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PERSPECTIVE

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The expanding landscape of inflammatory cells affecting cancer therapy

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Tumour-infiltrating myeloid cells (TIMCs) are critical regulators of cancer growth. The different phenotypes, functions and therapeutic effects of these phagocytes have, however, been difficult to study. With the advent of single-cell-based technologies, a new 'worldview' is emerging: the classification of TIMCs into subtypes that are conserved across patients and across species. As the landscape of TIMCs is beginning to be understood, it opens up questions about the function of each TIMC subtype and its drugability. In this Perspective, we outline the current map of TIMC populations in cancer and their known and presumed functions, and discuss their therapeutic implications and the biological research questions that they give rise to. The answers should be particularly relevant for bioengineers, materials scientists and the chemical and pharmaceutical communities developing the next generation of cancer therapies.

The makeup of most cancers involves immune cells and other inflammatory host cells. Tumour-infiltrating myeloid cells (TIMCs) are of particular interest because they are abundant in the stroma of a broad range of tumours, and because at least some TIMCs modulate key cancer-associated activities[1]. TIMCs also modulate the efficacy of virtually all types of cancer drugs, including nanomaterials and biologicals. Consequently, patients who do not respond to current treatment options, including immune-checkpoint-blockade therapies, may benefit from orthogonal approaches targeting TIMCs.

There are various subtypes of myeloid cells, operationally divided into mononuclear cells (macrophages, monocytes and dendritic cells) and polymorphonuclear cells (neutrophils, mast cells, basophils and eosinophils). This division can further diversify into a spectrum of activation states in response to exogenous stimuli. For a long time, it has remained unclear whether the lineage of each TIMC should be considered a broad continuum of states or rather a set of separate and targetable subsets of states. In fact, until recently, a comprehensive understanding of TIMCs has been lacking, in part because the approaches used to define them relied on a limited set of panels of markers that did not cover the full spectrum of TIMC states.

With the advent of single-cell-resolution methods and new bioinformatic tools, it has become possible to comprehensively map TIMCs in patient samples and to arrange these cells into defined populations without a priori knowledge of the markers that define them. The ability to perform these studies in any organism also permits unbiased comparisons of TIMCs across species including mice, which remain widely used preclinical models. Identifying discrete subsets of TIMCs opens up opportunities for defining therapeutic targets, for developing new pharmaceuticals and for testing them in both preclinical and clinical settings.

In this Perspective, we first outline the current map and known functions of populations of TIMCs, using lung cancer as a vantage point, and discuss unanswered research questions as a roadmap for future studies. We also consider emerging therapeutic implications, covering new therapeutic approaches being developed by bioengineering, materials science, and the chemical and pharmaceutical communities.

Known and presumed functions of myeloid cells in cancer

Single-cell-resolution methods are redefining the understanding of immunity in various human cancer types, particularly lung adenocarcinoma[2–4], melanoma[5,6], head-and-neck cancer[7], renal-cell carcinoma[8], breast cancer[9,10] and glioma[11,12]. At present, human TIMCs are perhaps best described in non-small-cell lung cancer. Single-cell RNA sequencing (scRNA-seq) and mass cytometry by time-of-flight (CyTOF) have revealed the presence and identity of defined populations of macrophages, monocytes, dendritic cells, neutrophils and mast cells in these tumours[2,4] (Table 1 and Fig. 1). The map of TIMC subtypes may be expanded as more tumour types are being analysed and functionally explored. In what follows, we discuss important TIMC subtypes and their known and presumed functions.

Macrophages. Macrophages are usually the most abundant immune population in tumour stroma (Box 1 and Fig. 1a). In non-small-cell lung cancer, spectral clustering of scRNA-seq data now allows for the division of macrophages into ten populations, namely $M\phi_{1–9}$ and the cycling macrophages $M\phi_{cycl}$ (Fig. 1b,c and Table 1). These population subsets show distinct transcriptional states; for example, $M\phi_{cycl}$ express genes involved in cell proliferation and are reproducibly found across individuals[2]. Compared with the other myeloid cell types discussed in this section, macrophages appear to be more heterogeneous, which might be explained in part by the diversity and plasticity of these cells[13,14]. Yet it remains unclear whether $M\phi_{cycl}$ is a specific macrophage subclass that is permanently undergoing cell proliferation within tumours, or a mixture of any of the other defined populations that happened to be cycling when the tumour was captured (in which case $M\phi_{cycl}$ could be defined as a transitory state).

When considering the genes that are enriched within each $M\phi_{1–9}$ subset (with respect to all other immune and non-immune cells within the tumour stroma), it becomes possible to interrogate bulk-mRNA-expression databases that include thousands of patients and to assess whether associations exist between patient survival and the abundance of defined macrophage subsets within tumours. Several methods exist to mine bulk RNA data from scRNA-seq methods[2,15–17]. These correlative analyses may help to discriminate between myeloid cells that promote or suppress cancer growth as

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Table 1 | Overview of TIMC classes in human lung cancer

Type	Subtype	Example genes	Association with clinical outcome	Comments
Macrophages	$M\phi_1$	*CXCL5, NT5E, IL1B*	Bad	M0-like
	$M\phi_2$	*FN1*	–	M0-like and M2-like
	$M\phi_3$	*APOE, CTSD*	–	M2-like
	$M\phi_4$	*MMP7, TIMP3*	Bad	M0-like
	$M\phi_5$	*CHIT1, CTSK*	Neutral	M0-like and M2-like
	$M\phi_6$	*CCL18, CD209*	Neutral	M2-like
	$M\phi_7$	*MARCO, PPARG*	Neutral	M0-like and M2-like
	$M\phi_8$	*CXCL12*	Bad	M2-like
	$M\phi_9$	*CXCL9, CXCL10*	Bad	M2-like (and M1-like)
	$M\phi_{Cycl}$	*MKI67, TOP2A*	–	M0-like and M2-like
Monocytes	$Mono_1$	*CD14, FCN1*	Bad	Similar to $CD14^+$ 'classical' monocytes
	$Mono_2$	*ITGAL, LILRB2*	–	Similar to $CD14^{int}$ and $CD16^+$ 'non-classical' monocytes
	$Mono_3$	*S100A8, IL1B*	Bad	Express neutrophil-associated genes (MonoN)
	$Mono_4$	*CCL17, CD74*	–	Express DC-associated genes (MonoDC)
Dendritic cells	DC_1	*CLEC9A, XCR1*	Good	Cross-present antigens to $CD8^+$ T cells (cDC1)
	DC_2	*CD1A, TNFSF12*	Good	Coordinate $CD4^+$ T-cell responses (cDC2)
	DC_3	*CD40, FSCN1*	Good	Can sense IFN-γ and produce IL-12
	pDC	*GZMB, TCF4*	Good	pDC
Neutrophils	N_1	*ARG1, PADI4*	Neutral	Present in healthy tissue in mice
	N_2	*IFIT1, IFIT2*	–	Rare subtype, also found in blood
	N_3	*CXCR1, CXCR2, IL1B*	Neutral	Likely an intermediate between N1 and N5
	N_4	*SIGLEC10, IL1B*	–	Likely an intermediate between N1 and N5
	N_5	*CCL3, PI3, IL1B*	Bad	Similar to pro-tumour Siglec-F^{hi} neutrophils in mice
Mast cells	$Mast_1$	*CLU*	Good	Mouse equivalent not yet identified
	$Mast_2$	*CMA1*	Neutral	Rare subtype

The information in this table has been extracted from ref. [2].

well as those that remain uninvolved (Table 1). In non-small-cell lung cancer, the presence of the macrophage subsets $M\phi_1$, $M\phi_8$ and $M\phi_9$ is strongly associated with poor patient survival, suggesting that these cells promote tumour outgrowth and are important immunotherapy targets. By contrast, the presence of $M\phi_5$, $M\phi_6$ and $M\phi_7$ correlates with neither better nor worse patient survival, positing these cells as possible bystanders in the tumour microenvironment. To date, other macrophage subsets have not been assessed.

