Myeloid-derived cells are key targets of tumor immunotherapy

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Abbreviations: CSF1, colony stimulating factor 1; DCs, dendritic cells; Gr-MDSCs, granulocytic myeloid-derived suppressor cells; iDCs, immature dendritic cells; NOS2, inducible nitric oxide synthase 2; IL-10, interleukin 10; mDCs, mature dendritic cells; Mo-MDSCs, monocytic myeloid-derived suppressor cells; MDSCs, myeloid-derived suppressor cells; NO, nitric oxide; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TAMs, tumor-associated macrophages; TANs, tumor-associated neutrophils; VEGF, vascular endothelial growth factor

Tumors are composed of heterogeneous cell populations recruited by cancer cells to promote growth and metastasis. Among cells comprising the tumor stroma, myeloid-derived cells play pleiotropic roles in supporting tumorigenesis at distinct stages of tumor development. The tumor-infiltrating myeloid cell contingent is composed of mast cells, neutrophils, dendritic cells, macrophages, and myeloid-derived suppressor cells. Such cells are capable of evading the hostile tumor environment typically prone to immune cell destruction and can even promote angiogenesis, chronic inflammation, and invasion. This paper briefly summarizes the different myeloid-derived subsets that promote tumor development and the strategies that have been used to counteract the protumorigenic activity of these cells. These strategies include myeloid cell depletion, reduction of recruitment, and inactivation or remodeling of cell phenotype. Combining drugs designed to target tumor myeloid cells with immunotherapies that effectively trigger antitumor adaptive immune responses holds great promise in the development of novel cancer treatments.

**The Tumor Microenvironment**

Tumors are more than simply masses of equivalent and proliferating cancer cells. Rather, they are heterogeneous by nature, being composed of multiple distinct cell types that participate in tangled interactions with one another (Fig. 1). Those cells which form the tumor-associated stroma are active contributors to tumor development. Over the last decade, accepted opinion has evolved from reductionism—perceiving a tumor as nothing more than a collection of relatively equivalent cancer cells—to the recognition of tumors as organs with interdependent cells whose complexity is somehow comparable to, or even exceeds that of, normal tissues. In fact, the tumor microenvironment serves as the key support system of a cancer, becoming the source of the 3-dimensional organization and architecture of the stroma, as well as providing all the protumorigenic factors that facilitate the growth, invasion, angiogenesis, and even metastatic ability of the neoplastic lesion. The tumor microenvironment contains malignant cells—those harboring genetic mutations—as well as other cell types that are activated and/or recruited such as fibroblasts, immune cells, and endothelial cells, many of which give rise to blood and lymphatic vessels. This heterogeneity of tumor cells is supported by tumor-derived factors that enhance the crosstalk between the cell populations and mediate tumor homeostasis.

The first link between inflammation and cancer was proposed by Rudolph Virchow in the 19th century who noticed leukocytes infiltrating tumors. Later on, at the beginning of the 20th century, Paul Ehrlich predicted that the immune system has the capacity to suppress the growth of cancerous lesions. Currently, researchers are convinced that an inflammatory microenvironment is an essential component of tumor development. Thus, neoplasms can be recognized and eliminated by the action of the host immune system. Nevertheless, most tumors continue to grow and progress.

This paradox may be accounted for by inefficient functioning of the host immune system toward a developing tumor. The immune system detects pathogenic insults through innate immune cell populations that subsequently mount a specific adaptive immune response aimed at responding appropriately to the damage. In this way, tumors are placed under natural selective pressures that lead them to evolve several mechanisms to bypass the immune recognition machinery and elude immune system checkpoints. As is the case for immune cells, the tumor microenvironment creates a milieu that inhibits antitumor immune reactivity. Thus, tumors modulate host immunity to remain as “invisible” as possible and so continue their path to invasiveness and metastasis.

