Tumour-associated macrophages as treatment targets in oncology

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Abstract | Macrophages are crucial drivers of tumour-promoting inflammation. Tumour-associated macrophages (TAMs) contribute to tumour progression at different levels: by promoting genetic instability, nurturing cancer stem cells, supporting metastasis, and taming protective adaptive immunity. TAMs can exert a dual, yin–yang influence on the effectiveness of cytoreductive therapies (chemotherapy and radiotherapy), either antagonizing the antitumour activity of these treatments by orchestrating a tumour-promoting, tissue-repair response or, instead, enhancing the overall antineoplastic effect. TAMs express molecular triggers of checkpoint proteins that regulate T-cell activation, and are targets of certain checkpoint-blockade immunotherapies. Other macrophage-centred approaches to anticancer therapy are under investigation, and include: inhibition of macrophage recruitment to, and/or survival in, tumours; functional re-education of TAMs to an antitumour, 'M1-like' mode; and tumour-targeting monoclonal antibodies that elicit macrophage-mediated extracellular killing, or phagocytosis and intracellular destruction of cancer cells. The evidence supporting these strategies is reviewed herein. We surmise that TAMs can provide tools to tailor the use of cytoreductive therapies and immunotherapy in a personalized medicine approach, and that TAM-focused therapeutic strategies have the potential to complement and synergize with both chemotherapy and immunotherapy.

Inflammatory cells are a key component of the ecological niche of cancer[1–4]. Indeed, an inflammatory microenvironment is now recognized to be an integral factor contributing to carcinogenesis — paradigms have shifted from a cancer-cell-centric view of carcinogenesis to one that encompasses the tumour microenvironment[3]. Therefore, suppression of effective anticancer immunity and tumour-promoting inflammation are now considered to be hallmarks of cancer. The formation of an inflammatory microenvironment within tumours can be driven by genetic events that cause cancer (aberrations involving oncogenes and tumour-suppressor genes), or by chronic nonresolving inflammatory conditions, such as inflammatory bowel disease, which increase the risk of cancer development[1]. In general, cancer-associated inflammation is characterized as being nonresolving[5].

Macrophages are a major component of the leukocyte infiltrate that is present, to a widely varying extent, in all tumours[6]. Dissection of the roles of tumour-associated macrophages (TAMs) in tumour progression has paved the way to defining the contributions of other inflammatory cells and mediators, such as inflammatory cytokines. In fact, TAMs have a dominant role as orchestrators of cancer-related inflammation (CRI). The nature of the CRI associated with tumours arising in different tissues is highly diverse[2,7]; although the cellular components of CRI differ in phenotype and quantity, and the mediators that coordinate immune function can vary considerably across cancers, TAMs regulate a common inflammatory pathway that ultimately drives CRI[8].

In the 1970s, studies demonstrated that macrophages activated by bacterial products and cytokines acquire the capacity to kill tumour cells[9–11]. On the other hand, investigators soon found that TAMs from malignant metastatic cancers promote tumour growth and metastasis[12]. Thus, early evidence suggested that macrophages could engage in a dual yin–yang relationship with cancer.

Herein, we review the current understanding of the roles of TAMs in determining the effectiveness of different anticancer treatment modalities, as well as emerging macrophage-targeting therapeutic strategies. A concise overview of the roles of macrophages in tumour initiation and progression is provided as a foundation for this discussion; however, a detailed description of CRI and, specifically, the functions of myeloid cells in tumours is beyond the scope of this article, and these aspects have been extensively reviewed elsewhere[1–3,6,13–15].

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Key points

- Tumour-associated macrophages (TAMs) are a key component of the cancer microenvironment, and influence tumour growth and progression
- TAMs can have a dual supportive and inhibitory influence on cancer, depending on the disease stage, the tissue involved, and the host microbiota
- TAMs can limit the antitumour activity of conventional chemotherapy and radiotherapy by orchestrating a tumour-promoting repair response to tissue damage, and by providing a protective niche for cancer stem cells
- Conversely, TAMs contribute to the antitumour activity of selected chemotherapeutic agents, such as doxorubicin (under certain conditions), and of monoclonal antibody therapies via antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP)
- Of note, macrophage depletion has a key role in the antitumour activity of the clinically approved anticancer agent trabectedin
- Therapeutic strategies targeting macrophages as tumour-promoting factors, and/or aimed at macrophage activation and re-education are undergoing clinical assessment; such strategies have the potential to complement cytoreductive, antiangiogenic, and immune-checkpoint-inhibitor treatments

