Coordinated regulation of myeloid cells by tumours

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Abstract
Myeloid cells are the most abundant nucleated hematopoietic cells in the human body and are a collection of distinct cell populations with many diverse functions. The three groups of terminally differentiated myeloid cells — macrophages, dendritic cells and granulocytes — are essential for the normal function of both the innate and adaptive immune systems. Mounting evidence indicates that the tumour microenvironment alters myeloid cells and can convert them into potent immune suppressive cells. Here, we consider myeloid cells as an intricately connected, complex, single system and we focus on how tumours manipulate the myeloid system to evade the host immune response.

Myeloid cells are the most abundant hematopoietic cells in the human body with diverse functions. All myeloid cells arise from multipotent hematopoietic stem cells (HSCs) that develop into mature myeloid cells through sequential steps of differentiation. However, myeloid progenitors do not form a hierarchical system but can instead be considered as a network of cells that can differentiate into various more specialized myeloid cell subsets (Fig. 1).

The three groups of terminally differentiated myeloid cells — macrophages (MΦ), dendritic cells (DCs) and granulocytes (G) — are essential for the normal functions of the innate and adaptive immune systems. Classically, they protect organisms from pathogens, eliminate dying cells, and mediate tissue remodeling. Although the contribution of myeloid cells to tumour pathogenesis has been recognized for over 100 years, only during the past two decades has their crucial role in promoting tumour angiogenesis, cell invasion, and metastasis been appreciated (reviewed in1–3). Mast cells were also implicated in regulation of tumor progression (reviewed in4). Mounting evidence indicates that the tumour microenvironment alters myeloid cells by converting them into potent immunosuppressive cells. In recent years the concept of myeloid-derived suppressor cells (MDSCs) (described below) has emerged. However, the wealth of new information concerning myeloid cells in cancer has also produced confusion. In most studies, individual myeloid cell populations were examined independently, generating fragmented information that contributed to a convoluted view of their role in immune responses in cancer. In addition, their expression of overlapping cell surface markers has made it difficult to distinguish between different myeloid cell populations, further obscuring the nature of specific myeloid cell subsets in cancer. These complications limit our understanding of myeloid cell biology and hamper attempts to develop and optimize therapeutic interventions.
In this Review, we present a cohesive view of the effects of the tumour on myeloid cells. Our goal is not to provide a comprehensive overview of changes in individual populations of myeloid cells as this has been accomplished in other recent reviews. Instead, we will briefly summarize the effects that tumours have on terminally differentiated myeloid cell subsets and will then focus on discussing myeloid cell interactions and responses during tumour development as an intricately connected single, albeit complex, system.

**Dendritic cells**

DCs are terminally differentiated myeloid cells that specialize in antigen processing and presentation. DCs differentiate in the bone marrow from various progenitors. They can also differentiate from monocytes under certain conditions, although most DCs in mouse lymphoid organs are not monocyte-derived. In contrast, monocytes are the major precursors of DCs in humans.

Two major subsets of DCs are currently recognized: conventional (cDCs) and plasmacytoid (pDCs). Although these cells share some common progenitors, they differentiate along distinct genetic programs and have different morphologies, markers, and functions (Box 1). The centerpiece of DC biology is the concept of functional activation and maturation in response to ‘dangerous’ stimuli. Differentiated DCs reside in tissues as ‘immature’ cells that actively take up tissue antigens, but are poor antigen presenters and do not promote effector T cell differentiation. Only functionally activated DCs can effectively stimulate immune responses. DCs are activated in response to stimuli associated with bacteria, viruses or damaged tissues; such stimuli are commonly referred to as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Activation of DCs leads to profound changes in their gene expression, resulting in increased expression of co-stimulatory molecules and cytokines that promote T cell activation, and also in upregulation of chemokine receptors that drive DC migration to lymphoid tissues. pDCs constitute a minor population of DCs that have a morphology reminiscent of plasma cells, express TLR7 and TLR9 (this receptor is not expressed on human cDCs) and produce large amounts of IFNα in response to activation of TLRs by viruses and self-DNA. A more detailed discussion of DC biology can be found in recent reviews.

**Box 1**

**Phenotypic definition useful for separation of different myeloid populations in lymphoid organs of mice**

- **Dendritic cells**: CD11c⁺F4/80⁻Gr-1⁻ MHC class II⁺
  - cDCs: CD11c⁺CD11b⁺MHC class II⁺CD205⁺F4/80⁻Gr-1⁻CD115low
    
      Expression of 33D or DEC205/CD205 is specific for DCs but these markers are not expressed on all cells
    
  - pDCs: CD11c⁺CD11b⁻B220⁺Siglec H⁺Gr-1⁻F4/80
- **Monocytes**: CD11b⁺ Ly6C⁺ Ly6G⁻ CD11c⁻ CD115⁺
  - **Resident monocytes**: CD11b⁺ Ly6ClowLy6G⁻ CD115⁺ MHC class II⁻ F4/80high CD11c⁻
  - **Inflammatory monocytes**: CD11b⁺ Ly6highLy6G⁻ CD115⁺ MHC class II⁻ F4/80⁺ CD11c⁻
- **Macrophages**: F4/80⁺CD11b⁺Gr-1⁻
- **M1 macrophages** iNOS\(^+\)IL-12\(^+\)CD86\(^+\)MHC class II\(^{\text{high}}\)
- **M2 macrophages** CD206\(^+\) CD163\(^+\) CD36\(^+\) ARG1\(^+\)MHC class II\(^{\text{low}}\) IL-10\(^+\)IL-4R\(\alpha\) FIZZ1\(^+\)YM1\(^+\)

- **Granulocytes**: CD11b\(^+\)Ly6G\(^+\)Ly6C\(^{\text{low}}\) F4/80\(^-\)CD11c\(^-\)

### Phenotypic definition useful for separation of myeloid populations in human blood

- **DCs** (*mononuclear fraction separated on standard ficoll gradient*) Lin\(^-\) (CD3\(^-\)CD14\(^-\)CD19\(^-\)CD56\(^-\)) HLA-DR\(^+\)BDCA-1\(^+\) CD209\(^+\)
  - **DCs**: Lineage (Lin) cocktail (CD3,CD14,CD19, CD56) \(^-\) CD11c\(^+\) CD11b\(^+\) CD33\(^+\) BDCA-1/CD1c\(^+\) BDCA-3/CD141, CD209/DC-SIGN\(^+\)
    
    Expression DEC205/CD205 is specific for DCs but this marker is not expressed on all cells
  - **pDCs**: Lineage cocktail (CD3,CD14,CD19, CD56) \(^-\) CD123\(^+\) BDCA-2/CD303\(^+\) BDCA-4/CD304

- **Monocytes** (*mononuclear fraction separated on standard ficoll gradient*) CD14\(^+\)HLA-DR\(^+\)CD15\(^-\)

- **Macrophages** CD14\(^+\)CD68\(^+\)
  - **M1 macrophages** iNOS\(^+\)IL-12\(^+\)CD86\(^+\)HLA-DR\(^+\)
  - **M2 macrophages** CD206\(^+\) CD163\(^+\) CD36\(^+\)HLA-DR\(^{\text{low}}\) IL-10\(^+\)CD124\(^+\)

- **Granulocytes** (*usually are not present in mononuclear fraction and require sedimentation after removal of mononuclear cells*): CD15\(^+\)CD14\(^-\) CD66b\(^+\) CD16\(^+\)

### Typical phenotype of mouse MDSCs

- **CD11b\(^+\)Gr-1\(^+\)CD11c\(^-\)F4/80\(^+\/-\)CD124\(^+\)**
  - **PMN-MDSCs**: CD11b\(^+\)Gr-1\(^{\text{hi}}\)Ly6C\(^{\text{low}}\)Ly6G\(^+\) CD49d\(^-\)
  - **M-MDSCs**: CD11b\(^+\)Gr-1\(^{\text{mid}}\)Ly6C\(^{\text{hi}}\)Ly6G\(^-\)CD49d\(^+\)