Invisibility in immunological terms is a complex issue. Tumors need to recruit immunosuppressive immune cells to...
control and overcome the host’s antitumor immune responses. As is the case with the systemic immune system, the tumor immune regulatory system is composed of both myeloid and lymphoid immune cells. Among a particular cell subset, there will be cells functionally specialized in specific duties, such as generating DNA damage through the release of toxic chemical molecules, recruiting suppressive cells by secreting chemokines and growth factors, or abrogating T cell proliferation. This hierarchic organization explains why different immunosuppressive cell subsets dominate in certain established tumors. Hence, a fuller and more detailed understanding of the interactions between the immunosuppressive cell subsets will open the gates to new therapeutic approaches.

**Tumor-Infiltrating Myeloid Cells**

Myeloid cells are an immune cell division that, along with natural killer (NK) cells, makes up the innate immune system. Innate immunity defends the organism against infection in a non-specific manner, responding to pathogens in a generic way. This arm of the immune system constitutes an evolutionarily...
older defense strategy and plays a pivotal role in both the onset and resolution of the tissue inflammatory process. However, when tissue homeostasis is chronically perturbed, the imbalance between innate and adaptive immunity can result in excessive tissue repair. This affects tissue architecture and produces several molecules such as free oxygen radicals which induce DNA damage in epithelial cells potentially leading to tumor development in some circumstances. Once neoplastic cells arise and persist, innate immune cells produce cytokines and chemokines—based on their physiological tissue remodeling machinery—helping epithelial cells and fibroblasts to create the tumor stroma. They also attract other immune cells to constitute an immunosuppressive milieu.

Apart from sharing a common progenitor cell, there is a panoply of myeloid immune cells that acquire their differentiated phenotype locally. For instance, blood monocytes migrate through the circulatory system reaching virtually all tissues where, dependent upon their ultimate location, these immature myeloid cells will differentiate into dendritic cells, macrophages, or osteoclasts. Mast cell progenitors also undergo differentiation when they reach their target tissue, adopting mast cell properties typically near vascular structures. The ability of myeloid cells to adapt their specialized functions depending on the tissue context is well manipulated by the tumor microenvironment to polarize myeloid cells through a paracrine or autocrine release of different molecules to promote a pro-tumorigenic myeloid phenotype. This property results in a plethora of myeloid-mediated tumor escape mechanisms. These include the predominance of immature myeloid cell subsets at the tumor site, the reduced antigen presentation and associated attenuation of T cell activation due to the loss of cell-to-cell contact between myeloid cells and T cells, and the interference with myeloid cell migration to secondary lymphoid organs. Thus, taken together, these tumor-guided myeloid functional deficiencies culminate in impaired antitumor immune response.

Myeloid cell populations play a pivotal role in tumor development. Tumor-associated myeloid cell subsets are comprised of myeloid-derived suppressor cells at different stages of differentiation as well as neutrophils, dendritic cells, or macrophages and mast cells. In this context, these latter cell populations, keen to resolve inflammation when a pathogenic threat is present, serve to render the host immune cell subsets tolerant to tumor growth as well as to strengthen tumor stroma development. As for tumor-induced tolerance, this is achieved by either direct—e.g., induction of T-cell anergy by cell-to-cell contact—or indirect mechanisms, including the release of diverse molecules, such as transforming growth factor β (TGFβ) or interleukin 10 (IL-10) that polarize effector T-cell differentiation toward a regulatory-like phenotype.

**Mast Cells**

Mast cells are a myeloid subset with canonical functions in regulating allergic events and in T-cell mediated immunity. However, mast cells also accumulate at the sites of tumor growth in response to chemokines, such as (C-C) chemokine ligands CCL5 and CCL2. Mast cells have also been noted to amass in human invasive melanoma. Mast cells are well known by their characteristic release of secretory granules upon activation. These granules may contain a variety of molecules, such as heparin, histamine, vascular endothelial growth factor (VEGF), interleukin 1, and serine proteases which, under certain conditions, can directly promote the angiogenic switch. In addition, the soluble mediators released by mast cells promote a proinflammatory tumor microenvironment prone to the generation of IL-17 producing T helper (Th17) cells.

