The role of myeloid cells in cancer therapies

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Abstract | Recent clinical trials have demonstrated the ability to durably control cancer in some patients by manipulating T lymphocytes. These immunotherapies are revolutionizing cancer treatment but benefit only a minority of patients. It is thus a crucial time for clinicians, cancer scientists and immunologists to determine the next steps in shifting cancer treatment towards better cancer control. This Review describes recent advances in our understanding of tumour-associated myeloid cells. These cells remain less studied than T lymphocytes but have attracted particular attention because their presence in tumours is often linked to altered patient survival. Also, experimental studies indicate that myeloid cells modulate key cancer-associated activities, including immune evasion, and affect virtually all types of cancer therapy. Consequently, targeting myeloid cells could overcome limitations of current treatment options.

Innate immune system
A system comprising various cell types that together provide defence to the host against infection and injury and orchestrate inflammatory responses. Unlike adaptive immune cells, innate immune cells express only germline-encoded pattern recognition receptors and generally they do not provide long-lasting immunity; however, they can activate the adaptive immune system through a process known as antigen presentation.

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doi:10.1038/nrc.2016.54
Published online 24 June 2016

Until the beginning of the twenty-first century, cancer was largely considered to be a cell-autonomous disease, with malignant growth driven by genetic mutations within tumour cells. More recently, it has become clear that heterotypic interactions between neoplastic and seemingly normal host cells also profoundly regulate cancer progression. Most notably, tumour-infiltrating T lymphocytes are now regarded as a key component of the tumour microenvironment because therapeutically activating these cells can durably control various cancer types[1]. These advances are not only revolutionizing cancer therapy but have also validated immune cell targeting as a relevant approach to fight human cancer. Considering that tumour microenvironments are home to diverse cell types[2] and that current immunotherapies benefit only a minority of patients[1], it is important to identify whether other components of the tumour microenvironment can be additional relevant therapeutic targets.

This Review focuses on myeloid cells, which belong to the innate immune system. Myeloid cells and their phagocytic activities were first discovered by Élie Metchnikoff[3] more than a century ago when his landmark starfish larvae microscopy studies showed that infection sites accumulate leukocytes that ingest and eliminate foreign material. In mammals, myeloid cells are among the most important defenders against infection. They are also essential in tissue homeostasis[4,5] and crucial in initiating, sustaining or inhibiting T cell immunity[6,7]. Emerging evidence indicates that myeloid cells affect cancer progression by interacting directly with tumour cells and indirectly by enabling a tumour stroma that promotes cancer growth[8–10]. The importance of myeloid cells in cancer is not entirely surprising: tumours do not use *de novo* mechanisms to interact with host components but instead employ pre-existing physiological programmes, particularly those involved with wound healing, that engage myeloid cells[11]. The interplay between myeloid cells and adaptive immunity is also emerging as an important regulator of cancer progression, with tumour-associated myeloid cells probably having an important role in cancer immune evasion.

Myeloid cells comprise various cellular subtypes and are operationally divided into mononuclear and polymorphonuclear cells (FIG. 1). Mononuclear phagocytes include macrophages, which reside in virtually all tissues, where they perform location-specific functions including the regulation of tissue homeostasis, immune surveillance and inflammation[12,13]. Mononuclear phagocytes also include dendritic cells (DCs), which consist of distinct subsets. Classic DCs (cDCs) form the predominant DC subset and are specialized to sample antigens in tissues and to migrate to local draining lymph nodes to induce antigen-specific T cell immunity or tolerance[6,7]; cDCs also control T cell responses within nonlymphoid tissues, including solid tumours[8,14]. Plasmacytoid DCs (pDCs) are another DC subset that is uniquely able to produce interferon-α (IFNα) and may also regulate cancer progression[15]. Macrophages and DCs can have various origins[5,16] but those that accumulate in tumours derive mostly from circulating precursors, called monocytes[17] and pre-DCs[18], respectively, which are themselves produced by bone marrow-derived haematopoietic stem cells (HSCs). Polymorphonuclear phagocytes,

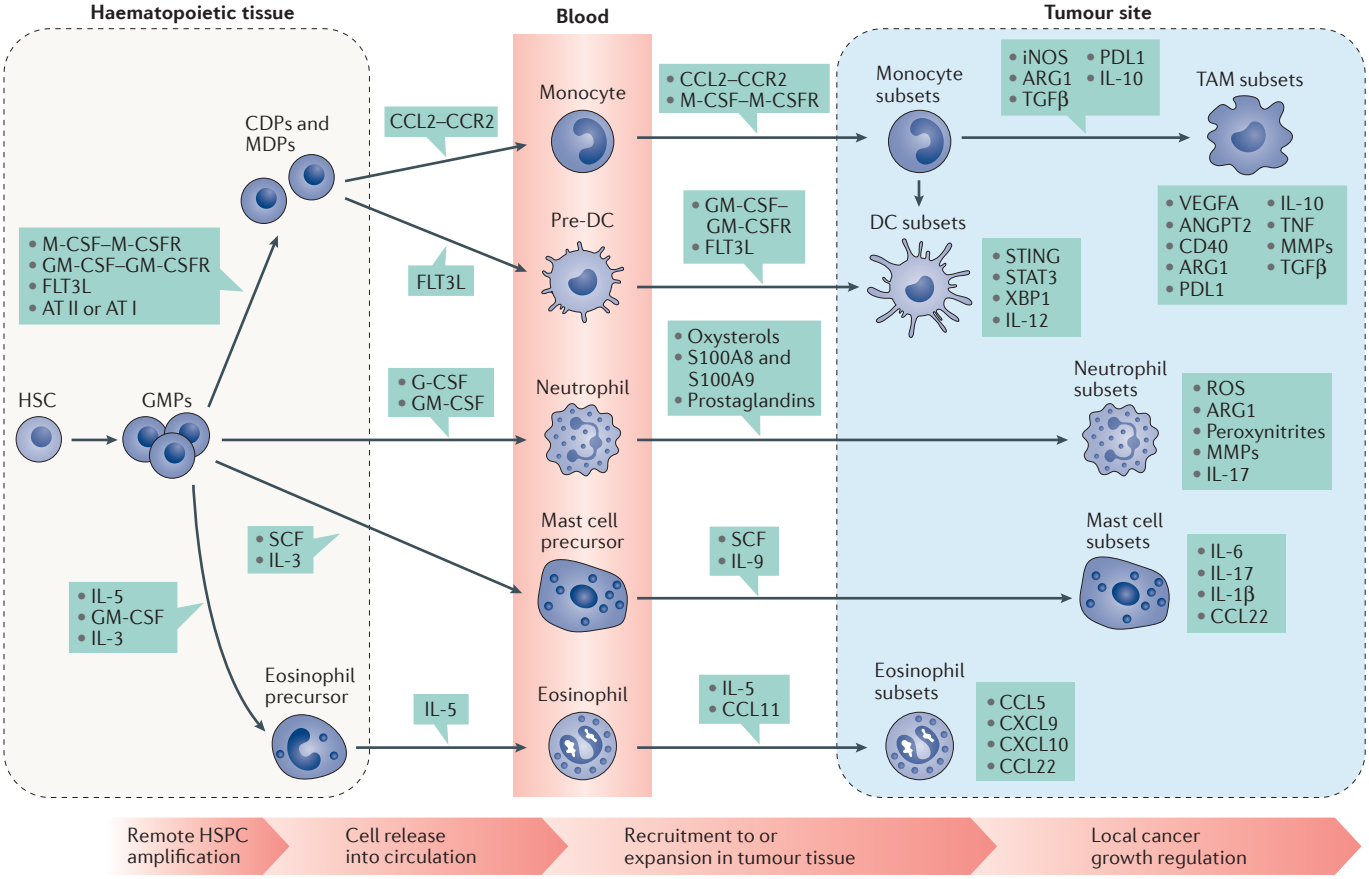

Figure 1 | **Developmental pathways of myeloid cells.** Discrete developmental activities control step-wise progression from haematopoietic stem cells (HSCs) to tumour-infiltrating myeloid cells. These activities occur in different body locations and include haematopoietic stem and progenitor cell (HSPC) amplification in haematopoietic tissues such as the bone marrow; release of the newly produced cells into peripheral blood; recruitment of the circulating precursors into the tumour stroma; and functional activities of the infiltrating cells within the tumour microenvironment. All these processes can be amplified or regulated by (distant) tumours. Molecular regulators of these processes are candidate drug targets to control myeloid cells as they progress along their developmental pathways. Myeloid-derived suppressor cells (MDSCs) can release factors shown here for neutrophils and/or monocytic cells. ANGPT2, angiopoietin 2; ARG1, arginase 1; AT, angiotensin; CCL, chemokine (C-C motif) ligand; CCR, C-C chemokine receptor; CDP, common dendritic cell progenitor; CXCL, chemokine (C-X-C motif) ligand; DC, dendritic cell; FLT3L, Fms-like tyrosine kinase 3 ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GM-CSFR, GM-CSF receptor; GMP, granulocyte–macrophage progenitor; IL, interleukin; iNOS, nitric oxide synthase 2, inducible; M-CSF, macrophage colony-stimulating factor; M-CSFR, M-CSF receptor; MDP, monocyte and dendritic cell progenitor; MMPs, matrix metalloproteinases; PDL1, programmed cell death 1 ligand 1; ROS, reactive oxygen species; SCF, stem cell factor; STAT3, signal transducer and activator of transcription 3; STING, stimulator of interferon genes; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; VEGFA, vascular endothelial growth factor A; XBP1, X-box binding protein 1.

Macrophages
Differentiated cells of the mononuclear phagocyte lineage that can clear dead cells and foreign particles through a process called phagocytosis. Macrophages assume tissue- and micro-environment-specific phenotypes to regulate tissue homeostasis, immunity and inflammation; they are essential protectors against injury and infections but also contribute to many diseases, including cancer.