Roles of TAMs in tumour progression

TAMs have long been hypothesized to originate from cells of the blood compartment, with chemotactic signals released from tumour cells or from nonmalignant cells present in the cancer microenvironment mediating recruitment of monocytic precursors to primary and metastatic tumour sites[11,15–19] (FIG. 1a); however, accumulating evidence calls this long-held view into question. In mice, resident macrophages in certain tissues (such as the microglia in the brain) originate from precursors that were seeded within the tissues during fetal and embryonic development, rather than from circulating monocytes[20,21] (BOX 1). In gliomas, TAMs constitute a mixed cell population that includes resident microglial cells, infiltrating blood monocytes, and macrophages[22]. The relative contribution of these populations to tumour progression has been investigated in a genetically engineered mouse model of glioma: recruitment of circulating Ly-6C^{hi} 'inflammatory' monocytes into tumour tissue was associated with an increased tumour incidence, decreased tumour latency, and shorter survival durations, with no contribution of microglial cells[22]. With regard to macrophage function in the tumour microenvironment, findings in mice indicate that the ontogenetic origin of TAM precursors does not have an appreciable effect on the macrophage phenotype that develops in response to tissue-derived cues[23]. Whether tissue macrophages derived from embryonic precursors contribute to the number, location, and diversity of TAMs remains an open question[24]. Of note, TAM proliferation has been observed in mouse models of sarcoma, and in mouse and human breast carcinomas, but this general mechanism does not seem to sustain the numbers of TAMs in growing tumours[25–27], suggesting that recruitment of circulating cells is required to maintain the TAM population. Circulating precursors that are recruited into tumour tissues and subsequently differentiate into TAMs include conventional inflammatory monocytes and monocyte-related myeloid-derived suppressor cells (M-MDSCs; BOX 1; FIG. 1a). Downregulation of the transcription factor STAT3 is a key process mediating the differentiation of M-MDSCs into mature TAMs[28]. In addition to contributing to the immunosuppressive tumour microenvironment, M-MDSCs are direct promoters of tumour metastasis[29]. Inflammatory monocytes, defined in mice as Ly6C$^+$/CCR2$^+$ cells, have been shown to contribute to TAM accumulation and maintenance in a mouse mammary tumour model[27], and to the establishment of pulmonary metastases derived from mouse or human breast cancer cells[19]. The differentiation of mouse inflammatory monocytes into TAMs is dependent on RBPJ (a transcriptional regulator of Notch signalling), and genetic deletion of this protein in TAMs reduces tumour burden in a mouse breast cancer model, indicating the nonredundant function of monocyte-derived TAMs in supporting tumour growth[27]. By contrast, a protective role of Ly6C$^-$/CX3CR1$^+$ 'nonclassical' patrolling mouse monocytes has been demonstrated. These cells, the differentiation of which is driven by the transcription factor NR4A1, patrol the lung microvasculature under steady-state conditions, but rarely extravasate into tissues and differentiate into macrophages; however, they rapidly accumulate within lung metastases, and inhibit tumour-cell seeding and growth in mouse models[30]. The antitumour functions of these nonclassical monocytes include scavenging of tumour debris, and recruitment and activation of natural killer (NK) cells[30].

Chemoattractants involved in monocyte recruitment include chemokines (such as CCL2 and CCL5), and cytokines (for example, CSF-1 and members of the VEGF family). TAMs themselves can be a source of CCL2 in cancer[1,5]. Genetic evidence from mouse models indicates that complement components, particularly C5a, are also important mediators of the recruitment and functional polarization of TAMs[31]. Indeed, such chemotactic factors act as more than attractants because they activate transcriptional programmes that contribute to the functional skewing of macrophages towards specific phenotypes[32]. CSF-1, in particular, is a monocyte attractant as well as a macrophage survival and polarization signal that drives TAM differentiation towards an immunosuppressive, tumour-promoting 'M2-like' phenotype[6,33] (BOX 1). Unlike CSF-1, GM-CSF activates macrophage functions related to antitumour activity[34].