**Tumor-Associated Neutrophils (TANs)**

Neutrophils are short-lived white blood cells derived from bone marrow precursors. They are among the first cells to arrive at the sites of infection, releasing chemokines and proteases to recruit innate and adaptive immune effector cells. However, in several transplantable tumor models TANs have been observed to both stimulate tumor angiogenesis (via the production of various proangiogenic factors) and suppress antitumor immunity. Furthermore, it has been shown that TANs also adopt a protumor polarized phenotype driven primarily by TGFβ signaling in established solid tumors, a polarization comparable to that of tumor-associated macrophages. Interestingly, intratumoral TGFβ blockade has been shown to alter the phenotype of TANs toward tumor-inhibitory properties. Thus, restricting TGFβ signaling may be critical to the maintenance of proinflammatory neutrophils that promote antitumor CD8+ T cell recruitment and activation via the secretion of T cell-attracting chemokines and proinflammatory cytokines.

**Dendritic Cells (DCs)**

Known as professional antigen presenting cells, DCs are strategically positioned for bridging innate and adaptive immunity. DCs are a highly heterogeneous population of cells with remarkable plasticity that share common features, such as cell morphology and functional characteristics. These cells can be subdivided into 2 fundamentally distinct developmental stages termed immature dendritic cells (iDCs) and mature dendritic cells (mDCs). Whereas iDCs are localized primarily in peripheral tissues and perform the specialized functions of antigen uptake and processing, mDCs reside in lymphoid organs, where they interact with antigen-specific T cells and initiate immune responses. The chemokine receptor repertoire differs between iDCs and mDCs, such that the signals they perceive direct migration and homing to new sites are correspondingly different. DCs along this developmental spectrum then directly sense pathogens or altered cell components (such as from transformed cells), or other danger signals, and conveys the antigen captured in peripheral tissues to the lymphoid organs in order to mount an immune response.

However, in the context of tumors DCs often remain dysfunctional due to tumor-induced abnormalities. For instance, mast cell progenitors also undergo differentiation when they reach their target tissue, adopting mast cell properties typically near vascular structures. The ability of myeloid cells to adapt their specialized functions depending on the tissue context is well manipulated by the tumor microenvironment to polarize myeloid cells through a paracrine or autocrine release of different molecules to promote a pro-tumorigenic myeloid phenotype. This property results in a plethora of myeloid-mediated tumor escape mechanisms. These include the predominance of immature myeloid cell subsets at the tumor site, the reduced antigen presentation and associated attenuation of T cell activation due to the loss of cell-to-cell contact between myeloid cells and T cells, and the interference with myeloid cell migration to secondary lymphoid organs. Thus, taken together, these tumor-guided myeloid functional deficiencies culminate in impaired antitumor immune response.2-6 Myeloid cell populations play a pivotal role in tumor development. Tumor-associated myeloid cell subsets are comprised of myeloid-derived suppressor cells at different stages of differentiation as well as neutrophils, dendritic cells, or macrophages and mast cells. In this context, these latter cell populations, keen to resolve inflammation when a pathogenic threat is present, serve to render the host immune cell subsets tolerant to tumor growth as well as to strengthen tumor stroma development. As for tumor-induced tolerance, this is achieved by either direct—e.g., induction of T-cell anergy by cell-to-cell contact—or indirect mechanisms, including the release of diverse molecules, such as transforming growth factor β (TGFβ) or interleukin 10 (IL-10) that polarize effector T-cell differentiation toward a regulatory-like phenotype.

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functional DCs may be eliminated in a tumor context either by abrogating their differentiation and maturation status or by the induction of apoptosis. These events have been clearly evinced by decreased numbers of circulating myeloid DCs detected in cancer patients.\textsuperscript{13} Moreover, tumor-associated DCs often appear to be incapable of migrating from the tumor site and further, have lost key components of their antigen processing and presentation machinery. Indeed, the tumor microenvironment abrogates the release of chemokines that normally attract functional DCs, thus reducing their numbers at the tumor site.\textsuperscript{15,16} Abnormal levels of circulating plasmacytoid dendritic cells in cancer patients and the marked increase of immature DC subsets both in patient and mouse tumor lesions highlight the pivotal role of tumor cells and stroma in polarizing tumor-associated immune cell subsets.\textsuperscript{17}