### Typical phenotype of human MDSCs

These cells are purified on standard ficoll gradient for isolation of mononuclear cells

- CD11b\(^+\)CD14\(^-\)CD33\(^+\) (*PMN-MDSCs in addition express CD15 and/or CD66b*)
- Lin\(^-\) (CD3, CD14, CD16, CD19) HLA-DR\(^-\)CD33\(^+\)
- CD14\(^+\)HLA-DR\(^{\text{low}}\/-\) = **M-MDSC**

### Effects of cancer on DCs

That cancer can have profound effects on the function of DCs has been known for more than 20 years. It is well established that DCs in tumour-bearing hosts do not adequately stimulate an immune response, which potentially contributes to tumour evasion of immune recognition. Significant evidence from numerous studies strongly indicates that abnormal myelopoiesis is the dominant mechanism responsible for DC defects in cancer\(^{14}\). This abnormal differentiation produces at least three main results: decreased production of mature functionally competent DCs; increased accumulation of immature DCs at the tumour site; and increased production of immature myeloid cells\(^{14}\). In recent years multiple clinical
studies have confirmed the findings of earlier studies and have indicated there is a decreased presence and defective functionality of mature DCs in patients with breast, non-small cell lung, pancreatic, cervical, hepatocellular, prostate cancers and glioma.

In addition to the many tumour-derived soluble factors previously implicated in abnormal DC differentiation, such as vascular endothelial growth factor (VEGF), macrophage colony-forming factor (M-CSF) and IL-6, recent studies have shown that other factors present in the tumour microenvironment impair normal DC functions. The tumour microenvironment is predominantly characterized by hypoxia, accumulation of extracellular adenosine, increased lactate, and a decreased pH. DC migration and function are severely impaired by hypoxia and adenosine. The transcription factor hypoxia-inducible factor (HIF) is upregulated by DCs in the hypoxic tumour environment and was shown to induce expression of adenosine receptor A2b by human DCs, causing these DCs to drive the development of T helper 2 (T<sub>H2</sub>) cells rather than more potent anti-tumour T<sub>H1</sub> cells. DCs differentiated in the presence of adenosine showed impaired allostimulatory activity in a mixed leukocyte reaction, and they expressed higher levels of the pro-angiogenic cytokine VEGF, the pro-inflammatory cytokines IL-6 and IL-8, and the immunosuppressive mediators IL-10, cyclooxygenase (COX2), transforming growth factor β (TGFβ) and indoleamine 2,3 dioxygenase (IDO). Addition of lactic acid during DC differentiation in vitro also induced a phenotype comparable with that of tumour-associated DCs. Blockade of lactic acid production reverted the tumour-induced DC phenotype to normal. DCs found in the peripheral blood and lymphoid organs of tumor-bearing mice and cancer patients, and especially those closely associated with the tumor, show increased accumulation of lipids. This is mediated primarily via up-regulation of macrophage scavenger receptor types I and II and impairs the ability of DCs to process soluble proteins and stimulate tumor-specific T cell responses.

Some DCs in tumour-bearing hosts actively suppress T cell function and both phenotypically immature and mature DCs may be conditioned by the environment to support immune tolerance or immunosuppression. MHCII<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>+</sup> tumour-infiltrating mouse DCs have been shown to suppress CD8<sup>+</sup> T cells and antitumour immune responses by producing arginase 1 (ARG1), an immunosuppressive mechanism previously attributed only to murine tumour-associated macrophages (TAMs) and MDSCs. Interestingly, pDCs infiltrating prostate cancer also use ARG1 and indoleamine 2,3-dioxygenase (IDO) to alter the functions of intratumoral CD8<sup>+</sup> T cells, suggesting that immunosuppressive programmes might be shared across different myeloid cells in cancer. Human lung tumour cells can convert mature DCs into TGFβ-producing cells and mouse lung cancer can drive DCs to express high levels of IL-10, nitric oxide (NO), VEGF, and ARG1. Accumulation of IDO-expressing DCs (most of which are pDCs) in tumour-bearing mice and in some cancer patients provides another possible mechanism of immune suppression by limiting T cell growth via depletion of L-tryptophan and promotion of T cell apoptosis by generating L-tryptophan metabolites and by altering redox potentials through consumption of superoxide radicals. Evidence supports the hypothesis that IDO-expressing DCs enhance the suppressive abilities of forkhead box protein P3 (FOXP3<sup>+</sup>) regulatory T (T<sub>Reg</sub>) cells in certain settings of chronic inflammation. As mentioned above, such immunosuppressive activities are primarily associated with DCs localized in tumour sites. However, abnormal DC differentiation and defective DC function is a systemic phenomenon that affects the myeloid cell lineage during cancer, as will be described further below.
Macrophages

MΦ are a group of terminally differentiated myeloid cells closely related to DCs. MΦ are tissue-resident cells derived from monocytes circulating in peripheral blood. They include a broad spectrum of cells whose markers and functions reflect their tissue microenvironment (Box 1). Their function in healthy individuals is to eliminate infectious agents, promote wound healing, and regulate adaptive immunity (reviewed in36). The terminology ‘M1’ and ‘M2’ was coined to describe the different functional states of MΦ and was originally based on studies of murine macrophages37. M1 or ‘classically activated’ MΦ are activated by IFNγ and bacterial products, express high levels of IL-12, low levels of IL-10, and are tumouricidal. In contrast, M2 or ‘alternatively activated’ MΦ are activated by IL-4, IL-13, IL-10 and glucocorticoid hormones, express high levels of IL-10, low levels of IL-12, and facilitate tumour progression. As discussed by Mantovani38, the M1/M2 nomenclature is useful, but oversimplified because MΦ form a continuum of phenotypes. Although there are some differences between M2-like mouse and human MΦ, phenotypically and functionally the MΦ in these two species are quite similar (Box 1).

Role of macrophages in promoting tumorigenesis

There is an extensive literature demonstrating that in both mouse and man MΦ are co-opted during malignancy to facilitate tumour growth (reviewed in 1, 238, 39) (Fig. 2). Their presence is associated with poor clinical outcome1, 40, and their pivotal role in cancer was recently highlighted by the demonstration that TAMs with a specific gene signature are associated with primary treatment failure in patients with Hodgkin’s lymphoma41. TAMs are M2-like MΦ and mediate their effects via both non-immune and immune mechanisms. Non-immune mechanisms include the promotion of angiogenesis42, facilitation of tumour cell invasion and metastasis43, and protection of tumour cells from chemotherapy-induced apoptosis44 (see also reviews by 1, 2, 38, 39, 45).

TAMs sabotage anti-tumour immunity by eliminating M1 macrophage-mediated innate immune responses and by impairing T cell activation. Studies with transgenic mice showed that IL-12 produced by M1 MΦ promotes the activation of natural killer (NK) cells and T H1 cells, which facilitate the activation of cytotoxic T lymphocytes (CTLs). However, because TAMs do not produce IL-12, they do not contribute to activation of NK cells and T H1 cells. Instead, they produce IL-10 and drive the development of T H2 cells. T H2 cells do not support the development of CTL responses, and their production of IL-4 drives the development of TAMs46. IL-10 produced by MΦ in inflamed lamina propria is required to maintain TReg cell activity and prevent autoimmune colitis47, raising the possibility that TAM-produced IL-10 may also promote tumor progression by enhancing TReg cell activity.