Dendritic cells
(DCs). Crucial antigen-presenting cells for immune control. DCs typically have a probing morphology and localize in T cell areas of lymphoid organs to activate specific $CD4^+$ and $CD8^+$ T cells, but they can also be found in nonlymphoid tissues, such as the tumour stroma.

often referred to as granulocytes, also derive from HSCs and include neutrophils, eosinophils, mast cells and basophils. These cells can accumulate in diseased sites where they release toxic and inflammatory agents that protect the host against various insults, including bacterial and parasitic infections[19–21]. So-called myeloid-derived suppressor cells (MDSCs) broadly include immature myeloid progenitors and monocyte- and granulocyte-like cells and are functionally defined based on their ability to suppress T cell activation *in vitro*[22]. MDSCs are viewed as distinct from terminally differentiated myeloid cells such as macrophages and DCs; however, it is important to recognize that macrophages can also exhibit T cell-suppressive activity[23,24]. The phenotypes of tumour-associated MDSCs can be distinct from those of resting monocytes and neutrophils; however, whether monocytes and neutrophils compared with MDSCs are different cell types or cellular states remains disputed and in appreciation of this complexity we discuss them together.

In this Review, we first summarize current knowledge on the various, and sometimes seemingly opposing, contributions of myeloid cells to cancer progression. We also highlight how these cells and their precursors are regulated locally and systemically by tumours. We then explore the ways in which myeloid cells are affected by anticancer drugs and influence treatment outcome. Finally, we discuss how myeloid cells can be targeted therapeutically and outline next-generation therapeutic

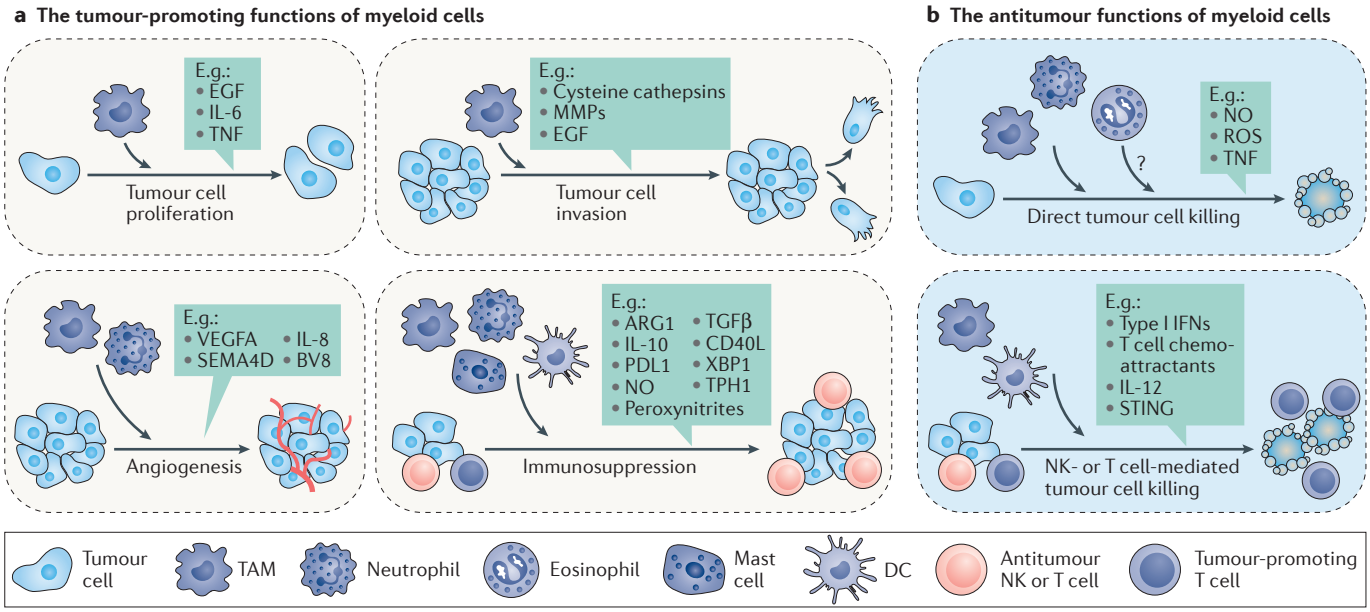

Figure 2 | **The tumour-promoting and antitumour functions of myeloid cells. a** | The tumour-promoting functions of myeloid cells include direct stimulation of tumour cell proliferation by cytokines and growth factors, enhanced tumour vascularization by angiogenic stimulators, increased tumour cell invasion by secreting enzymes and other factors and suppression of antitumour natural killer (NK) cells or T cells by various intracellular, cell surface and secreted factors. **b** | The antitumour functions of myeloid cells include: direct tumour cell killing by cellular signalling molecules and induction of tumour cell elimination by other cells, including NK and $CD8^+$ T cells. ARG1, arginase 1; DC, dendritic cell; EGF, epidermal growth factor; IL, interleukin; IFNs, interferons; MMPs, matrix metalloproteinases; NO, nitric oxide; PDL1, programmed cell death 1 ligand 1; ROS, reactive oxygen species; SEMA4D, semaphorin 4D; STING, stimulator of interferon genes; TAM, tumour-associated macrophage; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor; TPH1, tryptophan 5-hydroxylase 1; VEGFA, vascular endothelial growth factor A; XBP1, X-box binding protein 1.

Monocytes
Bone marrow-derived mononuclear phagocytes, crucial in protection against infections and in immune homeostasis, which when deployed to tissues can differentiate into a macrophage, and under certain conditions, a dendritic cell. Monocytes are typically divided into two subtypes: patrolling monocytes and inflammatory monocytes.

Neutrophils
Polymorphonuclear cells that develop and mature in the bone marrow, exist at high numbers in circulation and can be rapidly recruited to a site of injury or inflammation. Neutrophils can release potent biologically active antimicrobial enzymes, which are directly involved in clearance of infection.

Eosinophils
Granulocytic cells that are known mostly for their involvement in asthmatic disease and parasitic infections. Eosinophils are found primarily in the circulation, gut and thymic tissue but can be rapidly deployed into various tissues during inflammation to expel their granular content.

approaches that could better exploit myeloid cells to more effectively tailor treatment options for individual patients. Our overall goal is to guide readers, with nuance and cautious optimism, through the current understanding of myeloid cells in both human and mouse cancers.

Tumour-infiltrating myeloid cells

The definition of whether myeloid cells are relevant to cancer is currently largely based on two types of investigation. First, pathologists have begun to address whether the presence of tumour-infiltrating myeloid cells can predict patient outcomes. Typically, these studies are based on immunohistochemical analysis of tumour biopsies and are performed at the time of diagnosis (that is, in the absence of treatment). The results provide correlative, but not causative, links between myeloid cell content in the tumour stroma and disease progression. Furthermore, these data depend on a limited set of cellular markers that are seldom cell-type specific and may vary considerably across tissues and cancer types[25]. Nonetheless, these studies provide a window into myeloid cell involvement in human cancer, and the information obtained has diagnostic, prognostic and therapeutic potential. Second, animal and *in vitro* studies have started to investigate how myeloid cells regulate tumour progression. These investigations generally position myeloid cells as tumour-promoting components, with notable exceptions.

Macrophages. Tumour-associated macrophages (TAMs) frequently emerge as abundant immune cells in the tumour stroma in a broad range of cancers. However, the heterogeneity of macrophages[12,26,27] makes it challenging to define specific markers for these cells and additional methods are needed to accurately identify them. Currently, the intracellular glycoprotein CD68 is widely used in clinical studies as a TAM marker but it also detects other cell types[28,29] and does not identify the functional states of cells. The scavenger receptor CD163 (also known as M130 in humans) is also used to define TAMs, sometimes with concomitant CD68 staining. Studies using these markers revealed a significant association between high densities of TAM-like cells and poor prognosis in numerous cancer types including breast, thyroid, head and neck, liver, bladder, kidney, pancreatic, ovarian, oral, endometrial and lung cancer, as well as Hodgkin lymphoma[25,30,31].

These pathology findings are in accord with animal studies that show that TAMs can enhance cancer growth by producing mediators that shape the tumour microenvironment (FIG. 2a). Such mediators include growth factors and cytokines that support tumour cell survival and proliferation (for example, epidermal growth factor (EGF), interleukin 6 (IL-6) and tumour necrosis factor (TNF))[10,23]; extracellular matrix degrading enzymes (for example, matrix metalloproteinases (MMPs) and cysteine cathepsins) and other factors that modulate tissue architecture and favour tumour cell migration,

Mast cells
Crucial innate effector cells that are rich in granules that contain various immunoregulatory molecules. Upon stimulation by pathogens, allergens or endogenous factors, mast cells can rapidly degranulate and profoundly affect local and systemic tissue homeostasis, as exemplified by anaphylaxis.

Basophils
Circulating granulocytic cells known to mediate allergic responses and host defence against parasitic infections. Basophilic granules are a rich source of inflammatory mediators, including the vasodilator histamine and the anticoagulant heparin.

Tertiary lymphoid structures
Ectopic lymph node-like arrangements that form in tissues under pathophysiological conditions and that seem to facilitate local lymphocyte activation.

invasion and metastasis[32–34]; and pro-angiogenic agents that enable nutrient and oxygen delivery to tumours (for example, vascular endothelial growth factor A (VEGFA), IL-8 and semaphorin 4D (SEMA4D))[23,35,36]. TAMs can also promote cancer immune evasion by expressing cell surface proteins and releasing soluble factors that display immunosuppressive functions and blunt antitumour immunity (for example, arginase 1 (ARG1), IL-10, programmed cell death 1 ligand 1 (PDL1) and transforming growth factor-β (TGFβ))[23,24,37].