Thus, the production of tumor-derived factors which affect DCs at molecular and transcriptional levels might lead to abnormalities in DC maturation, suppress DC survival, and impair DC function at the tumor site. For example, tumor derived TGFB and IL-10 significantly reduce MHC class II and costimulatory surface molecule expression, diminish IL-12 production, abrogate DC maturation, and expand functional regulatory T cells (Tregs).\textsuperscript{18} The serum level of VEGF, another immunomodulatory tumor-derived factor, has been shown to inversely correlate with DC numbers in patients with colorectal cancer. Furthermore, monocyte-derived DCs cultured with exogenous VEGF are prone to apoptosis and an attenuated maturation status.\textsuperscript{19} VEGF has also been shown to be involved in recruiting immature DCs from the bone marrow to the tumor microenvironment.\textsuperscript{20} Catalysis of arachidonic acid, a member of the inflammatory-associated eicosanoid signaling cascade, by prostaglandin-endoperoxide synthase 2 generates prostaglandins such as prostaglandin E2, that impedes DC maturation and interleukin 12 production but considerably increases IL-10 levels in vivo.\textsuperscript{21} With regard to intrinsic transcription factors, activation of signal transducer and activation of transcription 3 (STAT3) by tumor-derived factors such as epidermal growth factor (EGF), VEGF, IL-10, and colony stimulating factor 2 (CSF2, also known as GMCSF) has been observed to block DC maturation and function.\textsuperscript{2,22}

Altogether, DCs dysregulation in tumors is important for tumor immune subversion, but DCs are not mere passive spectators of this process. Certain DCs subsets play crucial roles in tumor escape. For instance, tumor-induced immature myeloid DCs have been reported to promote the proliferation of Tregs in a TGFB-dependent manner in murine melanoma. Furthermore, the tumor microenvironment abrogates the native ability of DCs to present tumor antigens-thereby blocking their induction of tumor-specific cytotoxic T lymphocytes (CTLs)-and stimulates the upregulation of programmed cell death ligand 1 (PD-L1) on tumor DCs that further inhibits antitumor T cell-mediated immunity.\textsuperscript{23,24}

**Tumor-Associated Macrophages (TAMS)**

Macrophages are present in most solid tumors, representing up to 50% of the cell mass.\textsuperscript{25} Blood monocytes are recruited to the tumor stroma where they differentiate to macrophages.\textsuperscript{26} The soluble factors that promote the accumulation of macrophages and are produced by cancer and stromal cells of the tumor include both chemokines such as CCL2, CCL5, CCL7, CXCL8, and CXCL12, as well as cytokines such as VEGF, platelet-derived growth factor (PDGF), and CSF-1.\textsuperscript{27-29} Once present in the tumor stroma, macrophages promote all phases of tumorigenesis, such as tumor growth, invasion, and metastasis, as well as stimulating tumor-promoting processes such as angiogenesis and immune suppression. Clinical data reveal that macrophage infiltration strongly correlates with poor prognosis in a variety of human cancers.\textsuperscript{29} TAMs stimulate tumor growth through the release of soluble factors. Thus, invasion and metastasis are promoted by the release of proteins such as tumor necrosis factor (TNF), TGFB, IL-1, or matrix metallopeptidase 9 (MMP9).\textsuperscript{29,30} The process of angiogenesis required to sustain tumor growth is enhanced by the release of growth factors such as VEGF, PDGF, and TGFB.\textsuperscript{31} Finally, the suppression of antitumor immune responses is mediated by the pattern of cytokines released which is characterized by the production of high levels of IL-10 and low levels of IL-12.\textsuperscript{32} Inducible nitric oxide synthase 2 (NOS2) and arginase-1 also contribute to the TAM-mediated immune suppression. Nitric oxide (NO) production by the NOS2 pathway leads to T cell cytotoxicity and apoptosis, whereas arginase-1 converts arginine into putrescine and L-ornithine, metabolites used by cancer cells to proliferate and inhibit T cell proliferation. Both enzymes produce reactive oxygen species (ROS), suppressing tumor infiltration by lymphocytes.\textsuperscript{33}

Altogether, macrophages are the best example of how tumors manipulate their microenvironment during the process of malignant disease progression. The macrophages infiltrating tumors resemble “alternatively activated” (M2) macrophages but their tumor-promoting activity is reinforced by the expression of some M1-associated molecules typically associated with classically activated macrophages.\textsuperscript{30} This unique, tumor-associated subset of macrophages with amazing polarization properties is present at every stage of tumor development, continuously adapting to the tumor requirements for progression.