Signals originating from tumour cells, T lymphocytes and B lymphocytes, and stromal cells influence TAM function and diversity. Classically activated 'M1' macrophages (BOX 1) can kill tumour cells via extracellular mechanisms and thereby mediate tissue-destructive reactions involving the walls of blood vessels (haemorrhagic necrosis)[9–11,15]. Accordingly, evidence indicates that, in nascent tumours, macrophages contribute to the early 'elimination' phase of immunoediting orchestrated by T cells and interferons[35]. Subsequently, tumour progression is associated with skewing and subversion of macrophage function — in line with the 'equilibrium' and 'escape' phases of cancer immunoediting. In established, progressive tumours, such as mouse and human breast or pancreatic cancers, IL-4 and IL-13 derived from type 2 T-helper (T_H2) cells[36–38], eosinophils[39], or basophils[40] elicit alternative M2 activation of TAMs (BOX 1; FIG. 1b). In addition, signals originating from tumour cells (such as chemokines, CSF-1, and TGFβ),

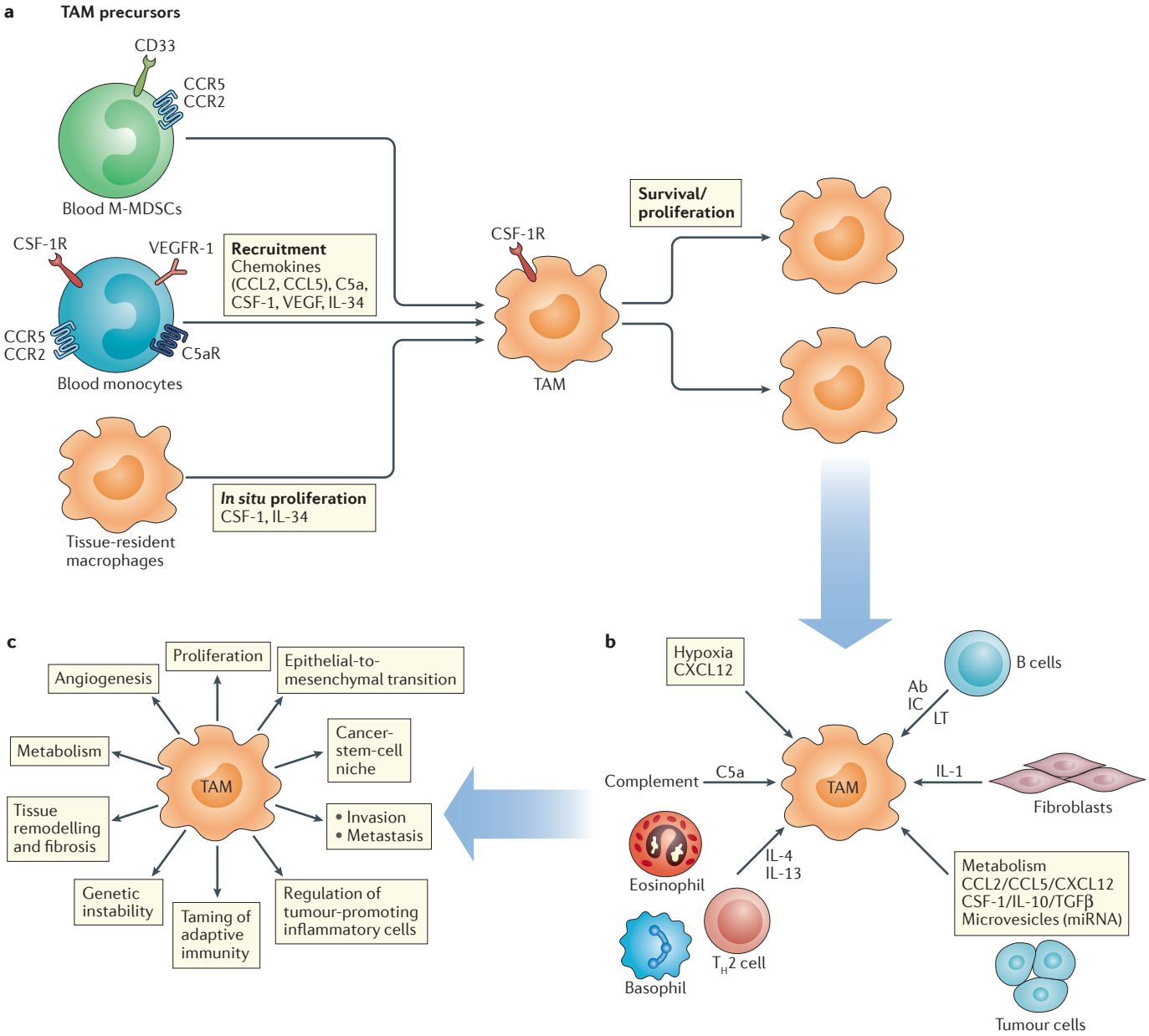

Figure 1 | **A schematic representation of the role of tumour-associated macrophages (TAMs) in tumour progression.** **a** | Monocytes and monocyte-related myeloid-derived suppressor cells (M-MDSCs) in the circulation can be recruited to tumours in response to diverse chemoattractants, including cytokines (such as CSF-1, VEGF, and IL-34), chemokines (for example, CCL2 and CCL5), and complement components (C5a). The tumour-infiltrating monocytes differentiate into TAMs. In some tumours, *in situ* proliferation can occur, and local tissue-resident macrophages of embryonic origin can contribute to the TAM population. **b** | Signals in the tumour microenvironment skew the function of TAMs. The pathways and molecules that influence TAM polarization vary between different tumours, and include: IL-4 and IL-13 derived from type 2 T-helper (T_H2) cells, eosinophils and basophils; cytokines, chemokines, metabolites, and other factors released from tumour cells; antibodies (Ab) secreted by B cells, and immune complexes (IC); stromal-cell-derived factors (such as, IL-1 and lymphotoxin (LT)). **c** | TAMs affect virtually all aspects of tumour-cell biology, including: angiogenesis; epithelial-to-mesenchymal transition; invasion and metastasis; cell proliferation; and genetic instability. Notably, TAMs also provide a protective niche for cancer stem cells. miRNA, microRNA.

B cells (immune complexes), and stromal cells (for example, IL-1) can cause phenotypic shifts in macrophages[8], imparting diverse functions that do not fit with those ascribed to classic M1/M2-polarized cell types (BOX 1). Indeed, evidence indicates that the relative importance of distinct pathways of macrophage differentiation varies between different tumours, resulting in heterogeneous TAM phenotypes and functions[2,7]. Moreover, distinct populations of TAMs within a given human or mouse tumour can also exhibit different phenotypes as a result of, for example, disparities in access to oxygen and activation of the HIF pathway[41–43] (FIG. 1b).

Box 1 | **Diversity and nomenclature of myelomonocytic cells**