However, clinical studies also suggest that TAMs may have divergent functions[25]. For instance, high densities of CD68$^+$ cells have been associated with improved survival in colon[38], gastric[39] and endometrial[40] cancer. The functional state of TAMs may help to define whether these cells promote or suppress tumours. Indeed, macrophages can display a spectrum of activation states that fulfil specific functions and are often catalogued as classically (M1) or alternatively (M2) activated cells. Both M1-like and M2-like cells can have protective functions, for example, by eliminating intracellular bacteria and controlling parasite infections, respectively. These two macrophage populations are often distinguished based on their inducing stimuli (for example, Toll-like receptor (TLR) ligands and IFNs for M1 cells and IL-4, IL-10, TGFβ and glucocorticoids for M2 cells) or their secretion profiles and transcriptional activity (for example, expression of *Il12*, nitric oxide synthase 2, inducible (*Nos2*; which encodes iNOS), macrophage receptor with collagenous structure (*Marco*), suppressor of cytokine signalling 3 (*Socs3*) for M1 cells; *Rela*, *Socs2*, Krüppel-like factor 4 (*Klf4*) and *Arg1* for M2 cells)[13,41,42]. Categorizing macrophages based on these signatures has limitations[27] — for instance, because M1 and M2 profiles are artificial *in vitro* extremes and TAM phenotypes typically go beyond simple M1 and M2 denominations — yet it has enabled an insight into the role of macrophages during physiological and disease processes. In growing tumours, TAMs often show M2-like phenotypes[24], which foster tumour cell growth and invasion, enhance tumour angiogenesis and blunt antitumour T cell functions. In mice, perivascular CD163$^+$ TAMs have been identified as cells expressing alternative activation markers, whereas some CD163^{lo} TAMs in necrotic tumour regions activate inflammatory pathways[43]. It is possible that some M1-like macrophages are involved in cancer control by directly killing tumour cells, producing angiostatic factors and/or stimulating antitumour T cell functions[44–46] (FIG. 2b). In support of these ideas, M2-like TAMs, compared with M1-like TAMs, were associated with less favourable prognosis in a pan-cancer analysis[47]. More sensitive characterization of TAM phenotypes and functions is needed to clarify whether the M1 and M2 classification is sufficient to predict pro-tumoural versus anti-tumoural TAM functions in various cancer types.

Dendritic cells. Unlike TAMs, DCs are not abundant at the tumour site but must be considered because they can efficiently present extracellular antigens on major histocompatibility complex (MHC) class I molecules to enable antitumour CD8$^+$ T cell activation[8] (FIG. 2b). Accordingly, high densities of tumour-infiltrating DCs, particularly cDCs, may be beneficial[48,49]. In lung cancer, high cDC densities are associated with increased T cell activation[48,50]. Additionally, activated cDCs and T cells can cluster within tertiary lymphoid structures in tumours from patients with lung cancer[49], a pattern that provides further evidence for DC–T cell interactions at tumour sites.

The relative abundance of anti-tumoural versus pro-tumoural DCs and the degree to which tumours co-opt DCs may vary between individuals and cancer types. In support of this idea, a rare tumour-infiltrating CD103$^+$ (CD103 is also known as integrin αE) cDC population seems poised to cross-present antigens and activate CD8$^+$ T cells in several mouse models[51]. Additionally, an elevated ratio of CD103^{hi}/CD103^{lo} expression in the tumour stroma is associated with increased overall survival for patients with breast, head and neck or lung cancer[51]. CD103$^+$ DCs, although sparsely distributed in the tumour stroma, may thus display distinct antitumour immune functions.

Simultaneously, tumours can suppress the antitumour activity of DCs[52]. For example, ovarian tumour cells in mice can activate the transcription factor X-box binding protein 1 (XBP1) in DCs, thereby rendering them dysfunctional[53]. XBP1 induces an endoplasmic reticulum stress response that enables oxidized lipids to accumulate in DCs and blunts T cell priming by these DCs[53] (FIG. 2a). DC functions can also be suppressed upon activation of the β-catenin signalling pathway in cancer cells[54], by tumour-derived cyclooxygenases[55] and by IL-10-producing TAMs[56].

Besides cDCs, pDCs can also accumulate in the tumour stroma; however, tumour-infiltrating cDCs and pDCs may have divergent functions. Interestingly, high CD123$^+$ (CD123 is also known as IL3Rα) pDC content in breast tumour biopsies correlated with decreased 5-year overall and relapse-free survival[57]. Similar results were reported for patients with melanoma[58].

There is an urgent need to be able to identify antitumour DCs in a broad range of cancer patients. Most likely, these DCs are rare and share attributes with other DC subsets; therefore, their characterization will require a careful assessment of DC functional states that goes beyond generic DC surface markers. Also, because mouse and human DCs express different markers[59,60] it will be important to define whether findings hold true across species.

Neutrophils, monocytes and MDSCs. The abundance of tumour-infiltrating neutrophils, monocytes and MDSCs is associated with advanced cancer stage and decreased disease-free and overall survival in patients with various tumour types, including lung adenocarcinoma, breast cancer and colorectal carcinoma[47,61]. Similarly, elevated levels of circulating monocytes[62,63], neutrophils[62,63] and MDSCs[64] often correlate with poorer clinical outcome. Mouse monocytes can be operationally divided into lymphocyte antigen 6 complex, locus C (Ly6C)^{lo} cells, which patrol the vasculature at steady state and clear damaged endothelial cells, and Ly6C^{hi} cells, which can differentiate into macrophages or DCs upon extravasation into

diseased tissues[17]. Many tumours in mice selectively expand Ly6C^{hi} monocytes, which seed tumour-promoting TAMs[37,65–67]. By contrast, Ly6C^{lo} monocytes are typically not expanded by tumours but may suppress metastasis, as discussed below.

In mice, both Ly6C^{hi} monocytic and granulocytic cells are positive for CD11b (also known as integrin αM) and Gr1 (a cell surface antigen that comprises both Ly6C and Ly6G epitopes). These markers are also used to define MDSCs, which can suppress antitumour T cells to varying degrees[22,68]. Key myeloid-mediated T cell suppressive mechanisms include secretion or expression of immunoregulatory factors, including ARG1 (REF. 69), nitric oxide (NO)[70], PDL1 (REF. 37), TGFβ[71] and cyclooxygenase 2 (COX2; also known as PTGS2)[72] among others[10]. These myeloid cells also produce reactive oxygen species (ROS) and peroxynitrite, which can interfere with MHC–T cell interactions[73], suppress T cell migration[74] and activate regulatory T cells (T_{reg} cells) via CD40–CD40L interactions to induce tolerance to tumour cell-expressed antigen[75] (FIG. 2a). Monocytic cells may preferentially use NO and cytokines to suppress immune responses, whereas granulocytic cells seem to preferentially use ROS[10].

In addition to suppressing antitumour immunity, neutrophils may promote tumour growth by limiting cancer cell senescence[76], promoting angiogenesis[77], triggering thrombosis via neutrophil extracellular traps[78], inducing genotoxic damage[79] and recruiting other tumour-promoting cells[80]. Because it is challenging to distinguish MDSCs from neutrophils and monocytes in both humans and mice[68], many functions attributed to MDSCs may apply to neutrophils and/or monocytes, and vice versa.

Neutrophils and monocytes may also limit tumour progression in certain settings[81] (FIG. 2b). For example, neutrophils can display anti-tumoural cytotoxic activities in some mouse models of cancer[82,83] and promote T cell activation when retrieved from patients with early-stage lung cancer[84]. Taken together, these studies demonstrate that neutrophils and monocytes are likely to be important modulators of tumour growth, but their tumour-associated functions may diverge. Our understanding of the mechanisms that determine whether neutrophils and monocytes will accelerate or restrict tumour growth in human cancer patients remains limited, but might depend on several factors, such as tumour stage and the local tissue microenvironment.

Other tumour-infiltrating myeloid cells. Although their tumour-associated functions remain much less studied, eosinophils and mast cells are other granulocytes that may regulate cancer progression. Intriguingly, the presence of tumour-infiltrating eosinophils, or their local degranulation, has been associated with favourable prognosis in oral squamous cell carcinoma and prostate and colon cancer[85,86], and tumour infiltration by eosinophils can suppress metastatic melanoma progression in mice[87]. Eosinophils may secrete cytotoxic proteins with direct anti-tumoural activities[85] and promote anti-tumour CD8$^+$ T cell immunity at least in some contexts[88] (FIG. 2b). In contrast to eosinophils, mast cells are often viewed as tumour-promoting cells[89]. For example, mast cells produce tryptophan 5-hydroxylase 1 (TPH1), which exhausts tryptophan. Such nutrient deprivation liberates immunoregulatory metabolites and thereby promotes immune suppression[90]. Mast cells may foster tumour outgrowth by activating cancer-promoting T_{reg} cells (which suppress antitumour immunity)[91–93], monocytes and neutrophils[93,94] and B cells[89] in mice (FIG. 2a). In support of these results, tumour infiltration by mast cells was linked to poorer disease outcome in patients with colorectal cancer[95]. However, other reports show conflicting results[96,97], which may reflect the divergent functions of mast cells during tumour progression or in different tumour microenvironments, as discussed above for other types of myeloid cell. Additionally, most mouse studies have used *Kit*-mutant genetic mouse models which, in addition to lacking mast cells, have KIT-dependent anomalies such as anaemia and sterility (*Kit*$^{W/Wv}$ mutant mice) or the less severe splenic myeloid and megakaryocytic hyperplasia (*Kit*$^{W-sh/W-sh}$ mutant mice)[98]. New Cre recombinase-based models of mast cell deficiency circumvent this issue[99,100]. Using one of these models, mast cells were reportedly dispensable for tumour growth in genetically induced oncogenic *Kras*^{G12D}-driven pancreatic cancer[101]. Whether this is true for other tumour types, or patients, requires investigation.