**Myeloid-Derived Suppressor Cells**

Myeloid-derived suppressor cells (MDSCs) comprise myeloid progenitors and immature myeloid cells at distinct stages along a spectrum of differentiation. In a physiological context, these cells would give rise to terminally differentiated DCs, macrophages, monocytes, or granulocytes. However, in response to a pathologic condition such as cancer, these cells become blocked in their maturation and tend to accumulate and expand precociously. Indeed, MDSCs are present in almost all cancer patients.\textsuperscript{34} However, myeloid cells which morphologically resemble MDSCs cell subsets have been found in healthy individuals, but do not have the same suppressive characteristics. Thus, it appears that MDSCs specifically act under certain pathological conditions where they accumulate in peripheral lymphoid organs and attenuate immunity.\textsuperscript{35}
MDSCs do not express canonical DC, monocyte or macrophage surface markers but their morphology resembles that of granulocytes (Gr-MDSCs) or monocytes (Mo-MDSCs). MDSC subsets can be identified by the surface expression of CD11b and Gr-1. The monoclonal antibody RB6–8C5, that recognizes Gr-1, binds to 2 members of the Ly6 family of leukocyte-expressed markers: Ly6C, a monocyte/macrophage marker, and Ly6G, a classical neutrophil marker. High Gr-1 expression that coincides with Ly6C<sup>bhi</sup>Ly6G<sup>low</sup> populations is associated with granulocytes, termed Gr-MDSCs. However, this marker is lowly expressed on mononcytic cells, such as monocyte and macrophage precursors, which can be alternatively defined by Ly6C<sup>bhi</sup>Ly6G<sup>low</sup> expression and are known as Mo-MDSCs. In the presence of the appropriate stimuli, Mo-MDSCs may differentiate to become DCs or macrophages in a hypoxia-dependent process both in vitro and in vivo. However, in cancer Mo-MDSCs preferentially differentiate toward Gr-MDSCs in a process governed by epigenetic silencing of the retinoblastoma (RB) gene controlled by histone deacetylase 2 (HDAC2). MDSC nomenclature in PBMCs from cancer patients is still a matter of debate. However, markers for the classification of Mo-MDSCs as CD14<sup>+</sup>CD11b<sup>+</sup>HLA-DR<sup>low/−</sup> cells and Gr-MDSCs as LIN-HLA-DR-CD33<sup>+</sup>CD11b<sup>+</sup> cells have been extensively reported throughout the literature.

The expansion and accumulation of MDSCs is governed by numerous tumor-derived factors. Most of these factors are principal components of the inflammatory response, such as CSF2, IL-6, IL-1, or VEGF. These cell-to-cell signaling molecules also function to regulate the expansion of other myeloid cell subsets, such as DCs, mast cells, TANs, or TAMs. Recently, cancer-stimulated microRNAs have been shown to be factors inducing MDSC accumulation and myeloid cell dysfunction. In the case of MDSCs, tumor-related signaling molecules trigger the phosphorylation and activation of STAT3, a key transcription factor regulating myeloid progenitor cell function and proliferation, and thus, MDSC expansion. STAT3 activation in myeloid progenitor cells lead to the production of S-100 calcium binding protein family members S100A8 and S100A9 proteins, which abrogate myeloid cell maturation and promote MDSC accumulation in the tumor bed as well as their migration to metastatic sites.