TAMs are ineffective APCs and produce CCL22, which chemotraacts TReg cells that inhibit T cell activation48. Secretion of prostaglandin E2 (PGE2) and TGFβ49 by TAMs further contribute to immune suppression. TAMs also can cause T cell apoptosis through their expression of programmed death ligand-1 (PD-L1), which binds to its receptor PD-L1 on activated T cells50, and murine MΦ produce ARG1, which deprives T cells of L-arginine51. A similar mechanism is also utilized by MDSC (see below). Inflammation is important for the recruitment of macrophages to tumour sites, with the pro-inflammatory mediators CCL252 and plasminogen53, 54 playing essential roles.

TAM polarization

Because areas within solid tumours contain distinct microenvironments, TAMs within an individual tumour will vary. Seven subsets of TAMs have been identified in mouse mammary carcinoma and lung adenocarcinoma based on expression of Ly6C, MHC class II,
CX3.CR1, CCR2, and CD62L. These subsets have different half-lives and their relative quantities change as the tumour microenvironment evolves with disease progression. Pro-angiogenic TAMs may express the angiopoietin receptor Tie2 and/or be MHC II^low and localize to hypoxic regions. TAMs that promote early tumour cell invasion are enriched for Wnt7b. Tumour-derived TGFβ and PGE2 promote the differentiation of MΦ to show high levels of Gr1 expression and express low levels of markers associated with M1-type MΦ.

T cells play an important role in MΦ regulation during tumorigenesis. In a mouse model of breast cancer driven by transgenic expression of the polyoma middle T (PyMT) antigen, mice with mammary adenocarcinomas developed CD4^+ T_{H}2 cells that produced IL-4 and polarized TAMs to an M2 phenotype. These TAMs produced epidermal growth factor (EGF), which initiates tumour cell invasion, migration, and metastasis by signaling through the corresponding receptor on the malignant mammary epithelial cells. TReg cells also regulate macrophages by orchestrating monocyte differentiation. A population of human CD4^+CD25^+CD127^low FOXP3^+ TReg cells was shown to induce monocytes to differentiate into M2 MΦ by inhibiting their responsiveness to lipopolysaccharide (LPS)-induced M1 polarization, and by increasing their expression of mannose (CD206) and scavenger (CD163) receptors. TReg cell production of IL-10, IL-4, and IL-13 promotes the non-responsiveness of MΦ to LPS. In contrast to TReg cells, Vα24-invariant NKT cells show cytotoxic activity towards TAMs and facilitate tumour rejection.

B lymphocytes also polarize macrophages towards a tumor-promoting phenotype. Recent study in a mouse B16 melanoma model has shown that B cells decreased macrophage production of TNFα, IL-1β, and CCL3 while increasing MΦ production of IL-10 which in turn facilitated pro-tumor macrophage activity as well as synthesis of the M2-like markers Ym1 and Fizz1. In another report, autoantibodies polarized CD45^+ leukocytes, including MΦ, towards a tumor-promoting phenotype by interacting with activating Fc receptors on the leukocytes.

Polarization of MΦ towards an M2 phenotype is also mediated directly by tumour cells. Human ovarian cancer cells cause increased MΦ production of IL-10, IL-1β, CCL5, CCL22, MMP7, MMP9, CD206 and CD163, with tumour cell-produced TNF being partially responsible for this polarization through its induction of MSR1.

Granulocytes

Granulocytes are myeloid cells that are characterized by the presence of cytoplasmic granules and specific nuclear morphology. The most abundant type of granulocytes in the body are polymorphonuclear neutrophils (PMNs) named after their poly-lobed nuclei. PMN possess a complex machinery to engulf and destroy bacteria. PMN are not released from bone marrow until they reach full maturity, but during inflammation, neutrophil precursors (myelocytes and promyelocytes) can be released.

Human tumours can be infiltrated with mature granulocytes whose numbers can also constitute independent prognostic factors for recurrence. Recent evidence has linked granulocytes, and particularly PMNs, with tumour angiogenesis and metastasis, and has provided initial clues about the immunoregulatory role of these cells in cancer. Tumor and tumor-associated stromal cells produce PMN-attracting CXC-chemokines and the orthologue of the secreted protein Bv8, prokineticin 2. Tumour-released G-CSF also mobilized granulocytes to pre-metastatic niches in the lung and supported subsequent metastasis formation, whereas prokineticin 2 aided tumour cell migration through activation of the Bv8 receptor, prokineticin receptor 1. It is likely that granulocytes facilitate the angiogenic switch by expressing matrix metalloproteinase 9 (MMP9), which promotes
tumour angiogenesis by inducing VEGF expression within neoplastic tissue. MMP9 also causes the release of elastase, which enters endosomal compartments of neoplastic cells and degrades insulin receptor substrate 1 (IRS1). Degradation of IRS1 facilitates interaction between phosphatidylinositol 3-kinase (PI3K) and the mitogen platelet-derived growth factor receptor, thus promoting tumour cell proliferation. In contrast to these observations, a recent study demonstrated that in 4T1 breast tumour-bearing mice, PMN inhibited tumor metastases via direct antitumor effects mediated by reactive oxygen species. These new data revisited the old concept of tumour cytotoxic PMN and suggest a possible dichotomic polarization of PMNs. Similar to M1 and M2 polarization in MΦ, PMNs have been shown to shift from an anti-tumoral ‘N1 phenotype’ to a pro-tumoral ‘N2 phenotype’ in the cancer environment. TGFβ drives the N2 phenotype, whereas TGFβ blockade promotes an N1 phenotype with antitumor activity. In lung adenocarcinoma and mesothelioma models, TGFβ favours a pro-tumour phenotype among tumour-infiltrating PMNs, which are characterized by ARG1 expression and low levels of TNF, CCL3, and ICAM1. In tumour-bearing animals, depletion of N2 PMN led to an increase in CD8+ T cell activity. In line with these findings, serum amyloid A1 protein induced the expansion of IL-10-secreting PMN that were able to suppress antigen-specific proliferation of CD8+ T cells in human melanomas. However, IL-10 production by activated human PMNs was not confirmed in a subsequent study.

**Myeloid cells as a single integrated system**

Neoplastic cells condition distant sites, such as the bone marrow and spleen, by releasing soluble factors that drive the accumulation of myeloid cells; these myeloid cells subsequently promote neovascularization and metastasis. This creates a tumour-driven ‘macroenvironment’. As discussed above this macroenvironment conditions DCs, MΦ and granulocytes to become immunosuppressive. However, the most prominent effect is accumulation of highly immunosuppressive, immature myeloid cells. These cells were named MDSCs to highlight their common myeloid origin and immunoregulatory properties. Immature myeloid cells with the same phenotype as MDSCs are continually generated in the bone marrow of healthy individuals and differentiate into mature myeloid cells without causing detectable immunosuppression. However, in cancer, normal myeloid cell differentiation is diverted from its intrinsic pathway of terminal differentiation of mature MΦ, DCs, or granulocytes and instead favours differentiation of pathological MDSCs (Fig. 3).

**Characteristics of MDSCs**

MDSCs were originally identified in tumor-bearing mice as cells that co-express CD11b and Gr1, however their phenotype in cancer is rather diverse. Currently, two main MDSC populations have been characterized: monocytic MDSCs (M-MDSC) and polymorphonuclear (also called granulocytic) MDSCs (PMN-MDSC) (Box 1). In tumour-bearing mice, PMN-MDSCs is the prevalent population of MDSC. They suppress antigen-specific CD8+ T cells predominantly by production of reactive oxygen species (ROS). PMN-MDSCs represent the major subset of circulating MDSCs; however, they are less immunosuppressive than M-MDSCs when assessed on a per cell basis. In human studies, the number of monocytic but not PMN-MDSCs correlated directly with suppression of in vitro T lymphocyte activation.