Although macrophages are often catalogued as classically activated cells (M1 type) or alternatively activated cells (M2 type; Box 1), categorizing tumour-associated macrophages (TAMs) as M1 or M2 has limitations (Fig. 1d and Table 1). For example, in non-small-cell lung cancer, many macrophage subsets simultaneously express an uncommitted M0 signature and an M2 signature; $M\phi_1$ are predominantly M0-like; $M\phi_3$, $M\phi_6$ and $M\phi_8$ are predominantly M2-like; and none of them are M1-like, perhaps with the exception of $M\phi_9$ macrophages, which are predominantly M2-like. Such a detailed analysis indicates that M2-like macrophages can be found in growing lung tumours and that M2 is not a distinct macrophage state. In breast cancers and endometrial cancers, bulk-RNA-sequencing studies revealed distinct gene-expression profiles for TAMs than for resident macrophages from homeostatic tissues (in particular, the genes *SIGLEC1* and *CD163*); yet the TAMs did not show a preferential enrichment for M2-associated genes[18]. Moreover, single-cell-resolution studies showed that breast-cancer-associated macrophages can simultaneously express M1-related and M2-related genes[10], further supporting the notion that macrophage activation within tumours does not follow a simple M1–M2 polarization model.

Mechanistic studies are warranted to further assess the functional relevance of TAM subsets and to clarify which ones promote or suppress tumours in various cancer types. For example, $CD163^+$ TAMs in experimental mouse models preferentially promote tumour growth when compared with their $CD163^-$ counterparts, and selectively removing $CD163^+$ TAMs promotes cancer regression, anti-tumour T-cell immunity and sensitivity to immunotherapy[19]. The $M\phi_3$, $M\phi_6$ and $M\phi_8$ subsets in human lung cancer express *CD163* at higher levels[2], making these subsets candidate therapeutic targets.

Monocytes. Monocytes are often operationally divided into so-called classical and non-classical cells (Box 1). Studies using scRNA-seq have revealed four distinct populations of monocyte-like cells in human tumours ($Mono_{1–4}$; Fig. 1b,c and Table 1). $Mono_1$ and $Mono_2$ correspond to classical and non-classical monocyte subsets[2], respectively, whereas $Mono_3$ and $Mono_4$ remain less understood. $Mono_3$ (or MonoN) and $Mono_4$ (or MonoDC) express neutrophil-associated and dendritic-cell-associated genes, respectively. All four subsets are reproducibly found across patient tumour samples and have also been detected in peripheral blood[2,20].

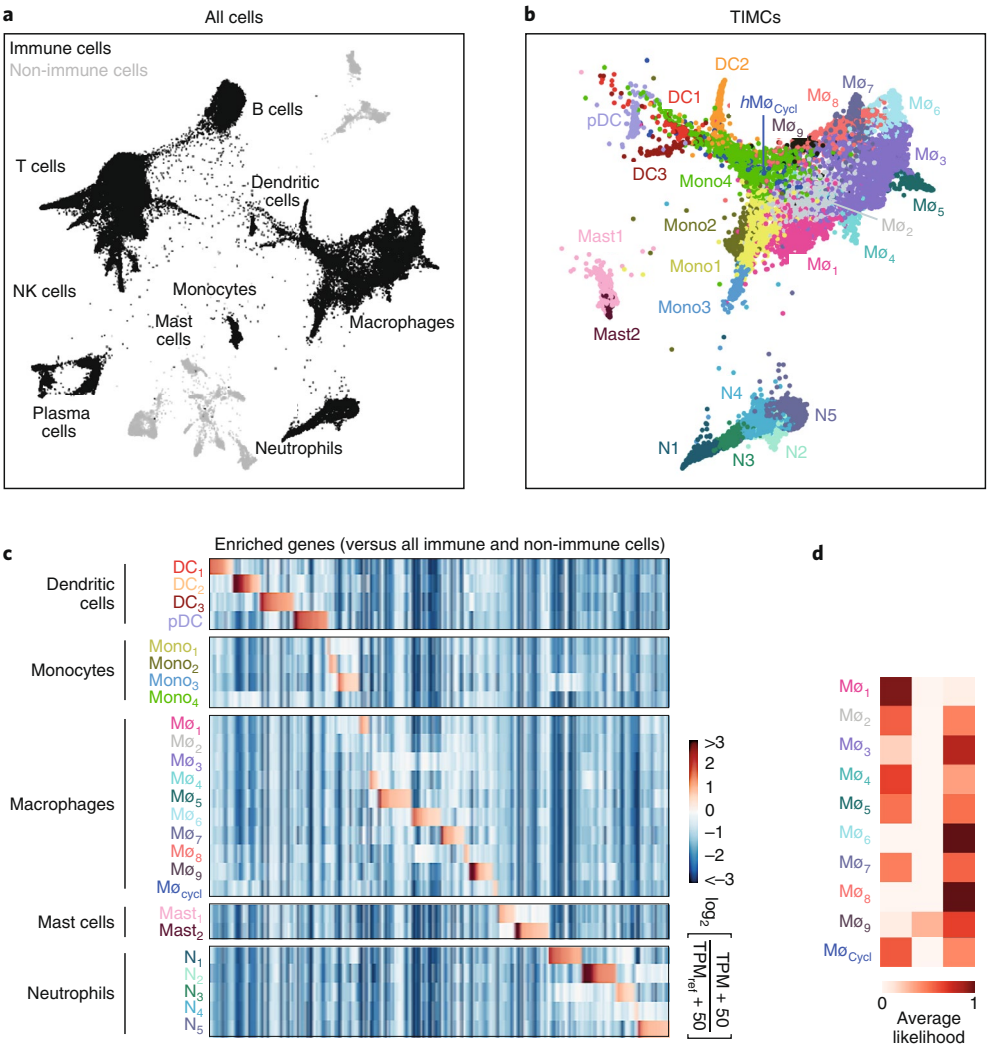

Fig. 1 | Tumour-infiltrating myeloid cell types. a, Two-dimensional visualization of immune and non-immune single-cell transcriptomes in lung tumours from patients. The data are shown using SPRING, a pipeline for data filtering, normalization and visualization using force-directed layouts[109]. Each dot represents a single cell. **b**, SPRING plots of TIMC subsets from the same patients. **c**, Identification of expression-enriched genes in each TIMC subset, as compared with all others in the microenvironment of the human lung tumour. NK cells, natural killer cells; TPM, gene transcripts per million; TPM_{ref}, second-highest expression value per gene transcripts per million (see ref. [2] for details). **d**, Classification of macrophage subsets by M0-like, M1-like and M2-like gene signatures. Fig. 1 adapted with permission from ref. [2], Elsevier.

Determining the precise tumour-related functions of these monocyte subsets requires more research. The presence of $Mono_2$ in lung tumours correlates with neither better nor worse survival; however, the presence of $Mono_1$ and $Mono_3$ is associated with poorer clinical outcomes (Table 1; $Mono_4$ could not be assessed). Also, bulk-RNA-sequencing studies showed altered gene-expression profiles for peripheral blood monocytes obtained from patients with breast cancer or endometrial cancer when compared to monocytes from healthy controls, which indicates that human solid tumours can amplify discrete monocyte states in the periphery[18]. This suggests that there are additional opportunities to study monocyte populations that presumably exhibit tumour-promoting functions. Interestingly, some experimental conditions trigger the accumulation of inflammatory monocyte-like cells that carry antitumour functions[19], which indicates that triggering tailored (inflammatory) monocyte responses may be therapeutically desirable. Considering that dendritic cells can exhibit antitumour activity, it is also relevant to study the functions of $Mono_4$ and their possible role in promoting antitumour immune responses.