Myeloid cells have emerged as crucial regulators of tumour progression, but our ability to distinguish tumour-promoting versus inhibitory cells in patients remains a major challenge for all myeloid cell subsets. Future analyses of clinical samples should aim to go beyond 'cell types' and instead focus on cellular functions; this may be achieved by harnessing recent technological advances, as discussed further in the Perspectives section below.

Systemic tumour–myeloid cell interplay

As cancer is a systemic disease, we extend our discussion of tumour–myeloid cell interactions to the entire body. We first examine how some tumours affect myeloid cell components well beyond the local tissue microenvironment and then consider how myeloid cell subsets in various body compartments can regulate tumour cell dissemination and metastasis.

The systemic impact of cancer on myeloid cells. Besides unravelling crucial anti- and pro-tumoural interactions between myeloid cells and cancer cells within the local tumour microenvironment, research during the past decade has revealed that tumours can regulate myeloid cells before they enter the tumour stroma (FIG. 3). Indeed, whereas many tissue macrophages are produced during embryogenesis and are maintained in adults[102], circulating monocytes contribute the vast majority of TAMs in mouse mammary[65] and lung[67] tumours and TAMs can be continually and rapidly replaced during cancer progression[65–67]. TAMs may proliferate in some tumours[103], although TAM amplification is contributed mostly by circulating precursor recruitment[65–67,103]. Animal studies further indicate that the macrophage colony-stimulating

Regulatory T cells
(T_{reg} cells). Specialized T cells that are functionally defined by their ability to confer peripheral tolerance to self, commensal and environmental antigens. T_{reg} cell accumulation in tumours can suppress antitumour immunity and is associated with poor prognosis in many cancers.

Degranulation
Release of cytotoxic and other molecules from secretory vesicles, also called granules, that are initially stored in some innate immune cells, for example, neutrophils, eosinophils and mast cells.

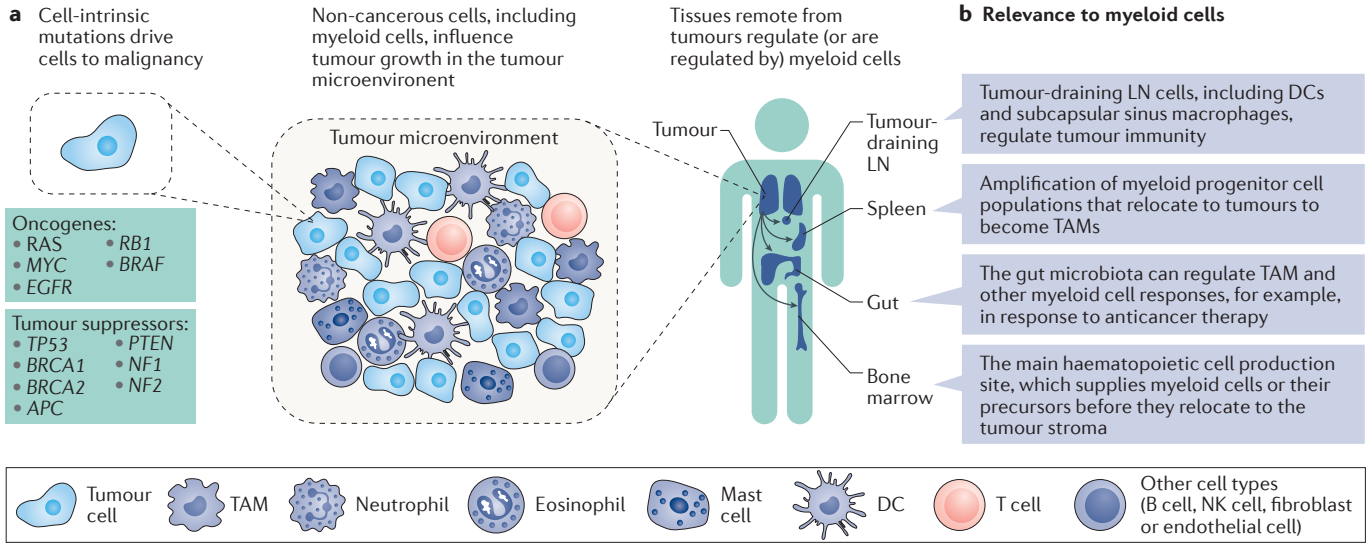

Figure 3 | **Cell-intrinsic, local and systemic regulation of cancer. a** | Cancer can be analysed at different levels and scales. Whereas tumour cell-intrinsic mutations are important drivers of tumour progression (left panel), tumour microenvironment host cells, including T cells and myeloid cells, also alter tumour growth (middle panel). Additionally, whole-body dynamics of anti- and pro-tumoural responses become important when considering that most tumour-infiltrating myeloid cells are continuously replaced by recruited circulating precursors and that immune responses are controlled in tissues distant from the tumour (right panel). Note that lung cancer is used here as a representative tumour stroma. **b** | Listed here are tissues that regulate myeloid cell production or participation in tumour-associated processes. APC, adenomatous polyposis coli; DC, dendritic cell; EGFR, epidermal growth factor receptor; LN, lymph node; NF, neurofibromin; NK, natural killer; RB1, retinoblastoma 1; TAM, tumour-associated macrophage.

factor (M-CSF)–M-CSF receptor (M-CSF–M-CSFR; also known as CSF1–CSF1R) and C-C motif chemokine ligand 2–C-C motif chemokine receptor 2 (CCL2–CCR2) pathways are crucial regulators of TAM recruitment and maintenance. The CCL2–CCR2 pathway is a central axis of TAM recruitment because it not only attracts circulating monocytes to tumours[104,105] but also mobilizes bone marrow-derived monocyte precursors to the periphery[66,106]. Similarly, M-CSF overexpression accelerates TAM recruitment and tumour growth, whereas genetic ablation of M-CSF reduces TAM density and delays tumour progression to malignancy[107,108]. Correspondingly, human studies revealed positive associations between CCL2 and/or M-CSF overexpression and poor prognosis in many cancer types, including breast[109,110], pancreatic[111], colorectal[112], hepatocellular[113] and endometrial[114] tumours. Also, myeloid chemoattractants are elevated in human tumours[110,113,115] and increased numbers of monocytes in peripheral blood correlate with poorer survival of cancer patients[63]. In contrast to TAMs, subcapsular sinus macrophages do not derive from circulating monocytes and can suppress melanoma progression in mice by physically limiting the systemic spread of tumour-derived vesicles in tumour-draining lymph nodes[116].

In comparison with macrophages, less is known about the origins and recruitment of other tumour-infiltrating myeloid cells, although many of them probably derive from circulating cells[7]. In the absence of disease, most DCs originate from circulating pre-DCs. In tumour-bearing mice, adoptively transferred pre-DCs can also produce tumour-infiltrating DCs[18]. Furthermore, mammary tumour DCs can be maintained independently of M-CSF[51], suggesting that these cells have a monocyte-independent origin. Different tumour-associated DC populations may be maintained by distinct developmental and amplification mechanisms. In mammary tumours, overexpressed Fms-related tyrosine kinase 3 ligand (FLT3L), a cytokine that is structurally similar to M-CSF, selectively expands CD103$^+$ DCs[51], whereas granulocyte–macrophage colony-stimulating factor (GM-CSF) amplifies tumour-infiltrating CD103$^-$ DCs. CD103$^+$ and CD103$^-$ DCs also depend on the expression of distinct transcription factors: zinc finger and BTB domain-containing protein 46 (*Zbtb46*), interferon regulatory factor 8 (*Irf8*) and basic leucine zipper transcriptional factor ATF-like 3 (*Batf3*) control CD103$^+$ DCs whereas *Irf4* expression controls their CD103$^-$ counterparts[117–120]. Accordingly, *Batf3* is required to produce DCs that promote antitumour immunity and control tumour progression in mice[119]. Monocytes also produce bona fide DCs in some inflammatory conditions[121]. Tumour-infiltrating neutrophils turn over within days in mouse models[122]; tumoural and/or systemic accumulation of these cells and other granulocytes is regulated by growth factors (for example, G-CSF, GM-CSF and VEGFA)[10,123,124] and various other components (for example, S100A8, S100A9, high mobility group protein B1 (HMGB1), oxysterols, IL-17 and prostaglandins)[10,122,125]. Some tumours also amplify myeloid cells or their precursors in remote body locations through various mechanisms, including the release of tumour-derived signals that extend far beyond the tumour stroma (BOX 1).

M-CSF–M-CSFR
(macrophage-colony stimulating factor and its receptor, also known as CSF1–CSF1R). A haematopoietic growth factor–receptor pair that is required for proper development, survival and maintenance of the monocyte and macrophage cell lineage.

CCL2–CCR2
(chemokine (C-C motif) ligand/receptor 2). A chemokine–receptor pair that mediates monocyte release from the bone marrow and, in the context of cancer, entry into the tumour microenvironment.

Premetastatic sites
Sites in which metastasis will occur. These sites are thought to be primed for tumour cell engraftment by factors that are secreted by the primary tumour and by bone marrow-derived haematopoietic cells that are recruited locally.

Natural killer (NK) cells
Cytotoxic lymphocytes that are crucial to the innate immune system and that provide rapid responses to eliminate abnormal cells, such as virus-infected cells and tumour cells.