M-MDSCs in addition to their specific markers (Box 1) co-express varying levels of classic monocyte markers, such as F4/80, CD115, 7/4 and CCR2. They suppress CD8+ T cells predominantly via expression of iNOS and ARG1 enzymes and through the production of reactive nitrogen species. This subset of MDSCs may also include progenitors that...
give rise to a subset of CD11b<sup>hi</sup>Gr-1<sup>low</sup>Ly6G<sup>−</sup>F4/80<sup>hi</sup>MHC class II<sup>+</sup>MΦ with potent immunosuppressive properties<sup>83, 86–88</sup>.

MDSCs with the phenotype LIN<sup>+</sup>HLA-DR<sup>−</sup>CD33<sup>+</sup>CD11b<sup>+</sup>have been isolated from the blood of patients with glioblastoma, breast cancer, colon cancer, lung cancer and kidney cancer<sup>80, 89–92</sup>. These cells share features and properties with progranulocytes<sup>91</sup>. The frequency of this immature cell population may reflect the tumour burden and correlates with a poor prognosis and radiographic progression in breast and colorectal cancer patients<sup>90, 91, 93</sup>. In addition, the frequency of each MDSC subset appears to be influenced by the type of cancer. Patients with renal cancer have immunosuppressive CD11b<sup>+</sup>CD14<sup>+</sup>CD15<sup>+</sup>CD66b<sup>+</sup>VEGFR1<sup>+</sup>PMN-MDSCs<sup>94</sup>, while CD14<sup>+</sup>CD11b<sup>+</sup>H<sub>LA</sub>-DR<sup>low/neg</sup>M-MDSCs circulate in the blood of patients with melanoma, multiple myeloma, prostate cancer, hepatocellular carcinoma, and head and neck cancer<sup>84, 95–98</sup>.

**Relationship of MDSCs to other myeloid cells**

Despite their morphologic similarity, PMN-MDSCs and PMNs are functionally and phenotypically different. PMN-MDSCs, but not PMNs, are immunosuppressive<sup>99</sup>. Expression of CD115 (also known as M-CSFR) and CD244 is up-regulated in PMN-MDSCs, whereas CXCR1 and CXCR2 are down-regulated<sup>99, 100</sup>. Compared with PMNs, PMN-MDSCs are less phagocytic, express higher levels of ARG1 and myeloperoxidase, show increased ROS production and reduced chemotaxis toward supernatants from human carcinomas<sup>99, 100</sup>. Similarly, although M-MDSCs and inflammatory monocytes share a common phenotype and morphology, these cell populations are functionally distinct. M-MDSCs are highly immunosuppressive, expressing, among other factors, high levels of both iNOS and ARG1. In contrast, these two proteins are not coordinately up-regulated in monocytes. Furthermore, although in M1 MΦ iNOS expression is a hallmark of a tumoricidal phenotype, in M-MDSCs iNOS expression promotes suppressive activities<sup>37</sup>. This shift in iNOS activity likely reflects the interplay of iNOS with other enzymes expressed by MDSCs, such as ARG1 and NADPH oxidase, as the coordinated activity of these enzymes was shown to promote the production of peroxinitrite that inhibits the proliferation, effector functions and migration of T cells<sup>101–104</sup>. Although differences exist in the expression of ARG1 and NOS2 among mouse and human myeloid cells (ARG1 is constitutively expressed in human granulocytes<sup>105</sup> but not monocytes), evidence indicates that human MDSCs can also co-express these enzymes<sup>98, 106</sup>.

MDSCs include direct progenitors of DCs, MΦ and granulocytes. Within 24 hours of culture, PMN-MDSCs phenotypically and functionally resemble PMNs<sup>99</sup>. Culture of tumour-derived MDSCs in the absence of tumour-derived factors or the transfer of MDSCs to tumour-free recipients results in the generation of mature MΦ and DCs<sup>107–109</sup>. In contrast, the presence of tumour-derived soluble factors or adoptive transfer into tumour-bearing hosts promotes the differentiation of MDSCs into immunosuppressive MΦ<sup>109, 110</sup>. MDSCs can also differentiate into DCs following transfer into tumour-bearing recipients<sup>111</sup>, but whether these DCs are immunosuppressive is not currently known. Furthermore, hypoxia in the tumor microenvironment drives the differentiation of MDSCs into TAM<sup>111, 112</sup> (Fig. 3).

**Immunomodulatory functions of MDSCs**

MDSCs exploit a plethora of redundant mechanisms to influence both innate and adaptive immune responses. Broadly speaking, these mechanisms can be grouped into four classes.
The first is lymphocyte nutrient depletion: L-arginine depletion by ARG1-dependent consumption\textsuperscript{51} and L-cysteine deprivation via its consumption and sequestration\textsuperscript{113}. These depletions cause down-regulation of the ζ-chain in the T cell receptor (TCR) complex and proliferative arrest of antigen-activated T cells.

The second is the generation of oxidative stress, which is caused by their production of ROS and reactive nitrogen species (RNS). Peroxynitrite and hydrogen peroxide are produced by the combined and cooperative activity of phagocytic oxidase, ARG1 and iNOS in different MDSC subsets and they drive a number of molecular blocks in T cells, ranging from the loss of ζ-chain expression\textsuperscript{114} and interference with IL-2 receptor signalling\textsuperscript{115}, to nitration and subsequent desensitization of the TCR \textsuperscript{103}.

The third set of mechanisms interferes with lymphocyte trafficking and viability. Plasma membrane expression of ADAM17 (a disintegrin and metalloproteinase domain 17) by MDSCs decreases L-selectin expression on the surface of naïve CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells, thereby limiting T cell recirculation to lymph nodes\textsuperscript{116}. Another example is the modification of CCL2 by MDSC-derived peroxynitrite, a process which impairs migration of effector CD8\textsuperscript{+} T cells to the tumour core\textsuperscript{117}. MDSCs express galectin 9, which binds to TIM3 on lymphocytes and induces T cell apoptosis\textsuperscript{118}. MDSCs mostly through membrane contact-dependent mechanisms, i.e. membrane bound TGF-β (mouse MDSCs) and interaction with the NK receptor NKp30 decrease the number and inhibit function of mouse and human NK cells\textsuperscript{119–121}.

The fourth is the activation and expansion of T\textsubscript{Reg} cells. MDSCs expand antigen-specific natural T\textsubscript{Reg} (nT\textsubscript{Reg}) cells and also promote conversion of naïve CD4\textsuperscript{+} T cells into induced T\textsubscript{Reg} (iT\textsubscript{Reg}) cells. The mechanisms are not completely understood, but may involve cell-to-cell contact, including CD40–CD40L interactions\textsuperscript{122}, production of soluble factors by MDSCs, such as IFNγ, IL-10, and TGFβ\textsuperscript{123}, and possibly also MDSC expression of ARG\textsuperscript{124} (Fig. 4). Human CD14\textsuperscript{+}HLA-DR\textsuperscript{−}/low MDSCs promote the transdifferentiation of Foxp3\textsuperscript{+} iT\textsubscript{Reg} from Th17 lymphocytes by producing TGF-β and retinoic acid\textsuperscript{125}.

In peripheral lymphoid organs MDSC-mediated suppression of CD8\textsuperscript{+} T cells is usually antigen-specific and requires the presentation of antigens by MDSCs and direct MDSC–T cell contact\textsuperscript{103, 126}. The activity of MDSCs is also enhanced by activated T cells in the periphery\textsuperscript{129} and at the tumour site\textsuperscript{111, 112, 127, 128}. As a result, MDSCs are able to suppress nearby T cells in an antigen-nonspecific manner. However, if T cells are activated and become FASL\textsuperscript{+}, they may induce apoptosis of FAS\textsuperscript{+} MDSCs\textsuperscript{129}.

