higher numbers of intratumor neutrophils severely affect overall survival of kidney cancer patients [23]. Results of Raccosta et al. identify an unanticipated protumor function of the oxysterol-CXCR2 axis and suggest that manipulating LXR ligands and their interaction with CXCR2 and immune cells could provide additional targets for the development of new antitumor therapies.

Monoclonal antibodies and molecular inhibitors targeting the VEGF and EGF pathways are already approved for treatment of various carcinomas alone or in combination, although none were specifically targeted on TAMs function, and their clinical efficacy has been variable [172].

TAMs can also be potential immunotherapeutic target [141, 167, 173, 174]. Cavnar and coworkers investigated their role in gastrointestinal stromal tumor (GIST) and demonstrated that tumor cell oncogene activity determined TAM phenotype and function. In mice, established GIST tumors contained M1-like TAMs, which were antitumoral, as proven by depletion studies. Imatinib therapy in mouse GIST polarized TAMs to become M2-like through the activation of CCAAT/enhancer binding protein (C/EBP)β. Human TAMs were also M1-like at baseline and became M2-like after imatinib therapy. In patients whose tumors developed resistance to imatinib, TAMs reverted to M1-like and had a remarkably similar gene expression profile as M1-like TAMs from untreated patients. These findings reveal the central importance of tumor cell oncogene activity in TAM polarization [175].

Progress in understanding the biology of TAM paves the way to development of different and alternative strategies to target these cells or modulate their function, although the careful definition of the immunobiology of the context in different tumors may be required for successful therapeutic exploitation.

8. Concluding Remarks

Resolution of inflammation, tissue repair, and remodelling involve the function of cells of the mononuclear phagocytes system. Tumor progression is modified by a wide variety of host myeloid cell types, including macrophages and neutrophils. Under the influence of multiple microenvironmental signals, macrophages as well as neutrophils polarize toward distinct phenotypes with protumoral or antitumoral activities. Moreover, elucidating the relationship between MDSC, TAM, and TAN needs further investigations. Early studies indicated a possible cytotoxic activity of neutrophils toward tumor cells, and more recent reports have shown that

neutrophils promote tumor progression via matrix degradation, tumor cell proliferation, increased metastasis, and enhanced angiogenesis, as it has been described in many studies for TAM. A better knowledge of the mechanisms that myeloid cells use to affect tumor growth and progression can be clinically relevant to develop new therapeutic strategies in cancer.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the Italian Ministry of Health and the Italian MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca).

References

[1] P. Allavena, A. Sica, G. Solinas, C. Porta, and A. Mantovani, "The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages," *Critical Reviews in Oncology/Hematology*, vol. 66, no. 1, pp. 1–9, 2008.

[2] L. M. Coussens and Z. Werb, "Inflammation and cancer," *Nature*, vol. 420, no. 6917, pp. 860–867, 2002.

[3] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[4] J. Condeelis and J. W. Pollard, "Macrophages: obligate partners for tumor cell migration, invasion, and metastasis," *Cell*, vol. 124, no. 2, pp. 263–266, 2006.

[5] A. Mantovani, B. Bottazzi, F. Colotta, S. Sozzani, and L. Ruco, "The origin and function of tumor-associated macrophages," *Immunology Today*, vol. 13, no. 7, pp. 265–270, 1992.

[6] Z. G. Fridlender, J. Sun, S. Kim et al., "Polarization of tumor-associated neutrophil phenotype by TGF-β: "N1" versus "N2" TAN," *Cancer Cell*, vol. 16, no. 3, pp. 183–194, 2009.

[7] A. Mantovani, "The Yin-Yang of tumor-associated neutrophils," *Cancer Cell*, vol. 16, no. 3, pp. 173–174, 2009.

[8] A. Mantovani, M. A. Cassatella, C. Costantini, and S. Jaillon, "Neutrophils in the activation and regulation of innate and adaptive immunity," *Nature Reviews Immunology*, vol. 11, no. 8, pp. 519–531, 2011.

[9] A. Mócsai, "Diverse novel functions of neutrophils in immunity, inflammation, and beyond," *The Journal of Experimental Medicine*, vol. 210, no. 7, p. 1283, 2013.

[10] D. I. Gabrilovich, V. Bronte, S.-H. Chen et al., "The terminology issue for myeloid-derived suppressor cells," *Cancer Research*, vol. 67, no. 1, article 425, 2007.

[11] E. Peranzoni, S. Zilio, I. Marigo et al., "Myeloid-derived suppressor cell heterogeneity and subset definition," *Current Opinion in Immunology*, vol. 22, no. 2, pp. 238–244, 2010.

[12] J.-I. Youn, V. Kumar, M. Collazo et al., "Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer," *Nature Immunology*, vol. 14, no. 3, pp. 211–220, 2013.

[13] A. Mantovani, P. Allavena, A. Sica, and F. Balkwill, "Cancer-related inflammation," *Nature*, vol. 454, no. 7203, pp. 436–444, 2008.

[14] B.-Z. Qian and J. W. Pollard, "Macrophage diversity enhances tumor progression and metastasis," *Cell*, vol. 141, no. 1, pp. 39–51, 2010.

[15] C. Gao, A. Kozlowska, S. Nechaev et al., "TLR9 signaling in the tumor microenvironment initiates cancer recurrence after radiotherapy," *Cancer Research*, vol. 73, no. 24, article 7211, 2013.