Diversity and plasticity are both hallmarks of cells of the monocyte–macrophage lineage[20,21,193–195]. In general, considerable differences exist between mice and humans in terms of markers defining monocyte–macrophage diversity. In humans, circulating monocytes, which originate from bone-marrow precursors, can be classified into two main subsets based on expression of CD14 and CD16: $CD14^{+}CD16^{-}$ and $CD14^{+}CD16^{+}$. In mice, two monocyte subsets have been identified based on the expression of Ly6C, CD11b, CCR2, and CX3CR1: $CD11b^{+}Ly6C^{hi}CCR2^{hi}CX3CR1^{lo}$ 'inflammatory' monocytes, and $CD11b^{+}Ly6C^{lo}CCR2^{lo}CX3CR1^{hi}$ 'patrolling' monocytes[20].

During cancer progression, immature bone-marrow-derived cells of the myeloid lineage become detectable in the circulation. These immature myeloid cells have immunosuppressive activity, as potent suppressors of adaptive immune responses, and have been operationally defined as 'myeloid-derived suppressor cells' (MDSCs) [48,57]. MDSCs are heterogeneous, being related to either monocytes (M-MDSCs) or neutrophils (PMN-MDSCs); both subtypes express the immature myeloid-cell marker CD33, but only M-MDSC express this marker at high levels[57]. M-MDSCs can infiltrate tumours and differentiate into tumour-associated macrophages (TAMs)[57].

Tissue macrophages originate either from embryonic precursors that seed peripheral locations and self-sustain over the lifetime of the host, or from circulating monocytes[21,196]. In tissues, cells of the monocyte–macrophage lineage undergo diverse forms of functional reprogramming in response to different signals[10,11,15]. In particular, bacterial products and interferons (IFNs), produced during type 1 immune responses driven by type 1 T-helper (T_H1) cells and type 1 innate lymphoid cells (ILCs), activate the tumour-cell killing and tissue-damaging properties of these cells. Cytokines produced during type 2 immune responses driven by type 2 ILCs and type 2 T-helper (T_H2) cells, such as IL-4 and IL-13, activate an alternative form of macrophage activation oriented to resistance against parasites, and to tissue repair and remodelling. Mirroring immunological nomenclatures in current usage (such as 'T_H1' and 'T_H2'), these two alternative forms of macrophage activation are often referred to as M1 (or classic) and M2 (or alternative). The extremes and continuum between the M1 and M2 phenotypes do not, however, recapitulate the full spectrum of macrophage plasticity; indeed, similar plasticity and flexibility of ILCs and T-cell phenotypes is now recognized. Such nomenclature issues in immunology have been discussed extensively elsewhere[34,197]. In neoplastic tissues, the signals orchestrating macrophage function are diverse and vary considerably between different tumours, or even different parts of the same tumour, resulting in varied TAM phenotypes, which in many cases do not fit the M1/M2 classification. We and others use 'M1' to concisely refer to macrophage phenotypes driven by IFNγ and bacterial products, and 'M1-like' to include those polarization states leading to antitumour responses and cytotoxicity, such as that induced by GM–CSF. We use 'M2' to concisely refer to macrophage phenotypes driven by IL-4 or IL-13 (REF. 34), and 'M2-like' to encompass a gamut of diverse phenotypes that share the functional outputs of tumour promotion and suppression of effective adaptive immunity. The inherent imperfection and limited utility of these oversimplified nomenclatures have been discussed elsewhere[197].