Dendritic cells. Although dendritic cells within tumours are typically rare, they can orchestrate antitumour functions (Box 1). In human lung tumours, scRNA-seq has revealed the presence of three conventional dendritic cell subsets, termed $DC_{1–3}$, in addition to plasmacytoid dendritic cells (pDC; Fig. 1b,c and Table 1). These cell populations were found in nearly all patients studied[2]. DC_1 and DC_2 express gene signatures that map well to those of conventional DC1 (cDC1) and cDC2. DC_3 do not express the canonical cDC1 markers XCR1 and CLEC9A, yet they do express the cDC1 factors basic leucine zipper ATF-like transcription factor 3 (BATF3) and interferon-regulatory factor 8 (IRF8). DC_3 present additional features that are relevant to antitumour immunity, including the production of the cytokine interleukin-12 (IL-12), which promotes the activation of tumour-infiltrating $CD8^+$ T cells and is necessary for effective immune checkpoint blockade in cancer mouse models[21]. DC_3 also upregulate the C–C chemokine receptor type 7, which can guide dendritic cell migration to T-cell areas in draining lymph nodes[22]; yet in the context of successful immunotherapy, at least some tumour-infiltrating DC_3 progressively lose their motility

Box 1 | Myeloid cell types

Macrophages. Macrophages localize within all organs of the body, are phenotypically and functionally diverse[14,99], and can have different origins[13,100]. Within tumours, these cells are frequently referred to as TAMs. In patients, TAMs are often defined as CD68$^+$, CD163$^+$ or both, and studies using these markers have revealed a strong association between the high densities of these cells and poor patient prognosis in numerous cancer types (with some exceptions)[1]. Conversely, experimental studies have described many tumour-promoting functions of TAMs, including stimulating tumour-cell proliferation, enhancing tumour vascularization, increasing tumour-cell invasion and suppressing anti-tumour immune cells. Some TAMs may also display anti-tumour functions, including directly killing tumour cells and activating other anti-tumour immune cells[1]. Macrophages have long been catalogued as M1-activated or M2-activated cells[101], with M1-like cells carrying anti-tumour functions and M2-like cells supporting tumour-promoting functions; however, single-cell-based studies indicate that macrophage activation within tumours does not follow a simple M1–M2 polarization model.

Monocytes. Monocytes are circulating cells that are often operationally divided into the so-called classical subsets (CD14$^+$ in humans and Ly6C^{hi} in mice) and non-classical subsets (CD14^{int} and CD16$^+$ in humans, and Ly6C^{lo} in mice)[102]. Non-classical monocytes patrol the vasculature and remove damaged endothelial cells. Although they typically remain low in number within tumours, non-classical monocytes may suppress tumour metastasis by removing cancer cells in the tumour vasculature and by recruiting antitumour immune cells locally[103]. By contrast, classical monocytes can be amplified under inflammatory conditions and might extravasate into tumours, where they may differentiate into macrophages and other cell types. Many inflammatory monocyte-derived cells within tumours are thought to promote cancer outgrowth[1]. Single-cell-based studies have revealed four distinct monocyte populations, some of which remain poorly understood.

Dendritic cells. Dendritic cells and related lineages can be partitioned into pDCs, cDCs and monocyte-derived dendritic cells. cDCs include at least two populations, named cDC_1 and cDC_2 (ref. [22]). cDC_1s are often defined with the cell-surface markers XCR1 and CLEC9A and are best known to cross-present antigens to CD8$^+$ T cells[23]. cDC_2s are often identified with the markers CD1A and CD172A; they do not cross-present antigens and are therefore inefficient activators of CD8$^+$ T cells but can drive antitumour CD4$^+$ T-cell responses[26]. Monocyte-derived dendritic cells can have an array of functions but may be difficult to distinguish from cDCs by surface-marker expression[22,92]. Single-cell-based studies have revealed three distinct populations of conventional dendritic cells in addition to pDCs.

Neutrophils. Neutrophils have been identified as the most significant prognostic populations across many cancer types[104], particularly because the number of neutrophils increases as tumours progress[4]. In animal models, neutrophils enhance tumour-promoting events, including cancer-cell proliferation, angiogenesis and immunosuppression. Neutrophils can also foster tumour progression by awakening dormant cancer cells, escorting and protecting circulating tumour cells, and driving cancer metastasis[105–107]. However, neutrophils can also take on opposing functions[33] and have eliminated tumour cells in some experimental settings. They can also promote CD8$^+$ T-cell activation and antagonize metastasis[76,108]. Clearly, a better understanding of neutrophil diversity is needed. Single-cell-based studies have revealed at least five subsets of neutrophils.

locally[21], suggesting that these cells can acquire aberrant trafficking behaviour and persist within tumours, where they mediate antitumour functions.

Analysing human cancer biopsies supports the potential for dendritic cells to benefit the host. As with T-cell infiltration metrics, having more dendritic cells within tumours correlates with better overall patient survival and improved responses to immune checkpoint blockade in various cancer types[23–25]. The presence of DC_1-associated markers has been associated with positive prognoses in various cancer types, including breast cancer, lung cancer and melanoma[2,24,25]. DC_2 (refs. [2,26]), DC_3 (ref. [2]) and pDC (ref. [2]) remain less studied, but their presence within tumours has also been connected to improved clinical outcomes (Table 1). These data suggest that tumour-infiltrating dendritic cells, although typically rare, include distinct subsets with non-overlapping functions; also, each subset may promote antitumour immunity locally and, at least in some settings, could be harnessed for immunotherapy.

Neutrophils. Neutrophils are increasingly understood to be a particularly important myeloid population in the tumour microenvironment (Box 1). As these cells show lower transcript counts than other immune cells, they are occasionally and inadvertently excluded when employing the data filters commonly used in scRNA-seq studies. This limitation can be circumvented by setting low filtering thresholds to allow neutrophil detection. It also appears important to evaluate neutrophils (as well as other myeloid cell types) directly from freshly obtained samples, and to avoid cryopreservation strategies. By using this approach, studies using single-cell technologies have started to reveal neutrophil heterogeneity within tumours. In human lung tumours, neutrophils form a continuum of states that, via spectral clustering, resolve into five subsets, namely $N_{1–5}$ (Fig. 1b,c and Table 1). N_1 and N_5 define edges in an N_1-to-N_3-to-N_4-to-N_5 continuum, with N_1 expressing high levels of the canonical neutrophil markers S100A8, S100A9 and ADAM8, and N_5 expressing the cytokines chemokine (C–C motif) ligand 3 (CCL3) and colony stimulating factor 1 (CSF1), which are associated with tumour progression[27,28]. N_2, which are typically rare, express type-I-interferon response genes, including *IFIT1* and *IFIT2*.

The relative abundance of each neutrophil state varies across patients. The presence of N_1 and N_3 correlates with neither better nor worse patient survival, yet the presence of N_5 strongly correlates with poorer survival[2], suggesting that these cells promote tumour progression and may be relevant immunotherapy targets (Table 1; N_4 could not be assessed, and the rarity of N_2 makes them difficult to study in correlative studies). Accordingly, mouse studies have determined that neutrophils defined by Siglec–F^{high} expression include N_5 and exhibit more potent tumour-promoting functions than their Siglec–F^{low} counterparts[2,29].

The analysis of a randomized trial of the role of the inhibition of IL-1β, involving more than 10,000 patients with atherosclerosis and followed up for several years (the canakinumab anti-inflammatory thrombosis-outcomes study), identified substantially decreased lung cancer incidence and mortality in the group that received anti-IL-1β therapy[30]. Interestingly, in lung tumours, *IL1B* is strongly upregulated in some myeloid cell types, including neutrophils—most notably N_5—as well as $M\phi_1$ and $Mono_3$. As the presence within tumours of any of these populations is strongly associated with poor patient survival[2], it is possible that IL-1β production by these TIMC populations directly contributes to tumour outgrowth.

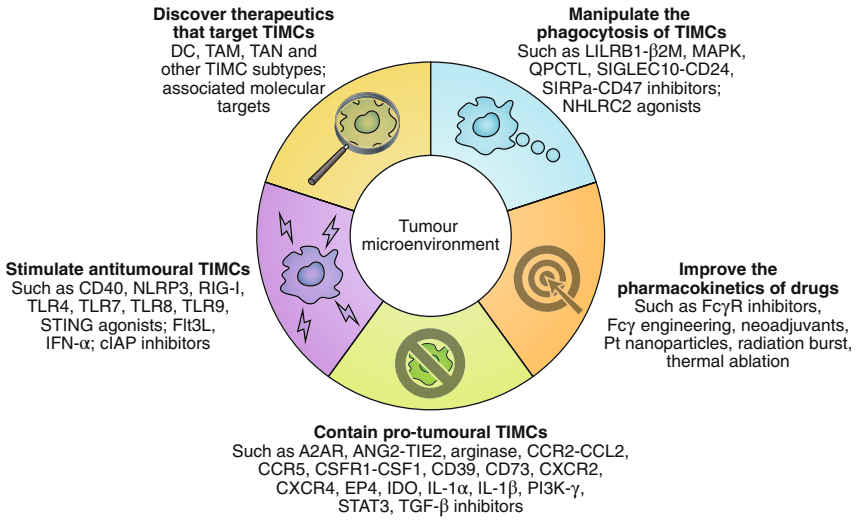

Fig. 2 | Five therapeutic opportunities relevant to TIMC populations. Greater understanding of the TIMC landscape can help discover new therapeutic targets (yellow), pharmacologically manipulate phagocytosis (blue), improve the delivery of drugs (orange), ablate or prevent the recruitment of pro-tumoural TIMC subsets (green) and unleash antitumoural TIMC responses (purple).