The activation of MDSCs mainly depends on factors derived from both activated T cells and tumor stromal cells, that regulate immunomodulatory molecules such as arginase-1, NOS2, ROS, and TGFβ through the action of STAT-family transcription factors STAT1 and STAT6 and nuclear factor-κ B (NF-κB) signaling pathways. Thus, downstream extracellular signaling factors, such as IL-4, IL-13, interferon-γ (IFNγ), and TGFβ mediate, the immunosuppressive activity of MDSCs. Furthermore, the STAT6 transcription factor has been shown to be involved in MDSCs turnover. In support, Suzanne Ostrand-Rosenberg’s group has shown that MDSCs express the cell surface death receptor Fas and can activate CD8<sup>+</sup> T cells expressing Fas ligand, such that MDSCs initiated T cell activation subsequently induces MDSC apoptosis.

In the same way as TAMs, MDSC–based T cell immune suppression at the tumor site is antigen non-specific, and therefore MDSCs also share the mechanisms by which they abrogate T cell proliferation and functionality, such as arginine metabolism through the action of STAT-family transcription factors, such as STAT1 and STAT6 and nuclear factor-κ B (NF-κB) signaling pathways. Thus, downstream extracellular signaling factors, such as IL-4, IL-13, interferon-γ (IFNγ), and TGFβ mediate, the immunosuppressive activity of MDSCs. Furthermore, the STAT6 transcription factor has been shown to be involved in MDSCs turnover. In support, Suzanne Ostrand-Rosenberg’s group has shown that MDSCs express the cell surface death receptor Fas and can activate CD8<sup>+</sup> T cells expressing Fas ligand, such that MDSCs initiated T cell activation subsequently induces MDSC apoptosis.

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### Modulation of Tumor-Infiltrating Myeloid Cells by Immunotherapy

Tumor-associated myeloid cells constitute one of the many barriers that antitumor immunotherapy must overcome to successfully eradicate established tumors. However, myeloid cells also play a part in effector immune responses. These cells are not only involved in innate immune responses but are also important effector cells in adaptive immune responses. Indeed, the relevance of myeloid cells in enhancing antitumor immunity has been underestimated due to the critical role of other effector cells such as T lymphocytes or B cells in adaptive immune responses. Therefore, in order to achieve potent antitumor effects and defeat cancer, all the “troops” must be recruited to the battlefield, including antitumor myeloid cells.

Conceptually, the immunosuppressive activity of tumor-associated myeloid cells can be abrogated by several distinct routes. These include methods to deplete myeloid suppressor cell levels via chemotherapy. Other approaches include agents to reduce myeloid recruitment to the tumor microenvironment, as well as means to attenuate their protumorigenic functions. This step paves the way for the generation of antitumor effector myeloid cells. These myeloid cells can originate by conversion of tumor-associated myeloid cells into antitumor myeloid cells or, alternatively, by recruitment of a new subset of myeloid cells into the tumor stroma (Fig. 2).

### Depletion of Myeloid Suppressor Cells

The most promising and feasible strategy to reduce the intratumoral numbers of myeloid suppressor cells is the use of low doses of approved chemotherapy drugs. Ugel et al. evaluated 12 widely used anticancer drugs for their ability to deplete myeloid suppressor cells and to restore immune responsiveness in tumor-bearing mice. They found that 7 of these drugs were able to effectively reduce tumor-infiltrating MDSCs: cyclophosphamide, 5-fluorouracil, fludarabine, gemcitabine, bortezomib, sorafenib, and sunitinib. This comparative study confirmed prior reports, since the majority of these anticancer drugs had been previously observed to reduce MDSCs tumor-infiltration in various tumor models. In addition to anticancer drugs, other small molecules have been found to block the expansion of MDSCs. Among these drugs are vitamin derivatives, amino-bisphosphonate, and antibodies or antagonists, such as an IL-4Rα RNA aptamer, that...
With regard to TAMs, clodronate-loaded liposomes are the most widely used drug to deplete macrophages in animal models.69 In breast cancer patients, treatment with these liposomal bisphosphonates reportedly reduced the formation of new metastases.70 Another compound that can be used to deplete TAMs is trabectedin, a recently approved chemotherapy drug originally isolated from a marine tunicate *Ecteinascidia*.71 This drug was originally developed due to its potent tumoricidal ability, but recently trabectedin has also been shown to possess potent immunomodulatory activities. Trabectedin appears to selectively deplete monocytes and macrophages and further, blocks angiogenesis and monocyte tumor recruitment via reducing the expression of VEGF and CCL2 in tumor vessels.72 This intriguing mechanism of a new chemotherapy drug highlights the importance of studying the effects of conventional chemotherapeutic agents on immunity in an attempt to maximize their antitumor efficacy.