The co-dependence of cells in the myeloid lineage is further demonstrated by the regulation of mature DCs and MΦ by MDSCs. Through an IL-10- and cell contact-dependent mechanism, MDSCs skew MΦ towards an M2 phenotype by decreasing MΦ production of IL-12\textsuperscript{130}. IL-12 down-regulation is exacerbated by the MΦ themselves, since MΦ increase MDSC production of IL-10 (Fig. 4). As MDSC potency is enhanced by inflammation\textsuperscript{131}, it is not unexpected that inflammation enhances the cross-talk between MDSCs and MΦ. Inflammation mediates these effects by increasing MDSC expression of CD14 and signaling through the TLR4 pathway\textsuperscript{132}. MDSCs similarly impair DC function by producing IL-10, which inhibits TLR-induced IL-12 production by DCs and reduces DC-mediated activation of T cells\textsuperscript{133}. 

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Common mechanisms of tumour impact on myeloid cell recruitment and function

Neoplastic and tumour-associated stromal cells release multiple tumour-derived soluble factors that perturb the myeloid compartment. Cytokines such as GM-CSF, G-CSF, M-CSF, SCF, VEGF, and IL-3 promote myelopoiesis and contribute, in part, to a blockade of myeloid cell maturation\(^{88, 107}\) (Fig. 5). Tumour-derived soluble factors that are pro-inflammatory, such as IL-1β, IL-6, and S100A8-9\(^{134–136}\), as well as cytokines released by activated T cells, such as IFN-γ, IL-4, IL-13, IL-10\(^{127}\), initiate the immunosuppressive pathways that commit immature myeloid cells to become MDSCs and then further promote MDSC differentiation towards immune suppressive MΦ and DCs (Fig. 5). The tumour-derived factors CCL2, prokineticin 2, CXCL5, S100A8-9, and CCL12 recruit immature myeloid cells to tumor stroma\(^{70, 137, 138}\). Immature myeloid cells are also chemoattracted by CCL2 that is nitrated/nitrosylated within the tumour environment. In contrast, effector CD8\(^+\) T cells are not recruited by modified CCL2, which may explain the selective enrichment of myelomonocytic cells within mouse and human tumours\(^{117}\). LPS, in combination with IFN-γ, promotes MDSC population expansion, probably by inhibiting DC differentiation\(^{139}\).

Tumor-derived TGF-β also regulates MDSC accumulation\(^{140}\) and neutrophil polarization\(^{75}\) (Fig. 5). Neoplastic cells and their associated stromal cells also release into the bloodstream subcellular components known as exosomes, which contain signal peptides, mRNAs, microRNAs, and lipids and promote MDSC expansion (reviewed in\(^{141}\)).

Tumour-derived soluble factors regulate myeloid lineage cells on multiple levels involving a variety of transcription factors\(^{88, 107, 131}\) (Fig. 5), with STAT3 playing a major role. Early studies identified STAT3 as a critical regulator of DC and MΦ defects\(^{142, 143}\) and MDSC expansion\(^{144, 145, 146}\). STAT3 not only prevents apoptosis and promotes cell proliferation via up-regulation of BCL-XL, MYC, cyclin D1, or survivin\(^{107, 147}\), but also regulates expression of multiple proteins critical for differentiation of myeloid cells. One such pathway involves the calcium-binding pro-inflammatory proteins S100A8 and S100A9\(^{148}\). STAT3-mediated up-regulation of these proteins in myeloid progenitors inhibits DC differentiation and promotes MDSC accumulation\(^{140}\). S100A8 and S100A9 also enhance MDSC suppressive activity and recruit MDSCs to the tumour site\(^{135}\). Myeloid cell NADPH-oxidase (NOX2) is another important target of STAT3. STAT3 up-regulation of the NOX2 components p47\(^{\text{phox}}\) and gp91\(^{\text{phox}}\) increases ROS levels, thereby making MDSCs more suppressive\(^{92}\). STAT3 also down-regulates PKCβII, which is required for DC differentiation and thus prevents the development of HPCs into mature cells\(^{150}\). In addition, STAT3 regulates the transcription factor CCAAT-enhancer-binding protein beta (C/EBPβ). C/EBPβ regulates myelopoiesis in healthy individuals\(^{151}\) and plays a crucial role in controlling differentiation of myeloid progenitors to functional MDSCs\(^{134}\). STAT3, at least partially, induces MDSC expansion via up-regulation of C/EBPβ and plays an indirect role in myeloid cell mobilization, accumulation, and survival\(^{152}\).

The transcription factor STAT1 regulates subsets of myeloid cells via its effects on NOS2 and is crucial for MΦ and MDSC-mediated immune suppression\(^{127, 153, 154}\). Other characteristics of MDSCs and MΦ, including the up-regulation of ARG1\(^{155–157}\), increased TGFβ production\(^{124, 158}\), and possibly expansion of MDSCs\(^{159}\), are controlled by STAT6. IL-4-induced polarization of TAMs activates STAT6, which binds to the promoter of the gene-encoding the demethylase Jumonji domain-containing 3 (JMJD3). Activated JMJD3 demethylates histone H3 lysine-27, which then increases expression of ARG1, YM1, and FIZZ1, resulting in M2 polarization\(^{160}\). However, the genetic ablation of JMJD3 gene in mice caused a defective and irf4-dependent M2 polarization in response to M-CSF, helminth infection or chitin administration, but not following IL-4 stimulation, suggesting a more complex regulatory network\(^{161}\).
The TLR family also plays a prominent role in myeloid cell development, primarily via the activation of MYD88 and the downstream induction of NF-κB. NF-κB signaling is important for mobilization of myeloid cells to sites of infection, injury, or tumour growth. TLR4 regulates inflammation-driven MDSC suppressive potency through an NF-κB-dependent mechanism. The pro-inflammatory mediators COX2 and PGE2, which enhance MDSC accumulation and suppressive activity, are also potential targets for NF-κB.

**Two-stage model of MDSC involvement in tumour progression**

Recent studies of autochthonous tumor formation in transgenic mice indicate that cells with an MDSC phenotype probably intervene in the very early stages of cancer progression. Mice with autochthonous pancreatic cancer undergo progressive waves of myeloid cell recruitment after initiation of the transforming programme driven by the Kras oncogene. Recruited myeloid cells contribute to the local production of IL-6 and IL-11 that activate STAT3. STAT3, in turn, induces anti-apoptotic and pro-proliferative genes, fuelling tumour initiation, promotion, and progression. During early events of colitis-associated cancer, myeloid cells act as tumour promoters by enhancing proliferation of tumour-initiating cells and by protecting premalignant intestinal epithelial cells from apoptosis.

The oncogenic fusion protein RET/PTC3 (RP3) in thyroid carcinomas directly regulates CCL2 and GM-CSF production, which recruits CD11b+Gr-1+ cells. An important question is whether these early recruited cells are immunosuppressive MDSCs. Unfortunately, only a few studies in autochthonous tumor models have determined the immunosuppressive activity of the tumour-associated CD11b+Gr-1+ cells. In models of spontaneous breast, pancreatic, or lung cancer, accumulated myeloid cells had both the phenotype and immunosuppressive features of MDSCs. In a recent study, conditional deletion of p120ctn in mice caused formation of invasive squamous cell cancer and desmoplasia associated with production of GM-CSF, CCL2, M-CSF, and TNF. These events resulted in accumulation of immunosuppressive CD11b+Gr-1+CD124+ MDSCs that promoted tumor progression by activating stromal fibroblasts.

In another model of multistep squamous carcinogenesis driven by the HPV16 early-region genes (including the E6/E7 oncogenes) under the control of the human keratin-14 promoter/enhancer, CD11b+Gr1+F4/80−CD11c− cells constituted the most abundant leukocyte subtype in premalignant skin, and accumulated progressively in the spleen. However, these cells failed to inhibit polyclonal activation of either CD4+ or CD8+ T cells and did not produce ROS.