Other classifications and cell types. In addition to the discussed immune populations, other TIMC subsets may be relevant targets for cancer treatment. For instance, among polymorphonuclear cells, mast cells and basophils substantially decrease in abundance when tumours arise[4]. Also, mast cells comprise two populations, namely $Mast_1$ and $Mast_2$, and the presence of $Mast_1$ in lung adenocarcinoma is strongly associated with better clinical outcomes[2]. It is possible that amplifying this mast-cell population could provide clinical benefit.

The term myeloid-derived suppressor cells (MDSCs) is frequently used to define a heterogeneous population of immature myeloid cells that suppress T-cell responses, promote cancer outgrowth and express CD11b and Gr1 in mice[31,32]. However, these markers indiscriminately label different subsets, including at least some neutrophils and monocytes[33]. This has limitations, in part because not all $CD11b^+$ $Gr1^+$ cells are immunosuppressive—other cells, such as TAMs, can also be immunosuppressive but are excluded when using the MDSC terminology—and because aside from immunosuppression, this group of cells can display other functions that should not be overlooked as they could also be relevant to cancer and its treatment. Overall, it appears more useful to identify myeloid cells as neutrophils, monocytes and other well-defined immune types—rather than as mixtures of cell populations—and to consider all tumour-associated functions for these cells.

Emerging therapeutic targets

The growing understanding of the landscape of TIMCs has important implications for developing new therapeutic approaches, for defining new and more specific drug targets and for understanding resistance mechanisms. Several review articles have focused on pharmacological parameters[34–37] and on screening nanomaterials[38]. Here we touch on five salient points relevant to the therapeutic targeting of TIMCs (Fig. 2).

Discovery of new therapeutic targets for TIMCs. To overcome limitations in the current treatment options for cancer, the targeting of the immune system beyond T cells should exploit the diversity of non-redundant immune components. The uncovering of the full repertoire of TIMC states and their functions is just beginning; accordingly, current treatments might affect both pro-tumoural and antitumoural populations in ways that are not yet understood. As many TIMC states exhibit distinct gene-expression profiles, the mining of scRNA-seq datasets can identify new cellular targets that selectively present pro-tumoural (or antitumoural) functions, and could thus be antagonized (or agonized) in a therapeutic setting. For example, a population of tumour-promoting neutrophils[29] and a population of antitumour dendritic cells[21] emerged as targets, but specific druggable proteins of these subsets have yet to be discovered. The search for new therapeutic targets may also benefit from considering genes or myeloid cell populations that are present within the stroma of growing tumours yet absent from healthy tissues. These and other efforts could have major implications for the design of new immunotherapies that are more efficient and less toxic than currently available treatments.

Manipulation of the phagocytosis of TIMCs. Phagocytosis plays a critical role in the surveillance of tumours, including the clearing of apoptotic cells, the elimination of cellular debris, the expelling of extravasated red blood cells and the removal of cancer-associated bacteria. Phagocytes use different surface receptors and signalling pathways to ingest nanoparticles and debris. Much of the understanding of the molecular basis of phagocytosis derives from genetic screens in model organisms. Genome-wide CRISPR screens investigating phagocytosis mechanisms for different magnetic nanoparticles in the macrophage-differentiated human myeloid cell line U937 revealed that the NHL repeat-containing protein 2 is a central player in phagocytosis, as well as the need for very long-chain fatty acids for the efficient phagocytosis of certain substrates[39]. Another study showed that a multi-receptor-tyrosine-kinase (MAPK)-inhibitor nanoformulation efficiently accumulated in TAMs to block bidirectional resistance pathways and thus that mitogen-activated protein kinase inhibitors affect TAM function[40]. Ongoing research suggests that there is a largely unexplored opportunity to pharmacologically manipulate the phagocytosis of TIMC subtypes.

Cancer cells can overexpress specific anti-phagocytic surface proteins, which prevent clearance by TIMCs, most notably macrophages. These proteins, often referred to as 'don't eat me' signals, include the membrane integrin CD47 (ref. [41]), beta-2 microglobulin of the major histocompatibility class I complex[42] and the sialoglycoprotein CD24 (ref. [43]). Interaction of these ligands with their receptors on TIMCs (namely SIRPα for CD47, LILRB1 for beta-2 microglobulin and SIGLEC10 for CD24) can inhibit phagocytosis and are thought to contribute to cancer-cell evasion from the

immune system. Drugs that antagonize the interaction of 'don't eat me' signals with their receptors may thus be used to raise tumouricidal TIMC functions. The clinical evaluation of a CD47-antibody checkpoint inhibitor showed promising activity in patients with non-Hodgkin's lymphoma when used in combination with rituximab[44]. Also, inhibitors of glutaminyl-peptide cyclotransferase-like protein, a modifier of the CD47–SIRPα checkpoint[45], are in development and could be used to enhance the immune blockade of antitumour TIMC responses.

Improvement of drug pharmacokinetics. Considering that TIMCs can substantially affect cancer drug pharmacokinetics, it is important to better understand this influence and harness it for therapy. In what follows, we discuss these unresolved questions: can one capitalize on TIMCs to improve drug accumulation within tumours? Can some TIMCs be further exploited as drug reservoirs? Conversely, can TIMCs interfere with the delivery of antibody drugs to their intended targets, and can this process be prevented to augment the efficacy of treatment?

The augmented delivery of drugs to tumours should improve therapeutic efficacy and could be achieved by harnessing TIMCs. For example, clinically approved chemotherapeutic-loaded therapeutic nanoparticles can be more effectively delivered to tumours when more TIMCs are present. Intravital imaging evidence has revealed that radiation therapy induces macrophages to accumulate near the tumour vasculature, where the cells elicit bursts of extravasation and enhance the uptake of a drug in neighbouring tumour cells[46]. Interestingly, TIMC numbers may significantly rise after therapy initiation, leading to an apparent increase in tumour size: a process often referred to as 'pseudoprogression'. Similar outcomes can be seen with cycles of neoadjuvant therapy or by mechanical means (in particular, radiofrequency or cryoablation). Overall, these findings suggest that manipulating TIMCs may facilitate the delivery of drugs to tumours, and that this is achievable therapeutically.

Aside from their ability to enhance the delivery of drugs to tumours, TIMCs may also serve as drug depots. For instance, phagocytic TIMCs can efficiently accumulate a number of therapeutic nanoparticles, such as Pt-poly(ethylene glycol)–poly(lactic acid-co-glycolic acid)[47]. Drug accumulation in macrophages is sometimes more pronounced than in tumour cells, even when nanomaterials are targeted to the latter with antibodies. Interestingly, however, TIMC-accumulated toxins are released in a gradient and can thus kill surrounding cancer cells over time[47]. When TIMCs are ablated (by zolendronate, for instance), nanotherapeutics are generally less effective[47]; conversely, the efficacy of therapeutic nanoparticles typically increases when more TIMCs are present. Moreover, delivering therapeutic nanoparticles to TIMCs could, in principle, affect the biological role that TAMs play in the intravasation of cancer cells into vasculature[48,49]. Irrespective of the method used to promote drug accumulation and release within tumours, it is clear that selectively harnessing TIMC subtypes with antitumour functions could prevent undesired effects mediated by other TIMC populations.