The impact of myeloid cells on metastasis. Some tumour cells acquire the ability to invade their surrounding tissue, enter and survive in the circulation, extravasate into distant tissue and grow[126]. Myeloid cells may be involved at each step of the metastatic cascade. For example, mouse macrophages at primary tumour sites can promote tumour cell migration and entry into blood (FIG. 4a) via a paracrine EGF–M-CSF macrophage–tumour cell loop[127]. The presence of macrophages next to endothelial cells and tumour cells also predicts cancer metastatic behaviour in at least some subtypes of human breast cancer[128]. Additionally, TAMs can enhance tumour cell intravasation by producing VEGFA, which increases vascular permeability[32]. Once in the circulation, tumour cells can be scavenged by liver-resident macrophages through phagocytosis, which could help to reduce metastases[9,129] (FIG. 4b). However, macrophage-mediated phagocytosis of circulating cancer cells is limited by tumour cell upregulation of CD47, which binds to signal regulatory protein-α (SIRPα) on macrophages and functions as a 'do not eat me' signal[130,131]. Some myeloid cells at premetastatic sites also foster tumour cell seeding by promoting aberrant vascular formation[132] (FIG. 4c). Ly6C^{lo} 'patrolling' monocytes, which largely remain in the circulation, may prevent lung metastasis by scavenging tumour-derived material in the tumour microvasculature and attracting anti-tumoural natural killer (NK) cells locally[133]. However, Ly6C^{hi} 'inflammatory' monocytes, which differentiate into tumour-promoting TAMs, enhance lung metastasis in a CCL2- and VEGFA-dependent manner[104]. These findings highlight the distinct functions and tropisms of subsets of monocytes, and their opposing roles in metastasis. Neutrophils can also support tumour cell intravasation and seeding. For example, neutrophil secretion of leukotrienes, controlled by arachidonate 5-lipoxygenase (ALOX5), promotes mouse lung colonization by metastasis-initiating breast cancer cells[134]. Neutrophils also augment tumour cell intraluminal survival by suppressing NK cell antitumour activity and further facilitate tumour cell extravasation by secreting IL-1β and MMPs[135]. Neutrophils[125] and macrophages[136] further regulate metastasis outgrowth; for example, by suppressing antitumour immunity, although the function of these cells may vary[81,82] (FIG. 4d). The underlying mechanisms that govern anti- versus pro-tumour myeloid cell functions in the development of human metastases require investigation, but potentially have crucial implications for therapy.

Box 1 | **Myeloid cell production away from the tumour stroma**

Some tumours produce soluble factors that act over extended distances in the body to actively induce myeloid cell production from haematopoietic stem and progenitor cells (HSPCs)[123,124,187]. Studies in mice have identified that granulocyte colony-stimulating factor (G-CSF) expands haematopoietic stem cells and myeloid progenitors in the bone marrow and leads to the amplification of tumour-associated macrophages (TAMs) and other myeloid cells[123]. In cancer patients, haematopoiesis is also typically enriched and skewed towards myelopoiesis[226]. Elevated levels of circulating granulocyte–macrophage progenitors (GMPs) are found across different tumour types and high blood levels of GMPs correlate with poor survival[226]. The cellular fate and tissue destination of expanded progenitors cannot be easily addressed in patients; however, adoptive cell transfer studies in mice have shown that bone marrow-derived GMPs produce TAMs[51,67] and tumour-infiltrating dendritic cells (DCs)[51] in mouse models of lung and breast cancer. The bone marrow is the main site of haematopoiesis in the adult[227], but extramedullary tissues such as the spleen also contribute TAMs in a genetic mouse model of lung adenocarcinoma driven by *Kras*^{G12D} and loss of *Trp53* (KP mice)[67]. The spleen is a monocyte reservoir in mice[228] and humans[67,229], and mouse splenic monocytes can be mobilized to distant injured tissues[228,230] and tumours[67,187], where they differentiate into macrophages. Some cancer patients and tumour-bearing mice also amplify splenic HSPCs that can produce monocytes[231]. These findings support the existence of monocyte production both inside and outside the bone marrow in humans, although whether extramedullary tissues contribute a substantial number of TAMs (and/or other myeloid cells) in human cancer is unknown. Also, the bone marrow contains dynamic microenvironments that produce monocytes in both the steady state and inflammation, and spleen-derived macrophages are predominantly products of inflammation; yet, whether these niches generate functionally different cells requires study. Interestingly, the peptide hormone angiotensin II, which is overexpressed in KP tumour-bearing mice, specifically augments extramedullary TAM progenitors[187], suggesting that different molecular pathways regulate TAM production from medullary and extramedullary tissue. Medullary and extramedullary tissues can also contribute tumour-promoting neutrophils in mouse models of metastatic breast cancer[135]. Combined, these studies support the notion of cancer as a systemic disease: tumours control and are controlled by processes that occur both within and outside the local tumour microenvironment. Investigations that further probe long-range mechanisms of myeloid cell-mediated tumour control should reveal additional tumour–host communication pathways that could serve as new clinical targets.

Myeloid cells in cancer therapies

The relevance of myeloid cells to current cancer treatments remains largely unexplored, particularly in the clinical setting, although virtually all therapeutic modalities, including surgery, chemotherapy, radiotherapy, immunotherapy and targeted therapy, probably involve these cells. Notably, myeloid cells are required to clear killed tumour cells and orchestrate the healing response that follows treatment-induced cancer regression. Furthermore, emerging evidence indicates that myeloid cells and cancer treatments are linked at other, sometimes unexpected, levels and that these connections can strikingly influence treatment outcome, either positively or negatively (TABLE 1). Below, we discuss recent discoveries, obtained mostly from experimental studies, related to the interplay between myeloid cells and various cancer treatments.

Myeloid cells and cytotoxic therapies. Decades ago, radiotherapies and chemotherapies were developed to kill dividing tumour cells. These treatments remain the primary therapies for many cancer types even though their efficacy is often limited. Tumour microenvironment analysis in experimental mouse models suggests that TAMs and other phagocytes can negatively influence the outcome of cytotoxic treatment. For example, TAMs can promote drug resistance by producing cysteine cathepsins that protect tumour cells from being killed by the chemotherapeutic agent taxol[137] and by secreting the immunoregulatory cytokine IL-10, which impairs drug-induced CD103$^+$ DC accumulation in tumours and antitumour CD8$^+$ T cell activity[56]. Consequently, suppressing TAMs can improve therapy: M-CSFR targeting enhances radiotherapies against mouse prostate[138] and mammary[139] tumours and

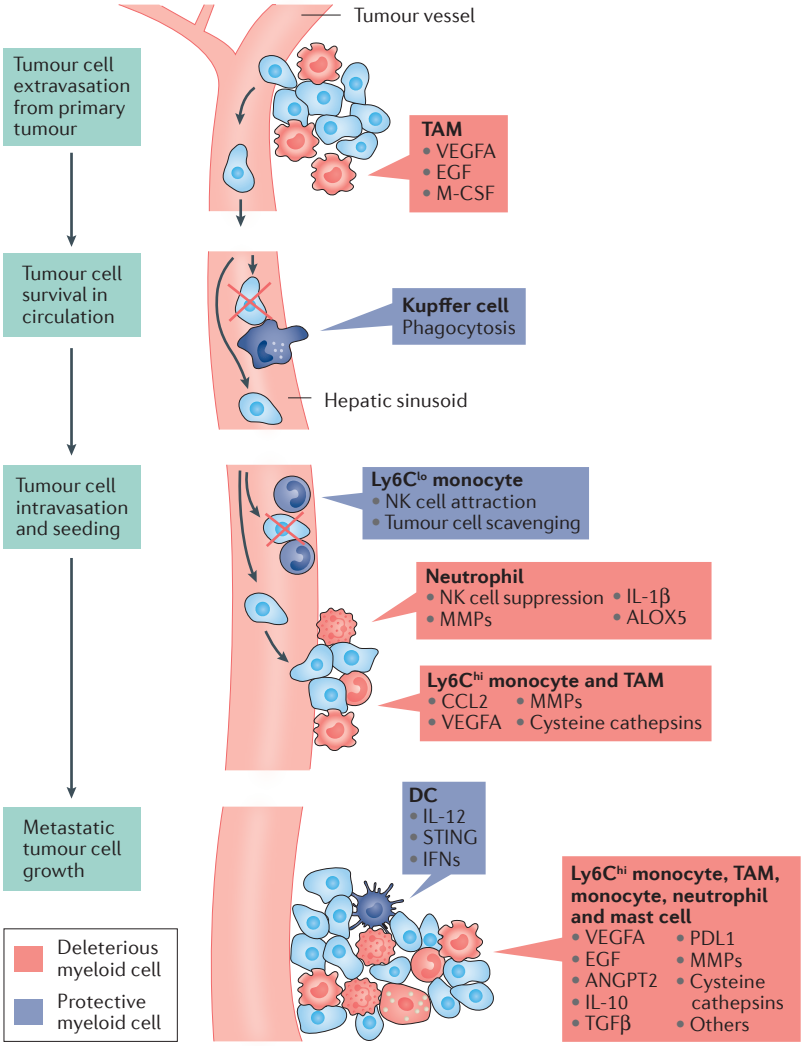

Figure 4 | **Myeloid cell regulation of metastasis.** To metastasize, tumour cells need to enter the bloodstream, survive in the circulation, extravasate and proliferate in distant tissues. Myeloid cells can both promote and limit these different steps. Some tumour-associated macrophages (TAMs) in primary tumours can increase vascular permeability and promote tumour cell migration and intravasation. Liver-resident macrophages (Kupffer cells) or other cells that filter blood, such as some macrophages in the spleen, may sample and reduce the number of circulating tumour cells. However, CD47 expression on tumour cells could at least in part explain the persistence of some tumour cells in circulation by acting as a 'do not eat me' signal to macrophages. Tumour cells exiting the bloodstream may interact with various myeloid cells. Lymphocyte antigen 6 complex, locus C ($Ly6C^{lo}$) monocytes may prevent metastasis by scavenging tumour-derived material and reducing tumour cell extravasation; $Ly6C^{lo}$ monocytes may not kill tumour cells directly but instead attract natural killer (NK) cells to accomplish this task. $Ly6C^{hi}$ monocytes and TAMs may instead favour metastasis by inducing the production of chemokines and growth factors. Neutrophils can also support metastasis initiation by suppressing antitumour NK cell activity and enhancing tumour cell extravasation and migration. Myeloid cells at metastatic sites can both promote and inhibit growth and persistence of metastatic cancer cells; similarly to their functions at primary tumour sites (FIG. 2). Cells in red and blue depict deleterious and protective myeloid cells, respectively. Specific factors or activities associated with defined myeloid cells are also listed. ALOX5, arachidonate 5-lipoxygenase; ANGPT2, angiopoietin 2; CCL2, chemokine (C-C motif) ligand 2; DC, dendritic cell; EGF, epidermal growth factor; IL, interleukin; IFNs, interferons; M-CSF, macrophage colony-stimulating factor; MMPs, matrix metalloproteinases; PDL1, programmed cell death 1 ligand 1; STING, stimulator of interferon genes; TGFβ, transforming growth factor-β; VEGFA, vascular endothelial growth factor A.