[16] R. Rotondo, G. Barisione, L. Mastracci et al., "IL-8 induces exocytosis of arginase 1 by neutrophil polymorphonuclears in nonsmall cell lung cancer," *International Journal of Cancer*, vol. 125, no. 4, pp. 887–893, 2009.

[17] S. Sangaletti, C. Tripodo, C. Vitali et al., "Defective stromal remodeling and neutrophil extracellular traps in lymphoid tissues favor the transition from autoimmunity to lymphoma," *Cancer Discovery*, vol. 4, no. 1, pp. 110–129, 2014.

[18] D. Laoui, E. Van Overmeire, G. Di Conza et al., "Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population," *Cancer Research*, vol. 74, no. 1, pp. 24–30, 2014.

[19] A. Mantovani, S. Sozzani, M. Locati, P. Allavena, and A. Sica, "Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes," *Trends in Immunology*, vol. 23, no. 11, pp. 549–555, 2002.

[20] C. Tecchio, P. Scapini, G. Pizzolo, and M. A. Cassatella, "On the cytokines produced by human neutrophils in tumors," *Seminars in Cancer Biology*, vol. 23, no. 3, pp. 159–170, 2013.

[21] A. Pommier, A. Audemard, A. Durand et al., "Inflammatory monocytes are potent antitumor effectors controlled by regulatory CD4+T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 32, pp. 13085–13090, 2013.

[22] F. Donskov, "Immunomonitoring and prognostic relevance of neutrophils in clinical trials," *Seminars in Cancer Biology*, vol. 23, no. 3, pp. 200–207, 2013.

[23] H. K. Jensen, F. Donskov, N. Marcussen, M. Nordsmark, F. Lundbeck, and H. Von Der Maase, "Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma," *Journal of Clinical Oncology*, vol. 27, no. 28, pp. 4709–4717, 2009.

[24] S. Trellakis, K. Bruderek, C. A. Dumitru et al., "Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease," *International Journal of Cancer*, vol. 129, no. 9, pp. 2183–2193, 2011.

[25] M. Wislez, N. Rabbe, J. Marchal et al., "Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death," *Cancer Research*, vol. 63, no. 6, pp. 1405–1412, 2003.

[26] D.-M. Kuang, Q. Zhao, Y. Wu et al., "Peritumoral neutrophils link inflammatory response to disease progression by fostering angiogenesis in hepatocellular carcinoma," *Journal of Hepatology*, vol. 54, no. 5, pp. 948–955, 2011.

[27] J. Wang, Y. Jia, N. Wang et al., "The clinical significance of tumor-infiltrating neutrophils and neutrophil-to-CD8+ lymphocyte ratio in patients with resectable esophageal squamous cell carcinoma," *Journal of Translational Medicine*, vol. 12, no. 1, article 7, 2014.

[28] G. Fossati, G. Ricevuti, S. W. Edwards, C. Walker, A. Dalton, and M. L. Rossi, "Neutrophil infiltration into human gliomas," *Acta Neuropathologica*, vol. 98, no. 4, pp. 349–354, 1999.

[29] M. D. Reid, O. Basturk, D. Thirabanjasak et al., "Tumor-infiltrating neutrophils in pancreatic neoplasia," *Modern Pathology*, vol. 24, no. 12, pp. 1612–1619, 2011.

[30] R. A. Caruso, R. Bellocco, M. Pagano, G. Bertoli, L. Rigoli, and C. Inferrera, "Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in Northern Italy," *Modern Pathology*, vol. 15, no. 8, pp. 831–837, 2002.

[31] N. Güngör, A. M. Knaapen, A. Munnia et al., "Genotoxic effects of neutrophils and hypochlorous acid," *Mutagenesis*, vol. 25, no. 2, pp. 149–154, 2010.

[32] A. M. Houghton, D. M. Rzymkiewicz, H. Ji et al., "Neutrophil elastase-mediated degradation of IRS-1 accelerates lung tumor growth," *Nature Medicine*, vol. 16, no. 2, pp. 219–223, 2010.

[33] M. M. Queen, R. E. Ryan, R. G. Holzer, C. R. Keller-Peck, and C. L. Jorcyk, "Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression," *Cancer Research*, vol. 65, no. 19, pp. 8896–8904, 2005.

[34] I. Mishalian, R. Bayuh, E. Eruslanov et al., "Neutrophils recruit regulatory T-cells into tumors via secretion of CCL17-A new mechanism of impaired antitumor immunity," *International Journal of Cancer*, 2014.

[35] J. Jablonska, S. Leschner, K. Westphal, S. Lienenklaus, and S. Weiss, "Neutrophils responsive to endogenous IFN-β regulate tumor angiogenesis and growth in a mouse tumor model," *Journal of Clinical Investigation*, vol. 120, no. 4, pp. 1151–1164, 2010.

[36] L. Raccosta, R. Fontana, D. Maggioni et al., "The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils," *The Journal of Experimental Medicine*, vol. 210, no. 9, p. 1711, 2013.

[37] L. Raccosta, R. Fontana, C. Traversari, and V. Russo, "Oxysterols recruit tumor-supporting neutrophils within the tumor microenvironment: the many facets of tumor-derived oxysterols," *Oncoimmunology*, vol. 2, no. 11, Article ID e26469, 2013.

[38] H. Nozawa, C. Chiu, and D. Hanahan, "Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 33, pp. 12493–12498, 2006.

[39] J. Liang, Y. Piao, L. Holmes et al., "Neutrophils promote the malignant glioma phenotype through S100A4," *Clinical Cancer Research*, vol. 20, no. 1, pp. 187–198, 2014.