Intravital imaging evidence has also shown that TIMCs can withhold anti-PD-1 monoclonal antibodies from their intended targets. Anti-PD-1 antibodies are designed to block the PD-1 receptor on the surface of $CD8^+$ T cells, enabling these cells to attack tumours; however, by simultaneously tracking macrophages, $CD8^+$ T cells and anti-PD-1 drugs in real time in mice, it was found that anti-PD-1 antibodies can be transferred from T cells to neighbouring macrophages within minutes after drug administration[50]. This process involves fragment crystallizable (Fc) receptors (FcγR), which are expressed on the surface of macrophages and other TIMC subsets and bind the antibody drug's Fc domain[50]. Blocking Fc–FcγR interactions prolongs the binding of anti-PD-1 antibodies to tumour-infiltrating $CD8^+$ T cells and enhances immunotherapy-induced tumour regression in mice. FcγR binding also affects the activity of other monoclonal antibodies. Therefore, a better understanding of the role of the various Fc receptors expressed by TIMC, and the leveraging of drug-action mechanisms, should facilitate the engineering of better therapeutics.

Containment of pro-tumoural TIMCs. Therapeutic approaches aimed at eliminating tumour-promoting TIMCs involve blocking their recruitment to tumours, ablating them and 're-educating' them to eliminate pro-tumour functions. Although clinical trials are underway, most of the knowledge currently derives from animal studies.

Because many myeloid cells within tumours originate from circulating precursors, controlling the numbers of TIMCs may be achieved by antagonizing molecules such as CSF1–CSF1R or CCL2–CCR2, which promote the production of TIMC precursors in haematopoietic organs or the recruitment of these cells to tumours[1,51]. For example, targeting CSF1R can reduce TIMC numbers, which improves T-cell responses and limits tumour progression in some animal models. However, such approaches are globally more effective when combined with orthogonal strategies, such as T-cell-targeting immunotherapies, radiotherapies or chemotherapies[1]. In the clinic, monotherapy with CSF1R antibodies has shown therapeutic efficacy against tenosynovial giant cell tumours[52], which are characterized by the overexpression of CSF1. Ongoing clinical trials should help evaluate the efficacy of various inhibitors of the above-mentioned pathways across cancer types and in combination with several other cancer drugs. Additional molecular pathways control neutrophil and monocyte production, and involve molecules such as the growth factor G-CSF[53] and the peptide hormone angiotensin II[54]. Targeting these molecules or associated pathways decreases the numbers of TIMCs and suppresses tumour outgrowth in animal models. Encouraging clinical data also indicate that adding an angiotensin II receptor blocker to cytotoxic therapy may benefit patients with locally advanced pancreatic cancer[55]. However, these approaches may have limitations. For instance, therapeutic agents that target macrophages indiscriminately could affect healthy tissues in which myeloid cells are involved with controlling normal tissue function and unselectively ablate TIMCs with opposing functions, including those that contribute to cancer control. An improved understanding of the heterogeneity of TIMCs should help define new therapeutic agents and strategies for the ablation of pro-tumoural TIMCs more selectively.

Considering that most TIMCs in growing tumours are immunosuppressive and pro-tumourigenic[56], an additional therapeutic strategy is to 're-educate' these cells so that they lose their tumour-enhancing functions and, ideally, gain immunosupportive and antitumourigenic activity. One promising approach is to stimulate toll-like receptors (TLRs) and cytosolic nucleic acid-sensing pathways[57–60]. Activating these pathways triggers the secretion of pro-inflammatory and antiviral cytokines. Most activators of these pathways are large and complex compounds—such as CpG DNA, lipopeptides, cyclic dinucleotides and double-stranded RNA, as well as their synthetic mimetics such as polyinosinic:polycytidylic acid—that require nanoformulations and/or specialized delivery systems. An exception are imidazoquinolines, which are small-molecule TLR agonists. For example, resiquimod, a TLR7/8 agonist, can be delivered to TIMCs in cyclodextrin nanoparticles and thus be used for immunotherapy[56]. Other small-molecule agonists of TLR and of the stimulator of interferon genes (STING) transmembrane protein are also emerging and are being tested clinically. Additional strategies for macrophage activation or 're-education' have also been explored[34]; for example, CSF1R may not only ablate macrophages or prevent their recruitment to tumours, but also alter their polarization[61,62]. Therapeutic agents aimed at 're-educating' tumour-promoting TIMCs may also activate antitumour TIMCs.

Unleashing antitumoural TIMCs. Considering that dendritic cells are obligate partners of T cells, the design of current and next-generation immunotherapies aiming to foster antitumour T-cell immunity should closely examine any effects of drugs on these cells. Characterizing dendritic cell populations and pathways of specific agonism could yield promising new therapeutic targets. Although recent efforts suggest that amplifying DC_1 within tumours promotes tumour control[23,24,63,64], increasing DC_2, DC_3 and pDC responses may also be rewarding therapeutically[2,21,26]. For example, DC_3 can sensitize tumours to immune checkpoint blockade via IL-12 production[21], and several therapeutic strategies may enhance this response, including the administration of cytokines intratumourally[65] or the provision of drugs that signal through the non-canonical nuclear factor κB pathway, which is upregulated in DC_3 (ref. [21]). Such drugs include agonistic antibodies specific to family members of the tumour necrosis factor receptor (for example, CD40, OX40 or LTBR)[66–69] and small molecules blocking cellular inhibitors of apoptosis (cIAP)[70]. These drugs can have unfavourable pharmacokinetics and show off-target toxicities when administered systemically, but these limitations may be circumvented when considering TIMCs. For example, myeloid-targeted nanoformulations incorporating a cIAP inhibitor preferentially induce IL-12 production by TIMCs in vivo and, as a monotherapy, slow down tumour growth without triggering significant systemic toxicity[71].

Additionally, the ability of dendritic cells to foster antitumour immunity indicates that tumours can develop evasion mechanisms, which could also be targeted therapeutically. For instance, some tumours keep away BATF3-dependent dendritic cells (likely both DC_1 and DC_3) either by suppressing the production of chemokines (such as CCL4) that attract dendritic cells[63] or by secreting inflammatory mediators (such as prostaglandin E2) that block dendritic-cell accumulation within tumours[72]. Both processes prevent T-cell recruitment into tumours and promote resistance to immunotherapy. Besides excluding dendritic cells, tumours can render them locally dysfunctional. For example, tumour-induced activation of the endoplasmic reticulum stress factor X-box binding protein 1 in intratumoural dendritic cells blunts antitumour immunity[73]. The immunosuppressive cytokine IL-10, which can be produced by TAMs, also suppresses the antitumour functions of dendritic cells[24]. Efficiently manipulating these different pathways in therapy should promote antitumour immunity.

Beyond dendritic cells, other TIMC populations may oppose tumour progression and can be therapeutically stimulated. For example, it is possible that antitumour macrophages can be expanded or further activated to eliminate tumour cells[74,75]. Some neutrophils may also participate in tumour-cell destruction[76,77] or promote various forms of antitumour T-cell immunity[78,79]. Since macrophages and neutrophils are globally associated with enhancing tumour outgrowth, it will be important to define more precisely which myeloid cell states functionally oppose tumour progression and thus could be harnessed for therapy.

Research questions

The increasingly understood and consequently expanding landscape of immune cells in cancers has given rise to a number of important biological questions.

TIMC subtypes are preserved across patients and species. TIMC mapping at the single cell level in human lung cancer has revealed that the same tumour myeloid populations are found across patients[2]. Tumour cells, by contrast, vary considerably across patients, indicating that the myeloid microenvironment, at least within lung tumours, is much more stereotyped than the tumour tissue that it infiltrates. Consequently, drugs that target defined myeloid subsets could have broad applicability in cancer immunotherapy.

Direct comparison of TIMCs in human and mouse lung cancers further indicates cross-species conservation. Specifically, all dendritic cells and all monocyte subsets, as well as the neutrophil subsets N_1, N_2 and N_5, are conserved, whereas macrophages show species-specific patterns[2]. This suggests that studying TIMCs in mice can help elucidate features of human cancers, and that mice are relevant experimental models for testing drugs that target TIMC subsets that are conserved across the two species.

Identifying the TIMC landscape in other cancer types requires further work. Interestingly, some tumour-infiltrating immune cell types may have unique conserved phenotypes regardless of the tumour type in which they reside. For instance, although tumour-infiltrating regulatory T cells—which can also promote cancer growth—express transcripts that are distinct to those of regulatory T cells in other tissues, the same regulatory T-cell phenotypes are reproducibly seen across tumour types in mice and humans[80].