M-CSF blockade ameliorates paclitaxel treatment efficacy against mammary tumours in mice[140], indicating that these TAMs promote cancer. Neutrophils and/or MDSCs also seem to limit the efficacy of radiotherapy as depletion of $Ly6G^+$ cells can improve the outcome of irradiated mice bearing colon carcinoma[141].

The interplay between cytotoxic therapies and myeloid cells goes both ways: myeloid cells can regulate treatment efficacy but cytotoxic therapies can also control myeloid cells. For example, some chemotherapeutic agents may reduce the numbers of myeloid cells by eliminating them directly or by limiting bone marrow-derived myeloid progenitors. Accordingly, trabectedin[142], doxorubicin[143] and 5-fluorouracil[144] can control tumour growth not only by killing cancer cells but also by reducing the number of TAMs and other tumour-promoting phagocytes. For example, trabectedin selectively induces caspase-8-dependent apoptosis in monocytes and TAMs, which is probably due to a unique expression pattern of TNF-related apoptosis-inducing ligand (TRAIL) receptors in these cells[142]. Instead, some chemotherapeutic agents may amplify myeloid cells. For example, paclitaxel increases TAM accumulation in mammary tumours in mice by stimulating M-CSF production by mammary epithelial cells[140]. This process is relevant to treatment because M-CSF blockade in combination with paclitaxel improves survival. Also, we have limited knowledge of the impact of drug treatment on the dynamics between TAMs and their progenitors but it is possible that reactivated haematopoiesis after drug clearance[145] transiently accelerates TAM production in myeloablated hosts.

Although these findings suggest that suppressing TAMs can improve cytotoxic chemotherapies, complete TAM elimination may be undesirable in some contexts. For example, local irradiation of tumours with low-dose ionizing radiation in a transgenic mouse model of pancreatic cancer stimulates $iNOS^+$ TAM accumulation; these cells may help to normalize the tumour vasculature and promote T cell influx into otherwise non-T cell-infiltrated tumours, thereby improving tumour control and mouse survival[146]. Furthermore, platinum-based oxaliplatin treatment against various mouse cancer models triggers TAMs and neutrophils to produce ROS[147], which mediate DNA damage and apoptosis. Oxaliplatin therapy response in a transplantable model of colon carcinoma requires both Toll-like receptor 4 (TLR4), which is expressed by myeloid cells, and an intact microbiota, suggesting the importance of myeloid cell–microbiota interactions in regulating drug effects[147]. Additionally, TAMs may be useful drug depots because they can accumulate large quantities of nanoparticles containing cytotoxic compounds and then release their payload to neighbouring tumour cells[148]. These TAMs are beneficial to the host because TAM depletion reduces treatment efficacy[148].

Tumour-infiltrating DCs are typically thought to improve cytotoxic therapies. For example, oxaliplatin and several anthracyclines can kill tumour cells through a process called immunogenic cell death, which involves ATP and HMGB1 release as well as calreticulin surface expression by dying tumour cells[149]. These effector

Table 1 | **Protective and deleterious contributions of TAMs and DCs to anticancer therapies**

	Protective myeloid cell contributions	Deleterious (but therapeutically targetable) myeloid cell contributions
Chemotherapy	• Chemotherapy promotes antitumour DC recruitment to tumours[150] • Chemotherapy-induced differentiation or infiltration of antigen-presenting cells stimulates antitumour T cell immunity[153,154] • Macrophages can be drug depots for nanotherapeutics[148]	• TAM suppression by IL-10R blockade improves chemotherapy[56] • TAM suppression by CCR2 or M-CSFR blockade improves chemotherapy[140,184] • Inhibition of TAM-derived cysteine cathepsins improves chemotherapy[137]
Radiotherapy	• Ionizing radiation induces antitumour adaptive immunity via STING and type I IFN-dependent signalling in DCs[156] • Local low-dose ionizing radiation instigates macrophage differentiation into iNOS$^+$ M1-like cells that support antitumour T cell immunity[146]	• TAM suppression by M-CSFR blockade improves radiotherapy[138,139] • Irradiation-induced intratumoural SDF1α recruits cancer-promoting TAMs[232]
mAb-based immunotherapy	• CD20 mAb-mediated removal of tumour cells depends on Fc receptors expressed by phagocytes[160,161,233] • CD40 mAb-mediated control of pancreatic tumours involves activation of tumoricidal macrophages[195] • Successful CTLA4 mAb therapy involves phagocyte-mediated T_{reg} cell depletion[164,165,234]	• TAM reprogramming by M-CSFR inhibition improves immune checkpoint blockade therapy[169] • PDL1 blockade on monocytes augments antitumour immunity[37] • Depletion of extratumoural macrophages enhances CD40 mAb therapy[235]
Adoptive cell immunotherapy	• Successful adoptive T cell therapy requires CD103$^+$ DCs[51] • DC adoptive transfer instigates tumour infiltration by T cells and sensitizes tumours to anti-PDL1 and anti-CTLA4 therapies[54]	TAM suppression by M-CSFR blockade improves adoptive cell therapy[236,242]
Small-molecule based therapy	• JAK2–STAT3 targeting causes DC activation and differentiation[178] • Histidine-rich glycoprotein-polarized TAMs promote antitumour immunity and vessel normalization[237] • KIT inhibition stimulates antitumour NK cell activation via DCs[238] • MEK and BRAF inhibitors revert BRAF-mutated melanoma-induced DC suppression[176,177] • Bortezomib stimulates DCs to induce antitumour T cell immunity[239]	• Inhibition of TNF production by macrophages improves therapies that target the MAPK pathway[171] • Inhibition of VEGFA production by macrophages improves BRAF inhibitor treatment[172] • TAM suppression by M-CSFR blockade improves BRAF inhibitor therapy by increasing antitumour immunity[173] • M-CSFR blockade controls TAM recruitment and improves antiangiogenic therapy[240] • TAM depletion improves tyrosine protein kinase inhibitor therapy[241]

CCR2, chemokine (C-C motif) receptor 2; CTLA4, cytotoxic T lymphocyte-associated antigen 4; DC, dendritic cell; IFN, interferon; IL, interleukin; iNOS, nitric oxide synthase 2, inducible; JAK2, Janus kinase 2; mAb, monoclonal antibody; M-CSFR, macrophage colony-stimulating factor receptor; NK cell, natural killer cell; PDL1, programmed cell death 1 ligand 1; SDF1α, stromal-derived factor 1α; STAT3, signal transducer and activator of transcription 3; STING, stimulator of interferon genes; TAM, tumour-associated macrophage; TNF, tumour necrosis factor; T_{reg} cell, regulatory T cell; VEGFA, vascular endothelial growth factor A.

molecules modulate DCs: ATP release results in DC recruitment into the tumour bed[150], calreticulin stimulates tumour antigen engulfment by DCs[151] and HMGB1 enhances the capacity for antigen presentation by DCs to T cells[152]. In mouse models, anthracyclines stimulate tumour infiltration by CD11b$^+$ CD11c$^+$ Ly6C^{hi} DC-like cells that are necessary for treatment efficacy[153]. Because these antitumour cells do not require *Batf3* (REF. 153), they instead may derive from inflammatory monocytes[121]. Similarly, chemotherapies can increase HMGB1 levels in mouse lung tumours and induce tumour infiltration of DC-like myeloid subsets[154]. Radiotherapies can also trigger tumour infiltration by DC-like cells that foster antitumour T cell immunity[155–157].

Myeloid cells and monoclonal antibody-based immunotherapies. Harnessing the immune system can durably control cancer in some patients while limiting side effects[1]. In the years to come, immunotherapy will probably become the backbone of cancer treatment, as either monotherapy or as part of combination therapies. Many immunotherapy approaches involve immunoglobulin G (IgG) monoclonal antibodies (mAbs) that can either target cancer cells (for example, rituximab targets CD20$^+$ non-Hodgkin lymphoma cells), stimulate antitumour T cell immunity (for example, nivolumab and pembrolizumab antagonize the T cell immune-checkpoint receptor programmed cell death protein 1 (PD1; also known as PDCD1)) or inhibit angiogenesis (for example, bevacizumab targets VEGFA)[158]. Some of these therapeutic mAbs may directly control tumours by inducing apoptosis or inhibiting cell proliferation, but others depend on additional host components, such as complement cascade proteins and Fcγ-receptor (FcγR)-positive cells[158]. Indeed, IgG mAbs contain a variable Fab domain that confers binding specificity but also a constant Fc domain that bridges antibody-coated targets with FcγR$^+$ cells, which include macrophages and DCs[158,159].