[40] J. Cools-Lartigue, J. Spicer, B. McDonald et al., "Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis," *Journal of Clinical Investigation*, vol. 123, no. 8, pp. 3446–3458, 2013.

[41] E. Pivetta, C. Danussi, B. Wassermann et al., "Neutrophil elastase-dependent cleavage compromises the tumor suppressor role of EMILIN1," *Matrix Biology*, vol. 34, pp. 22–32, 2014.

[42] Y. Pang, S. K. Gara, B. R. Achyut et al., "TGF-β signaling in myeloid cells is required for tumor metastasis," *Cancer Discovery*, vol. 3, no. 8, pp. 936–951, 2013.

[43] I. Mishalian, R. Bayuh, L. Levy, L. Zolotarov, J. Michaeli, and Z. G. Fridlender, "Tumor-associated neutrophils (TAN) develop pro-tumorigenic properties during tumor progression," *Cancer Immunology, Immunotherapy*, vol. 62, no. 11, pp. 1745–1756, 2013.

[44] D. I. Gabrilovich and S. Nagaraj, "Myeloid-derived suppressor cells as regulators of the immune system," *Nature Reviews Immunology*, vol. 9, no. 3, pp. 162–174, 2009.

[45] F. Shojaei, X. Wu, C. Zhong et al., "Bv8 regulates myeloid-cell-dependent tumour angiogenesis," *Nature*, vol. 450, no. 7171, pp. 825–831, 2007.

[46] Z. G. Fridlender, J. Sun, I. Mishalian et al., "Transcriptomic analysis comparing tumor-associated neutrophils with granulocytic myeloid-derived suppressor cells and normal neutrophils," *PLoS ONE*, vol. 7, no. 2, Article ID e31524, 2012.

[47] S. K. Biswas and A. Mantovani, "Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm," *Nature Immunology*, vol. 11, no. 10, pp. 889–896, 2010.

[48] A. Sica and A. Mantovani, "Macrophage plasticity and polarization: in vivo veritas," *Journal of Clinical Investigation*, vol. 122, no. 3, pp. 787–795, 2012.

[49] S. Gordon and P. R. Taylor, "Monocyte and macrophage heterogeneity," *Nature Reviews Immunology*, vol. 5, no. 12, pp. 953–964, 2005.

[50] M. Kurowska-Stolarska, B. Stolarski, P. Kewin et al., "IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation," *Journal of Immunology*, vol. 183, no. 10, pp. 6469–6477, 2009.

[51] J. Pesce, M. Kaviratne, T. R. Ramalingam et al., "The IL-21 receptor augments Th2 effector function and alternative macrophage activation," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 2044–2055, 2006.

[52] D. Tseng, J.-P. Volkmer, S. B. Willingham et al., "Anti-CD47 antibody-mediated phagocytosis of cancer by macrophages primes an effective antitumor T-cell response," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 27, pp. 11103–11108, 2013.

[53] W. Noël, G. Raes, G. H. Ghassabeh, P. De Baetselier, and A. Beschin, "Alternatively activated macrophages during parasite infections," *Trends in Parasitology*, vol. 20, no. 3, pp. 126–133, 2004.

[54] C. A. Dinarello, "Blocking IL-1 in systemic inflammation," *Journal of Experimental Medicine*, vol. 201, no. 9, pp. 1355–1359, 2005.

[55] F. O. Martinez, S. Gordon, M. Locati, and A. Mantovani, "Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression," *Journal of Immunology*, vol. 177, no. 10, pp. 7303–7311, 2006.

[56] A. Puig-Kröger, E. Sierra-Filardi, A. Domínguez-Soto et al., "Folate receptor β is expressed by tumor-associated macrophages and constitutes a marker for M2 anti-inflammatory/regulatory Macrophages," *Cancer Research*, vol. 69, no. 24, pp. 9395–9403, 2009.

[57] S. Recalcati, M. Locati, A. Marini et al., "Differential regulation of iron homeostasis during human macrophage polarized activation," *European Journal of Immunology*, vol. 40, no. 3, pp. 824–835, 2010.

[58] S. K. Biswas and A. Mantovani, "Orchestration of metabolism by macrophages," *Cell Metabolism*, vol. 15, no. 4, pp. 432–437, 2012.

[59] F. Rae, K. Woods, T. Sasmono et al., "Characterisation and trophic functions of murine embryonic macrophages based upon the use of a Csf1r-EGFP transgene reporter," *Developmental Biology*, vol. 308, no. 1, pp. 232–246, 2007.

[60] D. Duluc, M. Corvaisier, S. Blanchard et al., "Interferon-γ reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages," *International Journal of Cancer*, vol. 125, no. 2, pp. 367–373, 2009.

[61] F. Balkwill, "Cancer and the chemokine network," *Nature Reviews Cancer*, vol. 4, no. 7, pp. 540–550, 2004.

[62] B. Bottazzi, N. Polentarutti, A. Balsari et al., "Chemotactic activity for mononuclear phagocytes of culture supernatants from murine and human tumor cells: evidence for a role in the regulation of the macrophage content of neoplastic tissues," *International Journal of Cancer*, vol. 31, no. 1, pp. 55–63, 1983.

[63] A. Mantovani, P. Allavena, S. Sozzani, A. Vecchi, M. Locati, and A. Sica, "Chemokines in the recruitment and shaping of the leukocyte infiltrate of tumors," *Seminars in Cancer Biology*, vol. 14, no. 3, pp. 155–160, 2004.