These results, together with the data discussed above from transplantable tumour models, support a two-stage model of MDSC involvement in cancer. The almost universal feature of tumour progression is activation of abnormal myelopoiesis and recruitment of immature myeloid cells into tissues. This process is governed by diverse soluble factors and is dependent upon up-regulation of STAT3 and other key transcription factors. Myelopoiesis during acute infections, stress, or trauma results in rapid terminal differentiation of myeloid cells. In contrast, cancer myelopoiesis is associated with defective myeloid cell differentiation, which results in accumulation and persistence of immature myeloid cells. Although necessary, these events are not sufficient to generate immunosuppressive MDSCs: activation of cells via a network of regulatory mechanisms is also required. Activation of these mechanisms in mice with most transplantable tumours and many, but not all, spontaneous tumours, results in the accumulation of immunosuppressive MDSCs. In tumor sites these cells further differentiate into TAMs and possibly into suppressive DCs. In patients with cancer, cells with an MDSC phenotype are
almost universally immune suppressive, which may reflect their isolation from patients with advanced disease. If immunosuppressive activity is not a property of the first wave of immature myeloid cells recruited to tumors, continuous stimulation of myelopoiesis and activation of immature myeloid cells by tumour-derived soluble factors may drive the subsequent accumulation of immunosuppressive MDSCs that support tumor promotion and form the metastatic niche. Accordingly, the oncogenic programme may influence the functional immunosuppressive activity more than the accumulation of CD11b+Gr-1+ cells. Thus, the transition from immature myeloid cells to MDSCs might be defective in some experimental tumour models, and different oncogenic programmes may differentially affect the kinetics of immature myeloid cell to MDSC conversion. Combinations of GM-CSF, G-CSF, IL-6 and IL-13 induce the rapid differentiation of cells similar to MDSCs from human and mouse bone marrow precursors in vitro\textsuperscript{91, 134, 177, 140}. These studies may provide the framework for identifying key molecules governing the stages of MDSC maturation.

**Therapeutic targeting of myeloid cells**

Knowledge of the molecular mechanisms responsible for accumulation of MDSCs, immune suppressive macrophages and DCs in cancer has allowed for therapeutic targeting of these cells as it is increasingly clear that successful cancer immunotherapy will require limiting the immunosuppressive effects of myeloid cells. This targeting is focused on six main goals: first, inhibiting the molecular mechanisms used by myeloid cells to block lymphocyte reactivity and proliferation; second, inhibiting the expansion of MDSCs from bone marrow progenitors or inducing apoptosis of circulating MDSC; third, forcing MDSCs to mature into proficient APCs; fourth, preventing trafficking of myeloid cells from bone marrow to peripheral lymphoid organs and to tumors; fifth, repolarizing or eliminating TAMs and replacing them with M1 macrophages; sixth, restoring the antigen-presenting capabilities of DCs and macrophages (Table 1).

The proposed two-stage model for MDSC involvement might have implications for the further development of therapies. Some immunosuppressive mechanisms are common to all myeloid cells but others are unique to individual populations. Therefore, targeting common effector molecules is likely to be more effective than targeting individual suppressive pathways.

**Conclusions and perspective**

It is not clear whether abnormal myelopoiesis and pathological activation of myeloid cells are temporarily regulated. Do cancer cells first condition mature leukocytes, MDSCs, DCs, and macrophages that are then recruited in response to suppressor factors produced by the progressing tumor? Or, do the two processes occur concurrently and MDSCs are recruited as a pre-requisite to tumor progression? More sophisticated tumor models and techniques will be required to address this key question. It is clear that the myeloid lineage is globally altered in cancer as a single, closely integrated system involving all terminally differentiated myeloid cells and their pathologically activated immature progenitors. Although there are a multitude of phenotypic and functional changes in different myeloid cell subpopulations, these changes are governed by common tumour-derived suppressor factors and transcriptional programmes. These commonalities provide an opportunity for therapeutic interventions that may concomitantly normalize multiple myeloid cell abnormalities.
## Glossary terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td><strong>Myeloid-derived suppressor cells</strong> (MDSCs)**</td>
<td>A group of immature CD11b+GR1+ cells (which include precursors of macrophages, granulocytes, DCs and myeloid cells) that are produced in response to various tumour-derived cytokines. These cells have been shown to inhibit tumour-specific immune responses.</td>
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<tr>
<td><strong>Pathogen-associated molecular patterns (PAMPs)</strong>)</td>
<td>These are molecular motifs that are found in pathogens but not mammalian cells. Examples include terminally mannosylated and polymannosylated compounds, which bind the mannose receptor, and various microbial products that activate host Toll-like receptors, such as bacterial lipopolysaccharides, hypomethylated DNA, flagellin and double-stranded RNA.</td>
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<tr>
<td><strong>Danger-associated molecular patterns (DAMPS)</strong>)</td>
<td>As a result of cellular stress, cellular damage and non-physiological cell death, DAMPs are released from the degraded stroma (for example, hyaluronate), from the nucleus (for example, high-mobility group box 1 protein (HMGB1)) and from the cytoplasm (for example, adenosine triphosphate, uric acid, S100 calcium-binding proteins and heat-shock proteins). Such host-derived DAMPs are thought to promote local inflammatory reactions.</td>
</tr>
<tr>
<td><strong>Mixed leukocyte reaction</strong></td>
<td>A tissue-culture technique for testing T cell reactivity and APC activity. A population of T cells is cultured with MHC-mismatched APCs, and proliferation of the T cells is determined by measuring the incorporation of ³H-thymidine into the DNA of dividing cells.</td>
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<tr>
<td><strong>Indoleamine 2,3 dioxygenase (IDO)</strong>)</td>
<td>An intracellular haem-containing enzyme that catalyses the oxidative catabolism of tryptophan. IDO suppresses T cell responses and promotes immune tolerance in mammalian pregnancy, tumour resistance, chronic infection, autoimmunity and allergic inflammation.</td>
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<tr>
<td><strong>Regulatory T (T_{Reg}) cells</strong></td>
<td>A specialized subset of CD4⁺ T cells that can suppress both innate and adaptive immune responses. These cells provide a crucial mechanism for the maintenance of peripheral self tolerance, but may also limit the effectiveness of anti-tumour immune responses.</td>
</tr>
<tr>
<td><strong>Natural T_{Reg} (nT_{Reg}) cells</strong></td>
<td>A subset of T_{Reg} cells that undergoes maturation in the thymus where these cells acquire the ability to recognize with intermediate avidity self antigens presented by host MHC class II molecules before being released to the periphery.</td>
</tr>
<tr>
<td><strong>Induced (iTreg) cells</strong></td>
<td>A subset of T_{Reg} cells that derives from the direct conversion of CD4⁺ effector T cells in peripheral lymphoid organs under several situations, including the interaction with tumor-conditioned myelomonocytic cells in tumor-bearing hosts.</td>
</tr>
<tr>
<td><strong>T_{H}17 cell</strong></td>
<td>A subset of CD4⁺ T helper cells that produce IL-17 and that are thought to be important in mediating host defence against certain infections, particularly at mucosal tissues. They are also thought to drive pathology in certain inflammatory and autoimmune diseases, such as Crohn’s disease.</td>
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<tr>
<td><strong>Plasminogen</strong></td>
<td>Plasminogen is the inactive precursor of plasmin, a serine protease involved in the dissolution of fibrin blood clots. A causal role has</td>
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*Nat Rev Immunol. Author manuscript; available in PMC 2013 April 01.*
been advanced for plasmin generation in cancer cell invasion through the extracellular matrix remodeling

**Invariant NKT cells**
A lymphocyte thought to be particularly important in bridging innate and adaptive immunity. They express a particular variable gene segment, V14 (in mice) and V24 (in humans), precisely rearranged to a particular J (joining) gene segment. Typically, NKT cells co-express cell-surface markers encoded by the natural killer (NK) locus, and are activated by recognition of CD1d.