**Blockade of Macrophage Recruitment to the Tumor Microenvironment**

Another strategy to reduce TAM levels is to interfere with molecules that recruit macrophages to the tumor bed. Importantly, attenuated macrophage tumor-infiltration manifests as reduced tumor growth and metastatic spread. The most widely studied strategy to date is blockade of colony stimulating factor 1 (CSF1) signaling, an essential regulator of macrophage homeostasis. Blockade of the receptor for CSF1 was first described as reducing TAMs, and thereby increasing CD8+ T cells,28 but it can also effectively reduce the infiltration of MDSCs.66 CSF1 signaling can be abrogated using inhibitors of the protein tyrosine kinase...
activity of the CSF1 receptor or by exogenous application of molecules that block CSF1 ligand-receptor binding. Among the inhibitors of tyrosine kinase activity, Ki20227 has been shown to be relatively selective for the CSF1 receptor but most other tyrosine kinase inhibitors are broad spectrum and inhibit other tyrosine kinases. For instance, PLX3397, which shows potent antitumor activity when combined with chemotherapy, can block CSF1 and cKIT receptor tyrosine kinases. Other strategies that have been implemented to specifically prevent the binding of CSF1 to its cognate receptor include the use of small interfering RNAs against CSF1 and the use of blocking antibodies against CSF1 or its receptor. Investigations of therapeutic applications of blockade of CSF1 as a cancer treatment has reached clinical trials. A Phase I/II clinical trial of an anti-CSF1 antibody has been recently performed (NCT00757757) and several clinical trials with inhibitors of protein tyrosine kinase activity are ongoing (NCT01499043, NCT01349049 and NCT01349036).

Another cytokine that promotes macrophage infiltration into tumors is VEGF. Tumor-associated macrophages express the VEGF receptor variant VEGFR2 (also known as kinase insert domain receptor, KDR) and VEGF-blocking antibodies reduce the number of TAMs. Chemokines are another potential target that may be manipulated to interfere with macrophage recruitment. For instance, antibodies blocking CCL2 have been successfully used to reduce TAM migration and CXCR2 antagonists display antitumor activities and reduce metastatic dissemination by impeding Gr-1+ cell accumulation.

### Functional Inactivation of Protumorigenic Myeloid Suppressor Cells

The protumorigenic activity of myeloid cells can be attenuated not only by reducing the number of these subsets in the tumor but also by blocking the effector molecules that mediate the deleterious effects of myeloid cells. A common effector molecule used by several myeloid cell subsets are ROS. Acute release of ROS is among antitumor immune mediators, however chronic exposure to ROS actually promotes tumor progression by dampening effector immune cells and promoting DNA damage and chromosomal instability in tumor cells. An anti-inflammatory triterpenoid with the ability to reduce ROS release has been shown to dampen the activity of MDSCs. An alternative strategy to block the suppressor activity of these cells is to inhibit the catalytic enzymes overexpressed by MDSCs, namely arginase 1 and NOS2. These enzymes have been blocked by nitroaspirin and by inhibitors of phosphodiesterase-5 (such as sildenafil). Inhibition of both of these molecules has been shown to reduce the activity of MDSCs and enhance antitumor immunity. Finally, production of ROS by tumors can nitrate chemokines and promote the hyporesponsiveness of T cells to stimulation through the tyrosine phosphorylation of several proteins, including the CD3ζ chain of the T cell receptor complex. These immunosuppressive effects can be blocked by peroxynitrite-scavenging drugs.