FcγRs can be either activating or inhibitory. Cross-linking activating receptors (namely, FcγRI, FcγRIIA, FcγRIIC and FcγRIIIA in humans and FcγRI, FcγRIII and FcγRIV in mice) on the phagocyte surface can trigger cytotoxic or phagocytic elimination of mAb-coated target cells. This process probably contributes to the *in vivo* activities of many therapeutic mAbs[158,159]. For example, rituximab depletes non-Hodgkin lymphoma cells via FcγR-mediated monocyte and macrophage cytotoxicity[158,160]. Similar mechanisms occur with the mAbs trastuzumab (anti-ERBB2) and daratumumab (anti-CD38), which are used against ERBB2$^+$ breast

Fcγ-receptor
(FcγR). A surface-bound protein receptor expressed by phagocytes and other cell types, which binds to the constant heavy chain (Fc) region of an antibody and mediates cell clearance mechanisms. FcγRs, for which four different classes are known (FcγRI, FcγRII, FcγRIII and FcγRIV), bind to the Fc region of immunoglobulin G antibodies.

cancer[161] and multiple myeloma[162], respectively. In mice treated with antitumour mAbs, FcγR-mediated killing by Kupffer cells also eliminates circulating tumour cells that transit through the liver[129].

Similarly, engaging activated FcγRs expressed by myeloid cells influences the activities of T cell immune checkpoint blockers. Ipilimumab (anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)), which amplifies antitumour T cell activity while restricting immunosuppressive T_{reg} cells[163], reduces T_{reg} cell counts via FcγR-mediated phagocytosis[164]. Treatment-induced tumour control and T_{reg} cell depletion in mice both depend on FcγRIV, which is expressed by TAMs[164]. Also, patients with melanoma who respond to ipilimumab treatment have more FcγRIIIA$^+$ CD16$^+$ blood monocytes than non-responders[165]. The inhibitory receptor FcγRIIB, which is conserved in both humans and mice, also regulates mAb-based immunotherapies. Mice treated with agonistic CD40 mAbs require FcγRIIB crosslinking to stimulate myeloid cell maturation and activate CD8$^+$ T cells. This process controls cancer growth even in the absence of all activating FcγRs[166].

Taken together, these studies identify myeloid cells and FcγRs as important executors of responses to various immunotherapies, but FcγR-mediated activation may also have deleterious effects. As an example, endogenous IgGs can foster mouse squamous cell carcinoma progression by stimulating FcγRs on macrophages and mast cells[89]. Further investigation is needed to clarify how FcγR-mediated myeloid cell responses affect treatment outcomes and, by extension, how they can be harnessed for therapy. The results of FcγR engagement in a given microenvironment will probably be controlled by several variables, including the IgG Fc composition, the diversity of inhibitory and activating FcγRs engaged by IgGs, and the cell types expressing these FcγRs. Engineered mAbs that lack Fc domains, such as single-chain variable domain fragments[167], are considered for therapy in part because their reduced binding to FcγR$^+$ cells improves penetration into tumours; yet, how the absence of FcγR-mediated myeloid cell activation affects treatment outcome in humans requires study.

Myeloid cells may also influence mAb-based immunotherapies independently of FcγRs. First, Fab domains of some therapeutic mAbs can bind to tumour-infiltrating DCs and TAMs. PDL1 mAbs, for example, may control cancer growth at least in part by acting on PDL1$^+$ phagocytes[37,168]. Additionally, tumour-infiltrating DCs may be required for successful immunotherapies. In melanoma, active β-catenin signalling results in CD103$^+$ DC and T cell exclusion from the tumour stroma, but bone marrow-derived DCs injected into these tumours instigate T cell recruitment and sensitize tumours to anti-PDL1 and anti-CTLA4 therapy[54]. Also, immunogenic chemotherapeutics can upregulate TLR4 on tumour-infiltrating CD103$^+$ DC-like cells; TLR4$^+$ cells promote tumour infiltration by CD8$^+$ T cells, a process that sensitizes tumours to anti-CTLA4 or anti-PD1 therapy[154]. In contrast to tumour-infiltrating DCs, TAMs may antagonize immune checkpoint therapies. Indeed, inhibited M-CSFR signalling improves anti-CTLA4 and anti-PD1 therapy in mouse models of pancreatic cancer[169]. This indicates that agents that target myeloid cells and immune checkpoint blockers influence non-redundant mechanisms and can be more successful in combination. Manipulating TAM functions while boosting tumour-infiltrating DCs may further expand the proportion of patients who respond to current immunotherapies.

Myeloid cells and small-molecule based therapies. The development of small-molecule compounds that target cancers harbouring specific genetic alterations and/or molecular compositions has transformed cancer therapy. Genetic testing of somatic mutations is now a routine clinical procedure and an increasing number of molecularly targeted drugs are becoming available. These drugs can dramatically shrink tumours, but these effects are typically short-lasting. There is thus an urgent need to dissect the underlying tumour resistance mechanisms and find new avenues to improve the efficacy of small-molecule therapeutics. Myeloid cells may be relevant in both cases. Notably, in both human and mouse gastrointestinal cancers the tyrosine-kinase inhibitor imatinib induces M2 macrophage-associated genes and suppresses inflammatory cytokine production in TAMs[170], and genetically removing TNF in myeloid cells delays resistance to MAPK inhibitors in a mouse model of BRAF-driven melanoma[171]. Consequently, blocking M-CSFR signalling improves the efficacy of BRAF inhibitors[172] and extends survival in melanoma-bearing mice[173]. Blocking M-CSFR signalling similarly ameliorates antiangiogenic drug efficacy against mouse lung carcinomas[174]. Inhibition of the hepatocyte growth factor receptor MET, which is a molecular drug target for several cancers, including that of the lung[175], may also negatively influence neutrophil cytotoxicity and antitumour activities[82]. Interestingly, however, some targeted drugs may have positive effects on myeloid cells: MEK and BRAF inhibitors can revert BRAF-mutated melanoma-induced DC suppression *in vitro*[176,177], and molecular targeting of the Janus kinase 2–signal transducer and activator of transcription 3 (JAK2–STAT3) pathway can promote DC activation[178]. Additionally, long-term treatment with imatinib dramatically reduces the numbers of mast cells in patients with chronic myeloid leukaemia and in mice, although it is not clear if this is relevant to treatment outcome[179]. Future studies should reveal whether manipulating myeloid cells or molecular pathways involving these cells enables targeted therapies to produce more durable responses in patients.

Targeting myeloid cells to limit cancer

The relevance of tumour-infiltrating myeloid cells in cancer progression and therapy has spurred interest in therapeutically targeting these cells. One strategy is to reduce numbers of myeloid cells such as TAMs, which may be achieved by targeting TAMs themselves or their precursors (FIG. 3). Because myeloid cells can contribute

both anti- and pro-tumoural activities, modulating their functions, rather than depleting the cells, is another attractive option.

Manipulating myeloid cell numbers. Different myeloid cell depletion strategies, including pharmacological and genetic approaches, have been used to successfully control tumour progression in various mouse models[180]. In mice, broad depleting strategies that target CD11b-, Gr1- or Ly6G-expressing myeloid cells, delay tumour progression in several, but not all, experimental settings[125,140,181]. Rather than exclusively affecting myeloid cells at the tumour site, these strategies typically affect myeloid cell populations systemically; by extension, the observed antitumour effects could be driven at least partially by altered extratumoural myeloid cells. Because monocyte recruitment to tumours and TAM maintenance depend strongly on CCL2–CCR2 and M-CSF–M-CSFR signalling, these pathways are promising targets for depleting TAMs more selectively[23,25]. Different approaches using mAbs[104,169,182], small-molecule inhibitors[169,183,184] or nanoparticle-based gene expression silencing[67,185] can limit TAM accumulation and control disease progression in various mouse models of cancer. Drug regimen schedules, however, may profoundly affect clinical outcomes. Indeed, interrupting CCL2 blockade treatment in mice can result in sudden monocyte release from the bone marrow, which increases metastasis formation and accelerates death[145]. Furthermore, targeting the M-CSF–M-CSFR pathway can have different outcomes: it controls tumours in some cancer models[182,183,186], whereas others require combination with an additional treatment[56,138–140]. Accordingly, drugs that target the CCL2–CCR2 or M-CSF–M-CSFR pathway are being evaluated in patients both as monotherapies[182] and in combination with anticancer agents[25]. In mice, tumour phagocyte replenishment can also be controlled by limiting tumour-induced myelopoiesis. For instance, targeting production of GM-CSF[124] or angiotensin II[187] reduces monocyte and neutrophil-like cells or TAMs, respectively, and suppresses tumour progression. G-CSF inhibition may also reduce bone marrow production[123] and release[188] of tumour-promoting myeloid precursors.

Current myeloid cell depletion strategies have limitations: they can delay tumour progression but may be insufficient to eliminate or durably control cancer in mice on their own[125,140,169,187]. Also, myeloid cell ablation may have undesirable clinical side effects, such as increased risk of infections, that must be considered in translating these treatments for patient use.

Manipulating myeloid cell phenotypes. Strategies that modulate, rather than ablate, tumour-infiltrating phagocytes may not only harness their antitumour properties but also circumvent the drawbacks of phagocyte depletion strategies. Interestingly, in some cases, M-CSFR targeting induces tumour regression without depleting TAMs[186]. The drug-induced antitumour mechanisms probably involve changing TAM phenotypes, including downregulation of M2 macrophage-associated genes. A model of pancreatic cancer showed similar results[169], although M-CSFR blockade simultaneously reduced the TAM infiltrate. Besides M-CSF–M-CSFR targeting, other strategies may skew myeloid cell functions and exploit their antitumour potential. For instance, inhibiting the receptor tyrosine kinase MERTK triggers a pro-inflammatory TAM phenotype, increases $CD8^+$ T cell infiltration and improves tumour control in mice[189,190]. Several US Food and Drug Administration (FDA)-approved small-molecule compounds inhibit the MERTK pathway although they were not originally developed for this purpose[190]. Myeloid cell functions are also regulated at the epigenetic level; as an example, drugs that interfere with chromatin remodelling, such as bromodomain and extra-terminal motif proteins[191,192], or histone deacetylase (HDAC) inhibitors[193,194], can affect inflammatory macrophage phenotypes. Other approaches to the modulation of macrophage functions include local low-dose ionizing radiation[146] and CD40 targeting, which can trigger macrophages to kill tumour cells[195]. Whether these treatments repolarize individual TAMs or modulate the tumour microenvironment by reducing and expanding distinct TAM subtypes remains unclear, yet it seems that TAM 'reprogramming' without massive TAM depletion can effectively control cancer progression in at least some settings.