**Autochthonous tumor**
Differently from transplanted tumors, which arise from the experimental transfer of neoplastic cells or tissues, autochthonous tumors develop spontaneously in the host. Autochthonous tumors can derive from either chemical carcinogenesis or targeted tissue expression of oncogenes by genetic manipulation of the mouse.

**References**


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regulated epigenetically by chromatin/histone remodeling through an IL-4/STAT6-dependent mechanism. [PubMed: 19567879]


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References 203 and 204 show that it is possible to reprogram tumor environment by adoptive transfer of T lymphocytes recognizing a tumor antigen and engineered to release IL-12.


Online summary

- Tumours directly affect mature myeloid cells by converting some of them into immunosuppressive populations that facilitate tumour growth.

- In cancer, normal myeloid cell differentiation is also diverted from its intrinsic pathway of terminal differentiation to mature myeloid cells (dendritic cells, macrophages, and granulocytes) towards pathologically activated immature cells, which are known as myeloid-derived suppressor cells (MDSCs).

- MDSCs are immune suppressive, immature, and pathologically activated myeloid cells. However, in the absence of tumor-derived factors they are still able to differentiate into mature myeloid cells. MDSCs consist of two major populations: polymorphonuclear MDSCs and monocytic MDSCs. MDSCs suppress antigen-specific and non-specific immune responses via a variety of different mechanisms.

- Myeloid cell responses in cancer are regulated by common tumour-derived factors that activate a diverse set of transcription factors shared by myeloid cells. These transcription factors promote myelopoiesis and initiate the immunosuppressive pathways that commit immature myeloid cells to become MDSCs.

- A two-stage model of MDSC involvement in tumour development and progression is proposed. The universal feature of tumour progression is activation of abnormal myelopoiesis and recruitment of immature myeloid cells into tissues. These cells may or may not possess immunosuppressive features, depending on the activation signals provided by the tumor microenvironment. If immunosuppression is not a property of the first wave of immature myeloid cells that are recruited to tumors, continuous stimulation of myelopoiesis and activation of immature myeloid cells by tumor-derived factors drives the subsequent accumulation of immunosuppressive MDSCs, which support tumor growth and formation of the metastatic niche.
Myeloid cells are a subpopulation of hematopoietic cells and originate from a network of hematopoietic stem cells (HSC) and multi-potent progenitor cells (MPP). The network of progenitor cells that gives rise to various hematopoietic cells includes common myeloid progenitor cells (CMP); common lymphoid progenitor cells (CLP); MΦ and DC progenitors (MDP); common DC progenitors (CDP), granulocyte/macrophage progenitors (GMP), megakaryocyte/erythroid progenitors (MEP); - mast cell progenitors (MCP). cDC – conventional DCs, pDC – plasmacytoid DCs

Figure 1. Myeloid cell differentiation under normal physiological conditions
Myeloid cells are a subpopulation of hematopoietic cells and originate from a network of hematopoietic stem cells (HSC) and multi-potent progenitor cells (MPP). The network of progenitor cells that gives rise to various hematopoietic cells includes common myeloid progenitor cells (CMP); common lymphoid progenitor cells (CLP); MΦ and DC progenitors (MDP); common DC progenitors (CDP), granulocyte/macrophage progenitors (GMP), megakaryocyte/erythroid progenitors (MEP); - mast cell progenitors (MCP). cDC – conventional DCs, pDC – plasmacytoid DCs
Figure 2. The tumor microenvironment polarizes macrophages towards a tumor-promoting phenotype

Tumor cells produce factors that drive the generation of multiple regulatory cells, including CD4+ Th2, Tregs, B cells, and MDSC. Tumor cells also modify their microenvironment to produce hypoxia and inflammation (thin black arrows). The regulatory cells and the modified tumor microenvironment subsequently produce cytokines, chemokines, and other molecules that polarize MΦ by regulating MΦ gene expression (e.g. Tie2, HLA-DR, CD163, etc.), by modifying MΦ cytokine expression (e.g. IL-10, IL-12, etc.) and by enhancing MΦ recruitment to the tumor site (thick gray arrows). Tumors also produce factors (i.e. TNFα, etc.) that directly polarize MΦ. The resulting MΦ share some characteristics with alternatively activated MΦ and other characteristics unique to TAMs. Cross-talk between tumor cells and MDSC and between tumor cells and inflammation within the tumor microenvironment amplify the effects (thin black double-headed arrows). See the text for references and for which molecules and cellular interactions are known for murine macrophages vs. human macrophages.
Figure 3. Changes that occur in myeloid cells in cancer
Factors in the tumor microenvironment produced by tumor cells and stromal cells (including myeloid cells) modulate myeloid cell phenotype and function. iMC – denote immature myeloid cells, a combination of myeloid progenitors described in Figure 1. Thin dotted line - regular pathways of myeloid cell differentiation from iMC to DCs, MΦ and granulocytes. Solid thick lines – pathways of myeloid cell differentiation in cancer. Dotted thick line – suggested, not yet confirmed direction of myeloid cell differentiation. supDCs – DC with immune suppressive activity. TAM- tumor associated MΦ.
Figure 4. Mechanisms of myeloid cell-dependent inhibition of T cell activation and proliferation

Myeloid cells conditioned by tumors can induce paralysis of T lymphocytes by expanding/converting T\textsubscript{regs} (top left), depriving the environment of amino acids (top right), releasing oxidizing molecules (bottom left), and/or altering T cell migratory properties and viability (bottom right). Since induction of these pathways is regulated by common transcription factors, they can operate in more than one myeloid cell type, as reported in Figure 5. By binding to RAGE, S100A8/A9 also provides autocrine stimulation (middle left). TGF\textbeta, transforming growth factor-\textbeta; X\textsubscript{c}-, cystine/glutamate transporter; CAT2B, cationic amino acid transporter (L-arginine transporter); ASC, sodium-dependent neutral amino acid transporter (L-cysteine transporter); IFN\textgamma, interferon-\textgamma; IL, interleukin; MYD88, myeloid differentiation primary response protein 88; HIF-1\textalpha, hypoxia inducible factor 1\textalpha; CEBP\textbeta, CCAAT/enhancer-binding protein \textbeta; FOXP3, forkhead box protein P3; Phox, phagocyte oxidase; STAT, signal transducer and activator of transcription; NO, nitric oxide; ARG, arginase; NOS, nitric oxide synthase; Gal9, galectin 9; TIM3, T-cell immunoglobulin and mucin domain-containing protein 3; ADAM17, a disintegrin and metalloproteinase domain 17; CD62L, L-selectin. S100A8/A9, S100 calcium binding protein A8/A9; RAGE, receptor for advanced glycation end products.
Figure 5. Molecular mechanisms affecting myeloid lineage in cancer

The tumour microenvironment secretes many different cytokines (green circles) that affect myeloid progenitors as well as mature myeloid cells by regulating the activity of multiple transcription factors (blue). These transcription factors, in turn, regulate synthesis of their protein targets (yellow) affecting myeloid cell functions (black).
### Table 1
Pharmacological regulation of myeloid cells in cancer