[64] J. R. Reed, M. D. Stone, T. C. Beadnell, Y. Ryu, T. J. Griffin, and K. L. Schwertfeger, "Fibroblast growth factor receptor 1 activation in mammary tumor cells promotes macrophage recruitment in a CX3CL1-dependent manner," *PLoS ONE*, vol. 7, no. 9, article e45877, 2012.

[65] T. Ueno, M. Toi, H. Saji et al., "Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer," *Clinical Cancer Research*, vol. 6, no. 8, pp. 3282–3289, 2000.

[66] P. Allavena and A. Mantovani, "Immunology in the clinic review series; focus on cancer: tumour-associated macrophages: undisputed stars of the inflammatory tumour microenvironment," *Clinical and Experimental Immunology*, vol. 167, no. 2, pp. 195–205, 2012.

[67] B. Bierie and H. L. Moses, "Transforming growth factor beta (TGF-β) and inflammation in cancer," *Cytokine and Growth Factor Reviews*, vol. 21, no. 1, pp. 49–59, 2010.

[68] M. Gironella, C. Calvo, A. Fernández et al., "Reg3β deficiency impairs pancreatic tumor growth by skewing macrophage polarization," *Cancer Research*, vol. 73, no. 18, article 5682, 2013.

[69] G. Jin, H. I. Kawsar, S. A. Hirsch et al., "An antimicrobial peptide regulates Tumor-Associated macrophage trafficking via the chemokine receptor CCR2, a model for Tumorigenesis," *PLoS ONE*, vol. 5, no. 6, Article ID e10993, 2010.

[70] E. Y. Lin, V. Gouon-Evans, A. V. Nguyen, and J. W. Pollard, "The macrophage growth factor CSF-1 in mammary gland development and tumor progression," *Journal of Mammary Gland Biology and Neoplasia*, vol. 7, no. 2, pp. 147–162, 2002.

[71] N. Linde, W. Lederle, S. Depner, N. Van Rooijen, C. M. Gutschalk, and M. M. Mueller, "Vascular endothelial growth factor-induced skin carcinogenesis depends on recruitment and alternative activation of macrophages," *Journal of Pathology*, vol. 227, no. 1, pp. 17–28, 2012.

[72] J. Zhang, S. Sud, K. Mizutani, M. R. Gyetko, and K. J. Pienta, "Activation of urokinase plasminogen activator and its receptor axis is essential for macrophage infiltration in a prostate cancer mouse model," *Neoplasia*, vol. 13, no. 1, pp. 23–30, 2011.

[73] E. C. Keeley, B. Mehrad, and R. M. Strieter, "CXC chemokines in cancer angiogenesis and metastases," *Advances in Cancer Research*, vol. 106, pp. 91–111, 2010.

[74] G. Lazennec and A. Richmond, "Chemokines and chemokine receptors: new insights into cancer-related inflammation," *Trends in Molecular Medicine*, vol. 16, no. 3, pp. 133–144, 2010.

[75] A. Matsui, H. Yokoo, Y. Negishi et al., "CXCL17 expression by tumor cells recruits CD11b+Gr1highF4/80- cells and promotes tumor progression," *PLoS ONE*, vol. 7, no. 8, article e44080, 2012.

[76] S. L. Zhou, Z. Dai, Z. J. Zhou et al., "CXCL5 contributes to tumor metastasis and recurrence of intrahepatic cholangiocarcinoma by recruiting infiltrative intratumoral neutrophils," *Carcinogenesis*, vol. 35, no. 3, pp. 597–605, 2014.

[77] T. Jamieson, M. Clarke, C. W. Steele et al., "Inhibition of CXCR2 profoundly suppresses inflammation-driven and spontaneous tumorigenesis," *Journal of Clinical Investigation*, vol. 122, no. 9, pp. 3127–3144, 2012.

[78] Y. Wang, K. Wang, G. C. Han et al., "Neutrophil infiltration favors colitis-associated tumorigenesis by activating the interleukin-1 (IL-1)/IL-6 axis," *Mucosal Immunology*, 2014.

[79] S. I. Grivennikov, K. Wang, D. Mucida et al., "Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth," *Nature*, vol. 491, no. 7423, pp. 254–258, 2012.

[80] V. Cortez-Retamozo, M. Etzrodt, A. Newton et al., "Origins of tumor-associated macrophages and neutrophils," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 109, no. 7, pp. 2491–2496, 2012.

[81] V. Cortez-Retamozo, M. Etzrodt, A. Newton et al., "Angiotensin II drives the production of tumor-promoting macrophages," *Immunity*, vol. 38, no. 2, pp. 296–308, 2013.

[82] L. Gong, A. M. Cumpian, M. S. Caetano et al., "Promoting effect of neutrophils on lung tumorigenesis is mediated by CXCR2 and neutrophil elastase," *Molecular Cancer*, vol. 12, no. 1, article 154, 2013.

[83] S.-L. Zhou, Z. Dai, Z.-J. Zhou et al., "Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma," *Hepatology*, vol. 56, no. 6, pp. 2242–2254, 2012.

[84] C. A. Dumitru, H. Gholaman, S. Trellakis et al., "Tumor-derived macrophage migration inhibitory factor modulates the biology of head and neck cancer cells via neutrophil activation," *International Journal of Cancer*, vol. 129, no. 4, pp. 859–869, 2011.

[85] L. Y. Fang, K. Izumi, K. P. Lai et al., "Infiltrating macrophages promote prostate tumorigenesis via modulating androgen receptor-mediated CCL4-STAT3 signaling," *Cancer Research*, vol. 73, no. 18, article 5633, 2013.