The biological relevance of newly discovered cell states. Current scRNA-seq studies mostly focus on generating cell atlases that identify and enumerate different cell states present in clinical and preclinical samples. It is important that this information is used to generate models for assessing the functional relevance of newly defined cell states.

Identifying surface markers for immune populations of interest could help define how to purify these cells, which could then be further assessed phenotypically and functionally ex vivo or in vitro. Mining scRNA-seq datasets could help determine whether a population of interest preferentially transcribes genes encoding cell-surface proteins and thus which monoclonal antibodies could be used to enrich these cells from tumour tissues (notwithstanding the important caveat that mRNA expression does not always predict protein presence). Additionally, spatial transcriptomics and single-molecule tracking[81–83] should be useful to reveal the location of different cell populations and their interactions. Obtaining this information should further increase the understanding of the complexity and functional relevance of TIMCs in the context of the tumour architecture.

Immune cell states that are conserved across species could also be evaluated functionally in animal models. This offers the possibility of using a variety of approaches, including cell-fate-mapping tools[84], gene editing tools and intravital imaging setups[85], for assessing fundamental aspects of these cells (particularly their origin, their biological functions, and where and how they traffic) and to reveal if and how they respond to therapeutic interventions and other manipulations in vivo.

Additionally, identifying newly discovered myeloid-cell states could serve diagnostic purposes. For instance, a liquid-biopsy approach assessing a tumour-driven peripheral-blood-monocyte-derived transcriptomic signature[18] could be used to predict the presence of some cancers (at least those that foster a systemic monocyte response).

Whether subtypes of TIMCs are evolutionary intermediates or defined end populations is unclear. The ontogenic relationships between TIMC subsets remain incompletely understood. Many TAMs originate from circulating monocytes, which are themselves produced by bone-marrow-derived haematopoietic stem cells[84,86]. Experimental studies indicate the existence of unidirectional transitions from circulating monocytes that have arrived at the tumour site to sessile perivascular macrophages that promote tumour-cell intravasation[48]. Also, some TAMs originate from embryonic development independently from haematopoietic stem cells and monocytes[87–90]. These cells that reside in tissue may be relevant therapeutic targets, especially in the lung and liver, where they are particularly abundant. Having more information about the origins of macrophages, and making distinctions between resident and recruited

cell types as well as across lineage relationships and fates, will help annotate the Mø subsets identified by scRNA-seq and further reveal how overall macrophage responses could be manipulated.

Defining the origins of tumour-infiltrating dendritic cells also requires further work. For example, DC_1 cells, which provide antitumour functions and depend on BATF3 for their development, likely derive from cDC precursors but may also be produced by circulating monocytes[91,92]. Understanding intratumoural DC_1 generation may help define therapeutic approaches that amplify antitumour dendritic cell responses. The precise mechanisms that create anti-tumour dendritic cell diversity also need to be elucidated. Conceivably, DC_1 and DC_3 may derive from the same cDC1 progenitor considering that subsets both foster $CD8^+$ T-cell antitumour immunity and express the cDC1 transcription factors BATF3 and IRF8. Defining the cues that control DC_1 versus DC_3 trajectories might be relevant in therapy.

Because neutrophils form a continuum of states, it is possible that each subset is linked ontogenically to its immediate 'neighbour', with N_1 (which is a healthy tissue state in mice) progressing continuously to N_3, N_4 and N_5 (which are tumour-specific states in mice) in growing tumours. These considerations should help define new molecular and cellular targets that can be harnessed to limit tumour-promoting neutrophil responses in therapy.

By measuring the ratio of the spliced (mature) and unspliced (immature) versions of gene transcripts in scRNA-seq datasets[93], it is possible to infer whether the expression of each gene is increasing or decreasing. This method can predict cellular dynamics and trajectories, for example, from early progenitors to differentiated cells all collected and assayed at the same time. This method, among others, of inferring RNA velocities in single cells should facilitate the understanding of TIMC-lineage relationships in growing tumours and in their response to therapy in both humans and mice. Also, animal models can be used to directly map the fate of adoptively transferred TIMC progenitors in vivo[84] (provided that the transferred cells behave similarly to their endogenous counterparts).

Ways in which TIMC subsets can be identified more rapidly without using scRNA-seq. scRNA-seq is an extraordinarily powerful tool used to dissect the genetic heterogeneity of immune cells. However, the method is expensive, is not immune to errors and can be time consuming. Also, today's commonly used scRNA-seq approaches do not provide geographical information of gene expression. Therefore, there is a need to define and validate additional cell surface markers for all TIMC subtypes so that tissues can be more readily assessed by flow cytometry or immunohistochemistry. The use of validated markers is important, as some existing markers can be suboptimal and even inaccurate in defining TIMC populations[2]. To process samples more rapidly and more frequently, particularly in clinical contexts, new methods of cellular analyses are needed. One emerging technique relies on cyclic immunophenotyping of fine-needle aspirates, as blood analysis poorly reflects the immune cell composition of tumours[2]. The use of fine-needle aspirates obviates the need for core biopsies of primary tumours and relies instead on fine needles with much lower periprocedural complication rates and greater patient acceptance[94]. Moreover, it is well known that TIMCs shed extracellular vesicles into circulation and that sensitive and specific methods might be able to identify these rare vesicles in peripheral blood.

The phagocytic capacities of TIMC subtypes. Most research examining the toxicity and clearance of nanomaterials has focused on their organ distribution rather than their cellular distribution and differences in the rates of phagocytosis of different cells. Advances in intravital imaging[85] and careful flow-cytometry techniques (that avoid the loss of cell populations) are enabling more detailed studies. Two reports have shown that certain types of polymeric carbohydrate nanomaterials are preferentially internalized by TIMCs rather than by macrophages residing in tumour-free tissues[56,95]. However, considerable work remains to be done to define the cellular distribution of different nanomaterials and biologicals, particularly across all subtypes of TIMCs. This effort will lay the groundwork for increasingly selective phagocyte targeting. Efforts are also needed towards the complete understanding of the mechanisms of phagocytosis[39].

Outlook

Given the extraordinary power of studying TIMCs via single-cell-resolution methods—such as scRNA-seq[96–98], intravital imaging[85] and spatial transcriptomics[81,82]—and computational tools[83], we expect that the understanding of human and murine myeloid cell subtypes will improve rapidly over the next few years. Having a 'parts list' of cell types is the first step, and annotating them with functional information will be the next task. Given the central role of these cells in many diseases, new TIMC-directed therapeutics will invariably emerge. Beyond the discussed research questions, there are also important clinical questions: what new drug targets can be identified, and will they synergize with existing treatments? What kinds of studies are required to test and predict the human efficacy of new materials and biological drugs? What clinical readouts are available to quantitate the efficacy of these new drugs? We hope that the questions and discussion in this Perspective will inspire immediate research opportunities. Although the Perspective focuses on cancer, most of the knowledge discussed can be applied to other diseases involving myeloid cells, including autoimmune diseases, wound healing and cardiovascular conditions such as those derived from myocardial infarction, stroke and atherosclerosis.

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References

1. Engblom, C., Pfirschke, C. & Pittet, M. J. The role of myeloid cells in cancer therapies. *Nat. Rev. Cancer* **16**, 447–462 (2016).
2. Zilionis, R. et al. Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species. *Immunity* **50**, 1317–1334 (2019).
3. Lambrechts, D. et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nat. Med.* **24**, 1277–1289 (2018).
4. Lavin, Y. et al. Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses. *Cell* **169**, 750–765 (2017).
5. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **175**, 998–1013 (2018).
6. Tirosh, I. et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **352**, 189–196 (2016).
7. Puram, S. V. et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell* **171**, 1611–1624 (2017).
8. Chevrier, S. et al. An immune atlas of clear cell renal cell carcinoma. *Cell* **169**, 736–749 (2017).
9. Chung, W. et al. Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. *Nat. Commun.* **8**, 15081 (2017).
10. Azizi, E. et al. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. *Cell* **174**, 1293–1308 (2018).
11. Müller, S. et al. Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis for regional differences in macrophage activation in the tumor microenvironment. *Genome Biol.* **18**, 234 (2017).
12. Venteicher, A. S. et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. *Science* **355**, eaai8478 (2017).
13. Ginhoux, F., Schultze, J. L., Murray, P. J., Ochando, J. & Biswas, S. K. New insights into the multidimensional concept of macrophage ontogeny, activation and function. *Nat. Immunol.* **17**, 34–40 (2016).
14. Gordon, S. & Plüddemann, A. The mononuclear phagocytic system. Generation of diversity. *Front. Immunol.* **10**, 1893 (2019).
15. Baron, M. et al. A single-cell transcriptomic map of the human and mouse pancreas reveals inter- and intra-cell population structure. *Cell Syst.* **3**, 346–360 (2016).