Strategies to amplify the ability of DCs to stimulate effective antitumour T cell responses have long been considered[14]. GM-CSF is typically used to amplify DCs *in vitro* and can promote antitumour immunity *in vivo*[196]. For example, an FDA-approved vaccine, sipuleucel-T, uses *in vitro* GM-CSF-expanded patient DCs primed with prostate antigens to stimulate antitumour T cells[197]. However, GM-CSF may have pleiotropic effects on myeloid cells: it can expand T cell-activating DCs but also bone marrow-derived TAM progenitors and other immunosuppressive myeloid cells[124]. Defining when GM-CSF benefits or harms the host requires study, although evidence indicates that the microenvironment in which GM-CSF is produced can dictate the function of this cytokine[198].

Other approaches consist of modifying DCs to boost protective antitumour immunity and prevent tumour-induced exhaustion. Recent evidence indicates that cytosolic DNA sensing by stimulator of interferon genes (STING) induces type I IFN production and enables DCs to activate antitumour $CD8^+$ T cell responses[199]. STING agonists can potently activate this pathway in mice[200,201]. Encapsulating STING agonists into nanoparticles further amplifies DC-mediated antitumour immunity[202] and STINGVAX, a vaccine that uses GM-CSF-producing cells in combination with STING agonists, can regress mouse tumours that are otherwise poorly immunogenic[203]. The small-molecule compound DMXAA (also known as vadimezan), which was originally developed as a tumour-vascular disrupting agent, targets STING in mice[204]. Clinical trial results were disappointing but may be explained by the finding that human STING has an amino acid substitution that makes it insensitive to DMXAA[204]. New investigations involving human-specific STING agonists are under way.

Intriguingly, the absence of STING also improves tumour control and $CD8^+$ T cell activity in a mouse model of lung cancer[205].

Other strategies to activate DCs in tumours include upregulating the co-stimulatory molecules CD80, CD86 and CD40 (REF. 206), the T cell-stimulating cytokine IL-12 (REFS 56,207) or the immunostimulatory microRNA miR-155 (REF. 208); or suppressing the transcription factor STAT3 (REF. 209), the stress response factor XBP1 (REF. 53) or the β-catenin pathway[54]. Implanting physical scaffolds that incorporate a combination of immunoregulatory components into tumour-bearing subjects could be useful to optimize tumour-infiltrating DC activation *in situ*[210–212].

Perspectives

Although knowledge of phagocyte biology in cancer has exploded in recent years, we still have a limited understanding of how the various myeloid cell subtypes function *in vivo* during tumour progression and how drugs alter the activity of these cells. Functional imaging of intact microenvironments in mice[213] should help to address these questions and could be achieved by combining new mouse models and reporters, single-cell *in vivo* imaging technology and new computational analysis[32,214–216]. *In vivo* imaging is important because of its ability to define both temporally and spatially how different cells interact with their environment, respond to drugs and mediate immunosuppressive or tumoricidal actions[213]. Understanding these processes is crucial to defining how therapies fail or work within complex tissue environments.

Additionally, in the future we must uncover which myeloid cells are crucial to human disease, how cancer therapeutic agents modulate human myeloid cells and how these perturbations regulate treatment efficacy. We propose two major areas of emphasis to address these questions and obtain knowledge that can be harnessed to improve current treatment options (FIG. 5).

First, we need to decipher the complex repertoires of tumour-infiltrating myeloid cells in patients. Our increasing ability to analyse phagocyte content in tumours should help to uncover whether specific signatures can be used to reveal diagnostic and prognostic information, tailor treatments, monitor responses to therapy and/or predict drug resistance in individual patients. Human phagocyte studies remain limited by the scarcity of biopsy material, without which it is difficult to identify and manipulate tumour-infiltrating cell populations. However, techniques such as novel single-cell RNAseq approaches (for example, DropSeq)[217,218], single-cell mass cytometry (CyTOF)[219], protein mass spectrometry[220] and high-throughput protein analysis from fine-needle aspirates[221] permit the extraction of substantially more information from limited tissue samples, which should help to discriminate between tumour-promoting and tumour-suppressing cells and, in turn, improve patient stratification and survival prediction. Additionally, myeloid cells could be mapped noninvasively in patients. Given their naturally high endocytosis activity, macrophages efficiently accumulate nanomaterials, which can then be detected by commonly used clinical imaging technologies, such as magnetic resonance imaging (MRI) and positron emission tomography (PET)[222]. Imaging of peripheral TAMs could also be used to delineate tumour margins noninvasively and serve as an aid for planning surgery[223].

Second, we should learn whether clinical interventions that manipulate myeloid cells have therapeutic benefit. Clinicians have already launched clinical trials with promising therapeutics that target TAMs or tumour-infiltrating DCs[25,224]. These investigations are relevant not only because we urgently need new treatment options against many cancers, but also because they consider crucial cellular components in the tumour microenvironment that often remain overlooked. Given that most cancer therapies affect myeloid cells, it is important to evaluate whether myeloid cell-targeting agents mitigate the limitations of other treatments. These studies should test different regimens, such as simultaneous versus sequential drug administration, because these variables can affect the outcome of combinatorial treatments[225]. Manipulating myeloid cell responses could have varied positive

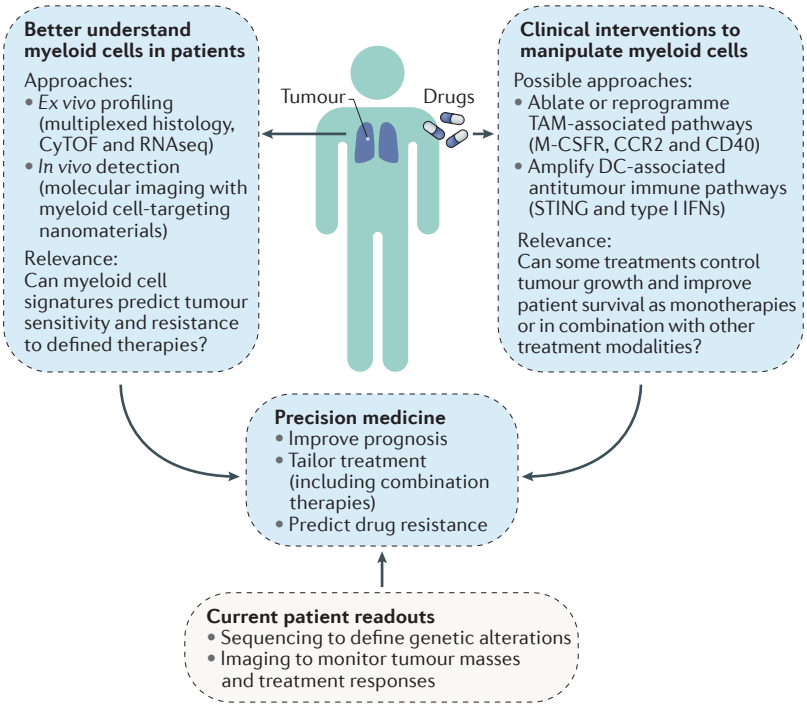

Figure 5 | **Towards a more comprehensive understanding of human tumours and relevant therapeutic targets.** Major areas of emphasis for research on myeloid cells in cancer include expanding our knowledge of the complex repertoires of these cells in cancer patients (top left panel) and uncovering whether interventions that affect these cells have therapeutic benefit (top right panel). Each box indicates possible approaches to address these issues, and their relevance. This knowledge, combined with information available from current patient readouts, should help clinicians to optimize precision medicine approaches (bottom blue panel). CCR2, chemokine (C-C motif) receptor 2; CyTOF, single-cell mass cytometry; DC, dendritic cell; IFNs, interferons; M-CSFR, macrophage colony-stimulating factor receptor; RNAseq, RNA sequencing; STING, stimulator of interferon genes; TAM, tumour-associated macrophage.

consequences: they may suppress some cancers that resist current treatments and/or sensitize tumours to other drugs or augment their therapeutic efficacy. For instance, current therapies with T cell immune checkpoint blockers can be highly successful but benefit only a small subset of patients. Myeloid cell-targeting agents could be used to increase the number of patients who respond to treatment and consequently trigger more pronounced antitumour effects in these patients.

The types of investigation mentioned above, whether their hypotheses are demonstrated or refuted, will help to define next-generation cancer treatments. The results may guide precision medicine approaches by revealing tumour microenvironmental components that vary drastically between tumours, direct tumour growth and influence drug responses. For example, tumours rich in TAMs or subsets thereof may be better controlled with therapies that incorporate phagocyte-targeting agents, whereas tumours that are poorly infiltrated by DCs may be suppressed more efficiently using drugs that foster DC recruitment to tumours and promote long-lasting antitumour T cell immunity. Overall, uncovering the intricate mechanisms that govern myeloid cells in human cancer is an ongoing challenge; nonetheless, the wealth of mouse data and the growing knowledge from human cancer studies suggest a central role for myeloid cells that warrants their consideration in future diagnostic and therapeutic approaches.

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Acknowledgements
The authors thank members of the Pittet laboratory and of the Massachusetts General Hospital (MGH) Center for Systems Biology for critical discussions and acknowledge all contributors to the field whose work we could not cite owing to space limitations. This work was supported in part by the Samana Cay MGH Research Scholar Fund, National Institutes of Health (NIH) grants P50-CA86355, R21 CA190344 and R01-AI084880 (to M.J.P.), the Boehringer Ingelheim Fonds (to C.E.) and the Deutsche Forschungsgemeinschaft (DFG) PF809/1-1 (to C.P.).

Competing interests statement
The authors declare no competing interests.