[86] P. Romagnani, A. De Paulis, C. Beltrame et al., "Tryptase-chymase double-positive human mast cells express the eotaxin receptor CCR3 and are attracted by CCR3-binding chemokines," *American Journal of Pathology*, vol. 155, no. 4, pp. 1195–1204, 1999.

[87] M. De Palma, R. Mazzieri, L. S. Politi et al., "Tumor-targeted interferon-α delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis," *Cancer Cell*, vol. 14, no. 4, pp. 299–311, 2008.

[88] S. Grenz, E. Naschberger, S. Merkel et al., "IFN-γ-driven intratumoral microenvironment exhibits superior prognostic effect compared with an IFN-α-driven microenvironment in patients with colon carcinoma," *American Journal of Pathology*, vol. 183, no. 6, article 1897, 2013.

[89] J. Galon, A. Costes, F. Sanchez-Cabo et al., "Type, density, and location of immune cells within human colorectal tumors predict clinical outcome," *Science*, vol. 313, no. 5795, pp. 1960–1964, 2006.

[90] M. Camus, M. Tosolini, B. Mlecnik et al., "Coordination of intratumoral immune reaction and human colorectal cancer recurrence," *Cancer Research*, vol. 69, no. 6, pp. 2685–2693, 2009.

[91] E. Naschberger, R. S. Croner, S. Merkel et al., "Angiostatic immune reaction in colorectal carcinoma: impact on survival and perspectives for antiangiogenic therapy," *International Journal of Cancer*, vol. 123, no. 9, pp. 2120–2129, 2008.

[92] X. F. Wang, H. S. Wang, F. Zhang et al., "Nodal promotes the generation of M2-like macrophages and downregulates the expression of IL-12," *European Journal of Immunology*, vol. 44, no. 1, article 173, 2014.

[93] E. Mira, L. Carmona-Rodríguez, M. Tardáguila et al., "A lovastatin-elicited genetic program inhibits M2 macrophage polarization and enhances T cell infiltration into spontaneous mouse mammary tumors," *Oncotarget*, vol. 4, no. 12, pp. 2288–2301, 2013.

[94] S. M. Pyonteck, L. Akkari, A. J. Schuhmacher et al., "CSF-1R inhibition alters macrophage polarization and blocks glioma progression," *Nature Medicine*, vol. 19, no. 10, p. 1264, 2013.

[95] S. K. Biswas, L. Gangi, S. Paul et al., "A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-κB and enhanced IRF-3/STAT1 activation)," *Blood*, vol. 107, no. 5, pp. 2112–2122, 2006.

[96] G. K. Katara, M. K. Jaiswal, A. Kulshrestha, B. Kolli, A. Gilman-Sachs, and K. D. Beaman, "Tumor-associated vacuolar ATPase subunit promotes tumorigenic characteristics in macrophages," *Oncogene*, 2013.

[97] A. Mantovani and A. Sica, "Macrophages, innate immunity and cancer: balance, tolerance, and diversity," *Current Opinion in Immunology*, vol. 22, no. 2, pp. 231–237, 2010.

[98] V. Gocheva, H.-W. Wang, B. B. Gadea et al., "IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion," *Genes and Development*, vol. 24, no. 3, pp. 241–255, 2010.

[99] Y. Nagakawa, T. Aoki, K. Kasuya, A. Tsuchida, and Y. Koyanagi, "Histologic features of venous invasion, expression of vascular endothelial growth factor and matrix metalloproteinase-2 and matrix metalloproteinase-9, and the relation with liver metastasis in pancreatic cancer," *Pancreas*, vol. 24, no. 2, pp. 169–178, 2002.

[100] C. Neyen, A. Plüddemann, S. Mukhopadhyay et al., "Macrophage scavenger receptor a promotes tumor progression in murine models of ovarian and pancreatic cancer," *Journal of Immunology*, vol. 190, no. 7, pp. 3798–3805, 2013.

[101] X.-Z. Ye, S.-L. Xu, Y.-H. Xin et al., "Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-β1 signaling pathway," *Journal of Immunology*, vol. 189, no. 1, pp. 444–453, 2012.

[102] D. Li, X. Wang, J.-L. Wu et al., "Tumor-produced versican V1 enhances hCAP18/LL-37 expression in macrophages through activation of TLR2 and vitamin D3 signaling to promote ovarian cancer progression in vitro," *PLoS ONE*, vol. 8, no. 2, article e56616, 2013.

[103] S. Huang, M. Van Arsdall, S. Tedjarati et al., "Contributions of stromal metalloproteinase-9 to angiogenesis and growth of human ovarian carcinoma in mice," *Journal of the National Cancer Institute*, vol. 94, no. 15, pp. 1134–1142, 2002.

[104] F. Granata, A. Frattini, S. Loffredo et al., "Production of vascular endothelial growth factors from human lung macrophages induced by group IIA and group X secreted phospholipases A2," *Journal of Immunology*, vol. 184, no. 9, pp. 5232–5241, 2010.

[105] R. Wang, J. Zhang, S. Chen et al., "Tumor-associated macrophages provide a suitable microenvironment for non-small lung cancer invasion and progression," *Lung Cancer*, vol. 74, no. 2, pp. 188–196, 2011.

[106] K. A. Hotchkiss, A. W. Ashton, R. S. Klein, M. L. Lenzi, G. H. Zhu, and E. L. Schwartz, "Mechanisms by which tumor cells and monocytes expressing the angiogenic factor thymidine phosphorylase mediate human endothelial cell migration," *Cancer Research*, vol. 63, no. 2, pp. 527–533, 2003.