16. Tsoucas, D. et al. Accurate estimation of cell-type composition from gene expression data. *Nat. Commun.* **10**, 2975 (2019).
17. Wang, X., Park, J., Susztak, K., Zhang, N. R. & Li, M. Bulk tissue cell type deconvolution with multi-subject single-cell expression reference. *Nat. Commun.* **10**, 380 (2019).
18. Cassetta, L. et al. Human tumor-associated macrophage and monocyte transcriptional landscapes reveal cancer-specific reprogramming, biomarkers, and therapeutic targets. *Cancer Cell* **35**, 588–602 (2019).
19. Etzerodt, A. et al. Specific targeting of CD163^{+} TAMs mobilizes inflammatory monocytes and promotes T cell-mediated tumor regression. *J. Exp. Med.* **216**, 2394–2411 (2019).
20. Villani, A. C. et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* **356**, eaah4573 (2017).
21. Garris, C. S. et al. Successful anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-γ and IL-12. *Immunity* **49**, 1148–1161 (2018).
22. Eisenbarth, S. C. Dendritic cell subsets in T cell programming: location dictates function. *Nat. Rev. Immunol.* **19**, 89–103 (2019).
23. Broz, M. L. et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. *Cancer Cell* **26**, 638–652 (2014).
24. Ruffell, B. et al. Macrophage IL-10 blocks CD8^{+} T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell* **26**, 623–637 (2014).
25. Barry, K. C. et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. *Nat. Med.* **24**, 1178–1191 (2018).
26. Binnewies, M. et al. Unleashing type-2 dendritic cells to drive protective antitumor CD4^{+} T cell immunity. *Cell* **177**, 556–571 (2019).
27. Lin, E. Y., Nguyen, A. V., Russell, R. G. & Pollard, J. W. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* **193**, 727–740 (2001).
28. Wu, Y., Li, Y. Y., Matsushima, K., Baba, T. & Mukaida, N. CCL3-CCR5 axis regulates intratumoral accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis process. *J. Immunol.* **181**, 6384–6393 (2008).
29. Engblom, C. et al. Osteoblasts remotely supply lung tumors with cancer-promoting SiglecF^{high} neutrophils. *Science* **358**, eaal5081 (2017).
30. Ridker, P. M. et al. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. *Lancet* **390**, 1833–1842 (2017).
31. Gabrilovich, D. I., Ostrand-Rosenberg, S. & Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253–268 (2012).
32. Fleming, V. et al. Targeting myeloid-derived suppressor cells to bypass tumor-induced immunosuppression. *Front. Immunol.* **9**, 398 (2018).
33. Coffelt, S. B., Wellenstein, M. D. & de Visser, K. E. Neutrophils in cancer: neutral no more. *Nat. Rev. Cancer* **16**, 431–446 (2016).
34. Ovais, M., Guo, M. & Chen, C. Tailoring nanomaterials for targeting tumor-associated macrophages. *Adv. Mater.* **31**, e1808303 (2019).
35. Miller, M. A. & Weissleder, R. Imaging of anticancer drug action in single cells. *Nat. Rev. Cancer* **17**, 399–414 (2017).
36. Miller, M. A. & Weissleder, R. Imaging the pharmacology of nanomaterials by intravital microscopy: toward understanding their biological behavior. *Adv. Drug Deliv. Rev.* **113**, 61–86 (2017).
37. Ng, T. S. C., Garlin, M. A., Weissleder, R. & Miller, M. A. Improving nanotherapy delivery and action through image-guided systems pharmacology. *Theranostics* **10**, 968–997 (2020).
38. Rodell, C. B., Koch, P. D. & Weissleder, R. Screening for new macrophage therapeutics. *Theranostics* **9**, 7714–7729 (2019).
39. Haney, M. S. et al. Identification of phagocytosis regulators using magnetic genome-wide CRISPR screens. *Nat. Genet* **50**, 1716–1727 (2018).
40. Wang, S. J. et al. Efficient blockade of locally reciprocated tumor-macrophage signaling using a TAM-avid nanotherapy. *Sci. Adv.* (in the press).
41. Willingham, S. B. et al. The CD47-signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. *Proc Natl Acad. Sci. USA* **109**, 6662–6667 (2012).
42. Barkal, A. A. et al. Engagement of MHC class I by the inhibitory receptor LILRB1 suppresses macrophages and is a target of cancer immunotherapy. *Nat. Immunol.* **19**, 76–84 (2018).
43. Barkal, A. A. et al. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature* **572**, 392–396 (2019).
44. Advani, R. et al. CD47 Blockade by Hu5F9-G4 and rituximab in non-Hodgkin's lymphoma. *N. Engl. J. Med.* **379**, 1711–1721 (2018).
45. Logtenberg, M. E. W. et al. Glutaminyl cyclase is an enzymatic modifier of the CD47- SIRPα axis and a target for cancer immunotherapy. *Nat. Med.* **25**, 612–619 (2019).
46. Miller, M. A. et al. Radiation therapy primes tumors for nanotherapeutic delivery via macrophage-mediated vascular bursts. *Sci. Transl. Med.* **9**, eaal0225 (2017).
47. Miller, M. A. et al. Tumour-associated macrophages act as a slow-release reservoir of nano-therapeutic Pt(IV) pro-drug. *Nat. Commun.* **6**, 8692 (2015).
48. Arwert, E. N. et al. A unidirectional transition from migratory to perivascular macrophage is required for tumor cell intravasation. *Cell Rep.* **23**, 1239–1248 (2018).
49. Linde, N. et al. Macrophages orchestrate breast cancer early dissemination and metastasis. *Nat. Commun.* **9**, 21 (2018).
50. Arlauckas, S. P. et al. In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. *Sci. Transl. Med.* **9**, eaal3604 (2017).
51. DeNardo, D. G. & Ruffell, B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat. Rev. Immunol.* **19**, 369–382 (2019).
52. Cassier, P. A. et al. CSF1R inhibition with emactuzumab in locally advanced diffuse-type tenosynovial giant cell tumours of the soft tissue: a dose-escalation and dose-expansion phase 1 study. *Lancet Oncol.* **16**, 949–956 (2015).
53. Spiegel, A. et al. Neutrophils suppress intraluminal NK cell-mediated tumor cell clearance and enhance extravasation of disseminated carcinoma cells. *Cancer Discov.* **6**, 630–649 (2016).
54. Cortez-Retamozo, V. et al. Angiotensin II drives the production of tumor-promoting macrophages. *Immunity* **38**, 296–308 (2013).
55. Murphy, J. E. et al. Total neoadjuvant therapy with FOLFIRINOX in combination with losartan followed by chemoradiotherapy for locally advanced pancreatic cancer: a phase 2 clinical trial. *JAMA Oncol.* **5**, 1020–1027 (2019).
56. Rodell, C. B. et al. TLR7/8-agonist-loaded nanoparticles promote the polarization of tumour-associated macrophages to enhance cancer immunotherapy. *Nat. Biomed. Eng.* **2**, 578–588 (2018).
57. Downey, C. M., Aghaei, M., Schwendener, R. A. & Jirik, F. R. DMXAA causes tumor site-specific vascular disruption in murine non-small cell lung cancer, and like the endogenous non-canonical cyclic dinucleotide STING agonist, 2'3'-cGAMP, induces M2 macrophage repolarization. *PLoS ONE* **9**, e99988 (2014).
58. Kather, J. N. & Halama, N. Harnessing the innate immune system and local immunological microenvironment to treat colorectal cancer. *Br. J. Cancer* **120**, 871–882 (2019).
59. Kawai, T. & Akira, S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* **34**, 637–650 (2011).
60. King, K. R. et al. IRF3 and type I interferons fuel a fatal response to myocardial infarction. *Nat. Med.* **23**, 1481–1487 (2017).
61. DeNardo, D. G. et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov.* **1**, 54–67 (2011).
62. Pyonteck, S. M. et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nat. Med.* **19**, 1264–1272 (2013).
63. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
64. Salmon, H. et al. Expansion and activation of CD103^{+} dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity* **44**, 924–938 (2016).
65. Momin, N. et al. Anchoring of intratumorally administered cytokines to collagen safely potentiates systemic cancer immunotherapy. *Sci. Transl. Med.* **11**, eaaw2614 (2019).
66. Jahan, N., Talat, H. & Curry, W. T. Agonist OX40 immunotherapy improves survival in glioma-bearing mice and is complementary with vaccination with irradiated GM-CSF-expressing tumor cells. *Neuro Oncol.* **20**, 44–54 (2018).
67. Ma, H. S. et al. A CD40 agonist and PD-1 antagonist antibody reprogram the microenvironment of nonimmunogenic tumors to allow T-cell-mediated anticancer activity. *Cancer Immunol. Res.* **7**, 428–442 (2019).
68. Sun, S. C. The non-canonical NF-κB pathway in immunity and inflammation. *Nat. Rev. Immunol.* **17**, 545–558 (2017).
69. Vonderheide, R. H. The immune revolution: a case for priming, not checkpoint. *Cancer Cell* **33**, 563–569 (2018).
70. Dougan, S. K. & Dougan, M. Regulation of innate and adaptive antitumor immunity by IAP antagonists. *Immunotherapy* **10**, 787–796 (2018).
71. Koch, P. D., Rodell, C. B., Kohler, R. H., Pittet, M. & Weissleder, R. Myeloid cell-targeted nanocarriers efficiently inhibit cellular inhibitor of apoptosis for cancer immunotherapy. *Cell Chem. Biol.* **16**, 95–104 (2020).
72. Zelenay, S. et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. *Cell* **162**, 1257–1270 (2015).
73. Cubillos-Ruiz, J. R. et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. *Cell* **161**, 1527–1538 (2015).