### Remodeling of Tumor-infiltrating Myeloid Cells

Several treatments have been shown to remodel the intratumoral myeloid cell compartment, switching from a suppressive myeloid cell milieu toward antitumor myeloid cell types. Among chemotherapy drugs, cyclophosphamide has a special ability to induce tumor myeloid cell remodeling. Ibe et al. reported that cyclophosphamide induces a fast switch in intratumoral macrophages from tumor-suppressive M2 to antitumor M1 subtypes. The antitumor activity of this drug is enhanced when the modulation of tumor-infiltrating myeloid cells is combined with immunostimulatory treatment. For instance, in combination with an antitumor vaccine and CpG, cyclophosphamide depleted MDSCs and promoted the appearance of antitumor neutrophils, thereby leading to the eradication of large tumors. Moreover IL-12 combined with cyclophosphamide modified the intratumoral myeloid cell compartment by a combination of pro-inflammatory monocytes and neutrophils that acquired direct tumor killing capabilities and promoted antitumor CD8+ T-cell infiltration. Interestingly, other chemotherapy components can trigger intratumoral myeloid cell remodeling. Recently, Ma et al. showed that recruitment of a DC-like CD11c+CD11b+Ly6G+CD11b+CD11c+ myeloid cell population was critical for the immunostimulatory effect of chemotherapies such as doxorubicin or mitoxantrone that trigger an immunogenic cell death.

Other therapeutic regimens can trigger this functional remodeling. For instance, the switch between N2 to N1-subtypes of intratumoral neutrophils can be achieved by blocking TGFβ. In addition, some reports have linked the antitumor activity of CSF2 to its ability to activate the killing activity of neutrophils. In regards to macrophages, the switch from M2 to M1 can be achieved by the use of zoleidronic acid or by the combination of CpG with an antibody to block the IL-10 receptor. In both cases, a potent antitumor innate immune response is exerted by remodeling the type of macrophages present within the tumor. Zoleidronic acid is an anti-resorptive agent that has been observed to exhibit antitumor effects both in vitro and in vivo. The proposed mechanism of action is the modulation of the mevalonate pathway that affects protein prenylation in both tumor cells and macrophages, thereby decreasing the viability of these cells. In addition, zoleidronic acid promotes the translocation of NF-kB to the nucleus, inducing the production of inflammatory cytokines. Disrupting another critical regulator of myeloid cell fate, STAT3 has been shown to be crucial for tumor-induced myeloid cell remodeling toward tumor-resolving inflammation. The STAT3 inhibitor JSI-124 induces antitumor immunity by maturing MDSCs toward DCs in vivo and there is an ongoing clinical trial investigating the use of an anti-sense STAT3 inhibitor in cancer patients. Finally, CD40 agonists alone or in combination with IL-2 have been shown to switch immunosuppressive TAMs into potent antitumor macrophages.
in preclinical settings and, more importantly, have been found to induce objective clinical responses in patients.\textsuperscript{101-103}

Conclusions

Myeloid cells are essential components of the tumor stroma that support all phases of tumor development. These cells promote tumor growth and metastasis by facilitating tumor transformation and angiogenesis, as well as by suppressing antitumor effector immune responses. Therefore, tumor-associated myeloid cells are excellent drug targets to therapeutically interfere with tumor development. Several strategies have been tested to deplete myeloid cells infiltrating tumors, including the inhibition of their recruitment, agents to abrogate their immunosuppressive functions or to remodel their tumor composition. These treatments usually achieve modest results in terms of tumor rejection but can have synergistic effects when combined with other cancer treatments such as chemotherapy, radiotherapy, monoclonal antibodies or other immunotherapies designed to boost the adaptive immune system. The clinical development of drug combinations is cumbersome and expensive, especially when single agents do not display potent antitumor effects. However, cancer is a complex disease with many players involved in the tumorigenic process. In order to achieve tumor rejections, several pathways will likely need to be simultaneously targeted.

The most feasible and inexpensive strategy to modulate tumor-associated myeloid cells is the use of low-doses of approved chemotherapy drugs. However, the doses and the timing of such drugs must be carefully examined in clinical trials in order to maximize their efficacy on myeloid cells and achieve the desired anticancer immunostimulatory effects. Positive clinical results obtained by modulating myeloid cells with such compounds will pave the way for the development of new drugs targeting these cells to boost the successful application of immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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