[107] C. Murdoch, M. Muthana, S. B. Coffelt, and C. E. Lewis, "The role of myeloid cells in the promotion of tumour angiogenesis," *Nature Reviews Cancer*, vol. 8, no. 8, pp. 618–631, 2008.

[108] A. Sica, A. Saccani, B. Bottazzi et al., "Autocrine production of IL-10 mediates defective IL-12 production and NF-κB activation in tumor-associated macrophages," *Journal of Immunology*, vol. 164, no. 2, pp. 762–767, 2000.

[109] T. Hagemann, J. Wilson, F. Burke et al., "Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype," *Journal of Immunology*, vol. 176, no. 8, pp. 5023–5032, 2006.

[110] D.-M. Kuang, Q. Zhao, C. Peng et al., "Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1," *Journal of Experimental Medicine*, vol. 206, no. 6, pp. 1327–1337, 2009.

[111] C. Chen, Y. Shen, Q.-X. Qu, X.-Q. Chen, X.-G. Zhang, and J.-A. Huang, "Induced expression of B7-H3 on the lung cancer cells and macrophages suppresses T-cell mediating anti-tumor immune response," *Experimental Cell Research*, vol. 319, no. 1, pp. 96–102, 2013.

[112] C. Chen, Q.-X. Qu, Y. Shen et al., "Induced expression of B7-H4 on the surface of lung cancer cell by the tumor-associated macrophages: a potential mechanism of immune escape," *Cancer Letters*, vol. 317, no. 1, pp. 99–105, 2012.

[113] C.-I. Chang, J. C. Liao, and L. Kuo, "Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity," *Cancer Research*, vol. 61, no. 3, pp. 1100–1106, 2001.

[114] W. G. Stetler-Stevenson and A. E. Yu, "Proteases in invasion: matrix metalloproteinases," *Seminars in Cancer Biology*, vol. 11, no. 2, pp. 143–152, 2001.

[115] F.-Q. Wang, J. So, S. Reierstad, and D. A. Fishman, "Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase," *International Journal of Cancer*, vol. 114, no. 1, pp. 19–31, 2005.

[116] L. R. Bohrer and K. L. Schwertfeger, "Macrophages promote fibroblast growth factor receptor-driven tumor cell migration and invasion in a Cxcr2-dependent manner," *Molecular Cancer Research*, vol. 10, no. 10, pp. 1294–1305, 2012.

[117] J.-Y. Lin, X.-Y. Li, N. Tadashi, and P. Dong, "Clinical significance of tumor-associated macrophage infiltration in supraglottic laryngeal carcinoma," *Chinese Journal of Cancer*, vol. 30, no. 4, pp. 280–286, 2011.

[118] W. Qing, W.-Y. Fang, L. Ye et al., "Density of tumor-associated macrophages correlates with lymph node metastasis in papillary thyroid carcinoma," *Thyroid*, vol. 22, no. 9, pp. 905–910, 2012.

[119] E. Gorelik, R. H. Wiltrout, M. J. Brunda, H. T. Holden, and R. B. Herberman, "Augmentation of metastasis formation by thioglycollate-elicited macrophages," *International Journal of Cancer*, vol. 29, no. 5, pp. 575–581, 1982.

[120] O. Vasiljeva, A. Papazoglou, A. Krüger et al., "Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer," *Cancer Research*, vol. 66, no. 10, pp. 5242–5250, 2006.

[121] G. Solinas, S. Schiarea, M. Liguori et al., "Tumor-conditioned macrophages secrete migration-stimulating factor: a new marker for M2-polarization, influencing tumor cell motility," *Journal of Immunology*, vol. 185, no. 1, pp. 642–652, 2010.

[122] D. G. DeNardo, J. B. Barreto, P. Andreu et al., "CD4+ T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages," *Cancer Cell*, vol. 16, no. 2, pp. 91–102, 2009.

[123] J. A. Joyce and J. W. Pollard, "Microenvironmental regulation of metastasis," *Nature Reviews Cancer*, vol. 9, no. 4, pp. 239–252, 2009.

[124] J. Zheng, M. Yang, J. Shao, Y. Miao, J. Han, and J. Du, "Chemokine receptor CX3CR1 contributes to macrophage survival in tumor metastasis," *Molecular Cancer*, vol. 12, no. 1, article 141, 2013.

[125] L. Wyler, C. U. Napoli, B. Ingold et al., "Brain metastasis in renal cancer patients: metastatic pattern, tumour-associated macrophages and chemokine/chemoreceptor expression," *British Journal of Cancer*, vol. 110, no. 3, article 686, 2014.

[126] E. Y. Lin, J.-F. Li, L. Gnatovskiy et al., "Macrophages regulate the angiogenic switch in a mouse model of breast cancer," *Cancer Research*, vol. 66, no. 23, pp. 11238–11246, 2006.

[127] T. Schmidt and P. Carmeliet, "Blood-vessel formation: bridges that guide and unite," *Nature*, vol. 465, no. 7299, pp. 697–699, 2010.

[128] S. F. Schoppmann, P. Birner, J. Stöckl et al., "Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis," *American Journal of Pathology*, vol. 161, no. 3, pp. 947–956, 2002.

[129] Q. Ebrahem, S. S. Chaurasia, A. Vasanji et al., "Cross-talk between vascular endothelial growth factor and matrix metalloproteinases in the induction of neovascularization in vivo," *American Journal of Pathology*, vol. 176, no. 1, pp. 496–503, 2010.