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of cancer tested In bold – cancer patients</th>
<th>Molecular events</th>
<th>Effect on myeloid cells</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroaspirin</td>
<td>Colon carcinoma</td>
<td>Downregulation of ARG1, NOS2, PNT</td>
<td>Inhibition of the MDSC suppressive effects</td>
<td>178</td>
</tr>
<tr>
<td>Phosphodiesterase-5 inhibitors (sildenafil and tadalafil)</td>
<td>Mammary, colon carcinomas and fibrosarcoma (mice)</td>
<td>Downregulation of ARG1, NOS2, and CD124 in MDSCs</td>
<td>Inhibition of the MDSC suppressive effects</td>
<td>98</td>
</tr>
<tr>
<td>AT38 (NO-donor based on furoxan molecule)</td>
<td>Fibrosarcoma, thymoma</td>
<td>Downregulation of ARG1, NOS2, PNT; nitrated/nitrosylated CCL2</td>
<td>Inhibition of the MDSC suppressive effects; MDSCs/CD8⁺ T cells ratio in tumors</td>
<td>104</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Colon, lung carcinomas, thymoma</td>
<td>Inhibition of ROS</td>
<td>Inhibition of the MDSC suppressive effects</td>
<td>179</td>
</tr>
<tr>
<td>Tyrosin kinase inhibitor (Sunitinib)</td>
<td>Fibrosarcoma, colon, breast, lung and kidney cancer: Renal cell carcinoma</td>
<td>Possible c-Kit blockade; STAT3 inhibition; GM-CSF confers resistance by activating STAT5 in intratumoral MDSCs</td>
<td>Inhibition of MDSC expansion in lymphoid organs but not in tumor stroma; modest inhibition of MDSC expansion in patients</td>
<td>180-184</td>
</tr>
<tr>
<td>Cyclooxygenase 2 inhibitor(SC58236, SC58125, celecoxib)</td>
<td>Mammary carcinoma, mesothelioma, lung carcinoma, glioma</td>
<td>Downregulation of PGE2, ARG1, ROS, CCL2. Increase in CXCL10</td>
<td>Inhibition of the MDSC suppressive effects</td>
<td>140, 168, 185</td>
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<tr>
<td>Anti-cKit antibody</td>
<td>Colon carcinoma</td>
<td>c-Kit-SCF interaction blockade</td>
<td>Inhibition of MDSC expansion</td>
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<td>CSF1 and cKit receptor tyrosine kinases inhibitor (PLX3397)</td>
<td>Mammary carcinoma</td>
<td>CSF1R and cKit blockade</td>
<td>Inhibition of TAM recruitment</td>
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<tr>
<td>Anti-CCL2 monoclonal antibody</td>
<td>Mammary carcinoma</td>
<td>Interference with CCL2/CCR2 binding and VEGFA upregulation</td>
<td>Inhibition of metastatic spread by targeting inflammatory monocytes and macrophages</td>
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<tr>
<td>Amino-bisphosphonate (zoledronate)</td>
<td>Mammary tumors, mesothelioma</td>
<td>Reduction in VEGF and pro-MMP9 serum levels</td>
<td>Inhibition of MDSC expansion</td>
<td>188, 189</td>
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<tr>
<td>Very small size proteoliposomes</td>
<td>Lymphomas and sarcoma</td>
<td>NOS2 downregulation</td>
<td>Changes in MDSC subset distribution</td>
<td>190</td>
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<tr>
<td>Antagonist of CXCR2- (S-265610) and CXCR4 (AMD3100)</td>
<td>Various cancer</td>
<td>Interference with SDF-1 and CXCL5 chemokines</td>
<td>altered recruitment of iMCs to tumor</td>
<td>137</td>
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<tr>
<td>Anti-BV8 antibody</td>
<td>Various human and mouse tumors in nude mice</td>
<td>Interference with the BV8 pleiotropic activity</td>
<td>Inhibition of PMN-MDSC expansion and recruitment to tumor and pre-metastatic niches</td>
<td>78, 71</td>
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<tr>
<td>CSF-1 receptor antagonist (GW2580)</td>
<td>Lung carcinoma</td>
<td>CSF-1R interference, ARG1 decrease in MDSCs, VEGF and MMP9 reduction in tumor</td>
<td>Inhibition of expansion and recruitment of MDSC and Φ to tumor</td>
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<tr>
<td>VEGF-trap, anti-VEGF antibody (avastin)</td>
<td>Various solid tumors Metastatic renal cell cancer</td>
<td>VEGF interference</td>
<td>Improvement of DC differentiation</td>
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<tr>
<td>Gemcitabine</td>
<td>Lung cancer, breast cancer</td>
<td>MDSC apoptosis</td>
<td>Inhibition of MDSC expansion</td>
<td>130, 194</td>
</tr>
<tr>
<td>Treatment</td>
<td>Type of cancer tested</td>
<td>Molecular events</td>
<td>Effect on myeloid cells</td>
<td>Ref.</td>
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<tr>
<td>5-fluorouracil</td>
<td>Thymoma</td>
<td>MDSC apoptosis</td>
<td>Inhibition of MDSC expansion</td>
<td>195</td>
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<tr>
<td>Doxorubicin</td>
<td>Breast cancer</td>
<td>MDSC apoptosis (?)</td>
<td>Weak inhibition of MDSC expansion</td>
<td>196</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Mammary carcinoma</td>
<td>MDSC apoptosis with differentiation to M1 MΦ of surviving cells</td>
<td>Inhibition of MDSC expansion, MΦ polarization</td>
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<tr>
<td>All trans retinoic acid</td>
<td>Sarcoma, colon carcinoma</td>
<td>Differentiation of iMCs to mature leukocytes</td>
<td>Inhibition of MDSC accumulation</td>
<td>198, 199</td>
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<tr>
<td>Vitamin D3</td>
<td>Head and neck cancer</td>
<td>Forced differentiation of CD34+ iMCs</td>
<td>Moderate effect on inhibition of MDSC expansion</td>
<td>200</td>
</tr>
<tr>
<td>IL-12, CCL16 + Cpg + anti-IL-10 receptor monoclonal antibody</td>
<td>Lung cancer, Breast cancer</td>
<td>Decrease in IL-10, MCP-1, and TGF-β and increase in TNFα, IL-15, and IL-18;</td>
<td>TAM reprogramming</td>
<td>201, 202</td>
</tr>
<tr>
<td>Tumor specific CTLs engineered to release IL-12; Chimeric antigen receptor (CAR)-redirected T-cells engineered to release IL-12</td>
<td>Melanoma, Colon carcinoma</td>
<td>Increased antigen cross-presentation and costimulation; acute inflammation signature (including IFN-γ); increased TNFα production</td>
<td>DC, MDSC, and TAM reprogramming</td>
<td>203, 204</td>
</tr>
<tr>
<td>IL-2 plus anti-CD40 monoclonal antibodies</td>
<td>Renal cell carcinoma</td>
<td>Increased NOS2 and tissue inhibitor of MMP 1</td>
<td>TAM reprogramming in lung metastasis but not in primary tumor</td>
<td>205</td>
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<tr>
<td>Agonist anti-CD40 monoclonal antibodies and gemcitabine</td>
<td>Pancreatic ductal adenocarcinoma</td>
<td>Targeting and activation of blood circulating macrophages</td>
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<tr>
<td>Histidine-rich glycoprotein (HRG)</td>
<td>Fibrosarcoma, pancreatic and breast cancer</td>
<td>Downregulation of placental growth factor</td>
<td>TAM reprogramming</td>
<td>207</td>
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<td>Inhibition of NF-κB signaling by targeting IκB kinase</td>
<td>Ovarian cancer</td>
<td>IL-12 production by NK cells; TAMs become IL-12&lt;sup&gt;high&lt;/sup&gt;, IL-10&lt;sup&gt;high&lt;/sup&gt;, MHC class I&lt;sup&gt;high&lt;/sup&gt;, arginase-1&lt;sup&gt;low&lt;/sup&gt;</td>
<td>TAM reprogramming to M1 phenotype</td>
<td>208</td>
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