74. Nathan, C. F., Silverstein, S. C., Brukner, L. H. & Cohn, Z. A. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. *J. Exp. Med.* **149**, 100–113 (1979).
75. Urban, J. L., Shepard, H. M., Rothstein, J. L., Sugarman, B. J. & Schreiber, H. Tumor necrosis factor: a potent effector molecule for tumor cell killing by activated macrophages. *Proc. Natl Acad. Sci. USA* **83**, 5233–5237 (1986).
76. Fridlender, Z. G. et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell* **16**, 183–194 (2009).
77. Matlung, H. L. et al. Neutrophils kill antibody-opsonized cancer cells by trogoptosis. *Cell Rep.* **23**, 3946–3959e6 (2018).
78. Singhal, S. et al. Origin and role of a subset of tumor-associated neutrophils with antigen-presenting cell features in early-stage human lung cancer. *Cancer Cell* **30**, 120–135 (2016).
79. Ponzetta, A. et al. Neutrophils driving unconventional T cells mediate resistance against murine sarcomas and selected human tumors. *Cell* **178**, 346–360 (2019).
80. Magnuson, A. M. et al. Identification and validation of a tumor-infiltrating Treg transcriptional signature conserved across species and tumor types. *Proc Natl Acad. Sci. USA* **115**, E10672–E10681 (2018).
81. Ståhl, P. L. et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* **353**, 78–82 (2016).
82. Vickovic, S. et al. High-definition spatial transcriptomics for in situ tissue profiling. *Nat. Methods* **16**, 987–990 (2019).
83. Finotello, F., Rieder, D., Hackl, H. & Trajanoski, Z. Next-generation computational tools for interrogating cancer immunity. *Nat. Rev. Genet.* **20**, 724–746 (2019).
84. Cortez-Retamozo, V. et al. Origins of tumor-associated macrophages and neutrophils. *Proc. Natl Acad. Sci. USA* **109**, 2491–2496 (2012).
85. Pittet, M. J., Garris, C. S., Arlauckas, S. P. & Weissleder, R. Recording the wild lives of immune cells. *Sci. Immunol.* **3**, eaaq0491 (2018).
86. Movahedi, K. et al. Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Res.* **70**, 5728–5739 (2010).
87. Schulz, C. et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. *Science* **336**, 86–90 (2012).
88. Zhu, Y. et al. Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression. *Immunity* **47**, 323–338 (2017).
89. Loyher, P. L. et al. Macrophages of distinct origins contribute to tumor development in the lung. *J. Exp. Med.* **215**, 2536–2553 (2018).
90. Laviron, M. & Boissonnas, A. Ontogeny of tumor-associated macrophages. *Front. Immunol.* **10**, 1799 (2019).
91. Sharma, M. D. et al. Activation of p53 in immature myeloid precursor cells controls differentiation into Ly6c^{+}CD103^{+} monocytic antigen-presenting cells in tumors. *Immunity* **48**, 91–106 (2018).
92. Marigo, I. et al. T cell cancer therapy requires CD40-CD40L activation of tumor necrosis factor and inducible nitric-oxide-synthase-producing dendritic cells. *Cancer Cell* **30**, 377–390 (2016).
93. La Manno, G. et al. RNA velocity of single cells. *Nature* **560**, 494–498 (2018).
94. Giedt, R. J. et al. Single-cell barcode analysis provides a rapid readout of cellular signaling pathways in clinical specimens. *Nat. Commun.* **9**, 4550 (2018).
95. Kim, H. Y. et al. Quantitative imaging of tumor-associated macrophages and their response to therapy using ^{64}Cu-labeled macrin. *ACS Nano* **12**, 12015–12029 (2018).
96. Stuart, T. & Satija, R. Integrative single-cell analysis. *Nat. Rev. Genet.* **20**, 257–272 (2019).
97. Stubbington, M. J. T., Rozenblatt-Rosen, O., Regev, A. & Teichmann, S. A. Single-cell transcriptomics to explore the immune system in health and disease. *Science* **358**, 58–63 (2017).
98. Heath, J. R., Ribas, A. & Mischel, P. S. Single-cell analysis tools for drug discovery and development. *Nat. Rev. Drug Discov.* **15**, 204–216 (2016).
99. Sica, A. & Mantovani, A. Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* **122**, 787–795 (2012).
100. Perdiguero, E. G. & Geissmann, F. The development and maintenance of resident macrophages. *Nat. Immunol.* **17**, 2–8 (2016).
101. Mantovani, A. & Allavena, P. The interaction of anticancer therapies with tumor-associated macrophages. *J. Exp. Med.* **212**, 435–445 (2015).
102. Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **14**, 392–404 (2014).
103. Hanna, R. N. et al. Patrolling monocytes control tumor metastasis to the lung. *Science* **350**, 985–990 (2015).
104. Gentles, A. J. et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat. Med.* **21**, 938–945 (2015).
105. Albrengues, J. et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science* **361**, eaao4227 (2018).
106. Szczerba, B. M. et al. Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* **566**, 553–557 (2019).
107. Wellenstein, M. D. et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature* **572**, 538–542 (2019).
108. Granot, Z. et al. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell* **20**, 300–314 (2011).
109. Weinreb, C., Wolock, S. & Klein, A. M. SPRING: a kinetic interface for visualizing high dimensional single-cell expression data. *Bioinformatics* **34**, 1246–1248 (2018).

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Competing interests

R.W. has received consultancy payments from ModeRNA, Tarveda Pharmaceuticals, Alivio Therapeutics and Accure Health, and is a shareholder of T2Biosystems, Lumicell and Accure Health. M.J.P. has received consultancy payments from Aileron Therapeutics, AstraZeneca, Elstar Therapeutics, KSQ Therapeutics, MPM Capital, Siamab Therapeutics, Third Rock Ventures and Tidal Therapeutics. All patents associated with R.W. and M.J.P. have been assigned to, and handled by, the Massachusetts General Hospital.

Additional information

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