[130] I. Avraham-Davidi, S. Yona, M. Grunewald et al., "On-site education of VEGF-recruited monocytes improves their performance as angiogenic and arteriogenic accessory cells," *The Journal of Experimental Medicine*, vol. 210, no. 12, article 2611, 2013.

[131] C. Murdoch, A. Giannoudis, and C. E. Lewis, "Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues," *Blood*, vol. 104, no. 8, pp. 2224–2234, 2004.

[132] A. Casazza, D. Laoui, M. Wenes et al., "Impeding macrophage entry into hypoxic tumor areas by sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity," *Cancer Cell*, vol. 24, no. 6, article 695, 2013.

[133] S. M. Zeisberger, B. Odermatt, C. Marty, A. H. M. Zehnder-Fjällman, K. Ballmer-Hofer, and R. A. Schwendener, "Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach," *British Journal of Cancer*, vol. 95, no. 3, pp. 272–281, 2006.

[134] Q. Zhao, D.-M. Kuang, Y. Wu et al., "Activated $CD69^+$ T cells foster immune privilege by regulating IDO expression in tumor-associated macrophages," *Journal of Immunology*, vol. 188, no. 3, pp. 1117–1124, 2012.

[135] V. Bronte and P. Zanovello, "Regulation of immune responses by L-arginine metabolism," *Nature Reviews Immunology*, vol. 5, no. 8, pp. 641–654, 2005.

[136] A. L. Doedens, C. Stockmann, M. P. Rubinstein et al., "Macrophage expression of hypoxia-inducible factor-1α suppresses T-cell function and promotes tumor progression," *Cancer Research*, vol. 70, no. 19, pp. 7465–7475, 2010.

[137] K. Movahedi, D. Laoui, C. Gysemans et al., "Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes," *Cancer Research*, vol. 70, no. 14, pp. 5728–5739, 2010.

[138] I. Kryczek, L. Zou, P. Rodriguez et al., "B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma," *Journal of Experimental Medicine*, vol. 203, no. 4, pp. 871–881, 2006.

[139] J. Sun, Y. Mao, Y. Q. Zhang et al., "Clinical significance of the induction of macrophage differentiation by the costimulatory molecule B7-H3 in human non-small cell lung cancer," *Oncology Letters*, vol. 6, no. 5, pp. 1253–1260, 2013.

[140] M. Ramakrishnan, S. R. Mathur, and A. Mukhopadhyay, "Fusion-derived epithelial cancer cells express hematopoietic markers and contribute to stem cell and migratory phenotype in ovarian carcinoma," *Cancer Research*, vol. 73, no. 17, p. 5360, 2013.

[141] G. L. Beatty, E. G. Chiorean, M. P. Fishman et al., "CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans," *Science*, vol. 331, no. 6024, pp. 1612–1616, 2011.

[142] J. P. Edwards and L. A. Emens, "The multikinase inhibitor Sorafenib reverses the suppression of IL-12 and enhancement of IL-10 by PGE2 in murine macrophages," *International Immunopharmacology*, vol. 10, no. 10, pp. 1220–1228, 2010.

[143] G. Germano, R. Frapolli, C. Belgiovine et al., "Role of macrophage targeting in the antitumor activity of trabectedin," *Cancer Cell*, vol. 23, no. 2, pp. 249–262, 2013.

[144] S. Rozel, C. J. Galbán, K. Nicolay et al., "Synergy between anti-CCL2 and docetaxel as determined by DW-MRI in a metastatic bone cancer model," *Journal of Cellular Biochemistry*, vol. 107, no. 1, pp. 58–64, 2009.

[145] H. Xin, C. Zhang, A. Herrmann, Y. Du, R. Figlin, and H. Yu, "Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells," *Cancer Research*, vol. 69, no. 6, pp. 2506–2513, 2009.

[146] Q.-W. Zhang, L. Liu, C.-Y. Gong et al., "Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature," *PLoS ONE*, vol. 7, no. 12, article e50946, 2012.

[147] L. Bingle, N. J. Brown, and C. E. Lewis, "The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies," *Journal of Pathology*, vol. 196, no. 3, pp. 254–265, 2002.

[148] E. P. Buddingh, M. L. Kuijjer, R. A. J. Duim et al., "Tumor-infiltrating macrophages are associated with metastasis suppression in high-grade osteosarcoma: a rationale for treatment with macrophage activating agents," *Clinical Cancer Research*, vol. 17, no. 8, pp. 2110–2119, 2011.

[149] S. Kayal, S. Mathur, A. K. Karak et al., "CD68 tumor-associated macrophage marker is not prognostic of clinical outcome in classical Hodgkin's lymphoma," *Leuk Lymphoma*, vol. 55, no. 5, pp. 1031–1037, 2014.

[150] S. Hasselblom, U. Hansson, M. Sigurdardottir, H. Nilsson-Ehle, B. Ridell, and P.-O. Andersson, "Expression of CD68+ tumor-associated macrophages in patients with diffuse large B-cell lymphoma and its relation to prognosis," *Pathology International*, vol. 58, no. 8, pp. 529–532, 2008.

[151] S. J. Nam, H. Go, J. H. Paik et al., "An increase of M2 macrophages predicts poor prognosis in diffuse large B-cell lymphoma patients treated with Rituximab-CHOP," *Leukemia & Lymphoma*, 2014.

[152] E. C. Dun, K. Hanley, F. Wieser, S. Bohman, J. Yu, and R. N. Taylor, "Infiltration of tumor-associated macrophages is increased in the epithelial and stromal compartments of endometrial carcinomas," *International Journal of Gynecologic Pathology*, vol. 32, no. 6, pp. 576–584, 2013.

[153] S. Ohno, H. Inagawa, D. K. Dhar et al., "The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients," *Anticancer Research*, vol. 23, no. 6D, pp. 5015–5022, 2003.

[154] M. Erreni, A. Mantovani, and P. Allavena, "Tumor-associated macrophages (TAM) and inflammation in colorectal cancer," *Cancer Microenvironment*, vol. 4, no. 2, pp. 141–154, 2011.

[155] S. Edin, M. L. Wikberg, J. Rutegård, P. A. Oldenborg, and R. Palmqvist, "Phenotypic skewing of macrophages in vitro by secreted factors from colorectal cancer cells," *PLoS One*, vol. 8, no. 9, article e74982, 2013.

[156] H.-L. Rao, J.-W. Chen, M. Li et al., "Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients'adverse prognosis," *PLoS ONE*, vol. 7, no. 1, article e30806, 2012.

[157] J.-J. Zhao, K. Pan, W. Wang et al., "The prognostic value of tumor-infiltrating neutrophils in gastric adenocarcinoma after resection," *PLoS ONE*, vol. 7, no. 3, article e33655, 2012.

[158] T. Hagemann, T. Lawrence, I. McNeish et al., ""Re-educating" tumor-associated macrophages by targeting NF-κB," *Journal of Experimental Medicine*, vol. 205, no. 6, pp. 1261–1268, 2008.

[159] N. Luheshi, G. Davies, E. Poon, K. Wiggins, M. McCourt, and J. Legg, "Th1 cytokines are more effective than Th2 cytokines at licensing anti-tumour functions in CD40-activated human macrophages in vitro," *European Journal of Immunology*, vol. 44, no. 1, p. 162, 2014.

[160] J. Zhang, L. Patel, and K. J. Pienta, "CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis," *Cytokine and Growth Factor Reviews*, vol. 21, no. 1, pp. 41–48, 2010.

[161] B.-Z. Qian, J. Li, H. Zhang et al., "CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis," *Nature*, vol. 475, no. 7355, pp. 222–225, 2011.

[162] K. Garber, "First results for agents targeting cancer-related inflammation," *Journal of the National Cancer Institute*, vol. 101, no. 16, pp. 1110–1112, 2009.

[163] C. L. Manthey, D. L. Johnson, C. R. Illig et al., "JNJ-28312141, a novel orally active colony-stimulating factor-1 receptor/FMS-related receptor tyrosine kinase-3 receptor tyrosine kinase inhibitor with potential utility in solid tumors, bone metastases, and acute myeloid leukemia," *Molecular Cancer Therapeutics*, vol. 8, no. 11, pp. 3151–3161, 2009.

[164] S. Aharinejad, P. Paulus, M. Sioud et al., "Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice," *Cancer Research*, vol. 64, no. 15, pp. 5378–5384, 2004.

[165] P. Paulus, E. R. Stanley, R. Schäfer, D. Abraham, and S. Aharinejad, "Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts," *Cancer Research*, vol. 66, no. 8, pp. 4349–4356, 2006.

[166] S. Goswami, E. Sahai, J. B. Wyckoff et al., "Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop," *Cancer Research*, vol. 65, no. 12, pp. 5278–5283, 2005.

[167] D. G. DeNardo, D. J. Brennan, E. Rexhepaj et al., "Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy," *Cancer Discovery*, vol. 1, no. 1, pp. 54–67, 2011.

[168] G. Germano, R. Frapolli, M. Simone et al., "Antitumor and anti-inflammatory effects of trabectedin on human myxoid liposarcoma cells," *Cancer Research*, vol. 70, no. 6, pp. 2235–2244, 2010.

[169] E. Giraudo, M. Inoue, and D. Hanahan, "An amino-bisphosphonate targets MMP-9—expressing macrophages and angiogenesis to impair cervical carcinogenesis," *Journal of Clinical Investigation*, vol. 114, no. 5, pp. 623–633, 2004.

[170] I. Daurkin, E. Eruslanov, T. Stoffs et al., "Tumor-associated macrophages mediate immunosuppression in the renal cancer microenvironment by activating the 15-lipoxygenase-2 pathway," *Cancer Research*, vol. 71, no. 20, pp. 6400–6409, 2011.

[171] E. J. Villablanca, L. Raccosta, D. Zhou et al., "Tumor-mediated liver X receptor-α activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses," *Nature Medicine*, vol. 16, no. 1, pp. 98–105, 2010.

[172] F. Ciardiello and G. Tortora, "Drug therapy: EGFR antagonists in cancer treatment," *The New England Journal of Medicine*, vol. 358, no. 11, pp. 1096–1174, 2008.

[173] S. L. Shiao, A. Preethi Ganesan, H. S. Rugo, and L. M. Coussens, "Immune microenvironments in solid tumors: new targets for therapy," *Genes and Development*, vol. 25, no. 24, pp. 2559–2572, 2011.

[174] D. A. Hume and K. P. A. MacDonald, "Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling," *Blood*, vol. 119, no. 8, pp. 1810–1820, 2012.

[175] M. J. Cavnar, S. Zeng, T. S. Kim et al., "KIT oncogene inhibition drives intratumoral macrophage M2 polarization," *The Journal of Experimental Medicine*, vol. 210, no. 13, article 2873, 2013.