At present, dissecting TAM diversity at the single-cell level and integrating this information remains challenging. In spite of the phenotypic plasticity of TAMs and the associated intratumour and intertumour diversity in CRI, ultimately, TAM polarization toward an immunosuppressive phenotype seems to be a common feature of most cancers. TAMs in progressing neoplasms typically express characteristic surface molecules, such as the haemoglobin scavenger receptor CD163 and macrophage mannose receptor 1 (also known as CD206)[44,45], and demonstrate properties related to stimulation of angiogenesis, suppression of adaptive immunity, and promotion of cancer growth and metastasis[6–8]. In line with a consensus recommendation[34], we limit use of the 'M2' designation to settings in which IL-4 or IL-13 are major drivers of macrophage polarization; for simplicity, we and others refer to the diverse immunosuppressive TAM populations as 'M2-like' (BOX 1).

TAMs influence the intrinsic properties of tumour cells, as well as those of the tumour microenvironment (FIG. 1c). For example, TAMs produce growth factors, such as EGF, which stimulates proliferation of breast carcinoma cells[1–5,11,17]. By producing proteolytic enzymes that digest the extracellular matrix, TAMs also pave the way to tumour-cell dissemination from the primary tumour site, thereby contributing to metastasis. Moreover, macrophages produce factors, such as IL-1, that promote the accumulation of tumour cells at distant sites, and TAMs present at metastatic sites can provide a supportive niche for metastatic cells[1,6,8]. In addition, reactive oxygen and nitrogen intermediates generated by TAMs contribute to cancer-cell genetic instability[31] — a hallmark of cancer that limits the effectiveness of chemotherapy and targeted therapies. Furthermore, TAMs promote angiogenesis and lymphoangiogenesis, as well as tissue remodelling, for example, by stimulating the deposition of fibrous tissue[1,46,47]. All of these processes can support tumour development and progression.

Myelomonocytic cells also contribute to the suppression of effective adaptive immunity — a key feature of cancer[3] — at various levels and through multiple mechanisms. MDSCs, and in particular M-MDSCs, suppress the development of antitumour immunity in lymphoid organs, as well as immune effector responses in the tumour itself[29,48]. TAMs can also promote the immunosuppressive activity of regulatory T (T_{reg}) cells through a bidirectional interaction[15] (FIG. 2). With regard to the mechanisms underlying these effects, in the tumour context, macrophages produce immunosuppressive cytokines (IL-10 and TGFβ)[6,7]. In addition, the profile of amino acid metabolism by M2 or M2-like macrophages and TAMs results in metabolic starvation of T cells owing to the activity of arginase and/or the production of immunosuppressive metabolites via the indoleamine 2,3-dioxygenase (IDO) pathway[34]. Moreover, prostaglandins produced by TAMs via arachidonic acid metabolism have immunosuppressive effects[14]. Finally, TAMs often express PD-L1 and PD-L2, which trigger the inhibitory PD-1-mediated immune checkpoint in T cells, as well as B7-H4 (REF. 49) and VISTA[50], which might have similar functions (FIG. 2).

Progress has been made in defining the molecular pathways involved in orchestration of macrophage function in tumours, including members of the STAT and NF-κB family[8]. Among these, MYC is interesting in that it mediates the tumorigenic mechanisms of both cancer cells and macrophages. Expression of the *MYC* oncogene accounts of approximately 40% of the transcriptional fingerprint of human M2-macrophage activation, and MYC is overexpressed in human TAMs[51]. In cancer cells, the oncogenic protein MYC has been shown to induce expression of the 'don't eat me' signal CD47, and the immune-checkpoint protein PD-L1 (REF. 52); thus, expression of MYC seems to enable tumour cells to suppress innate immunity in the form of macrophage-mediated phagocytosis, via CD47 expression, as well as activation of effective adaptive antitumour immunity, through induction of PD-L1 expression.

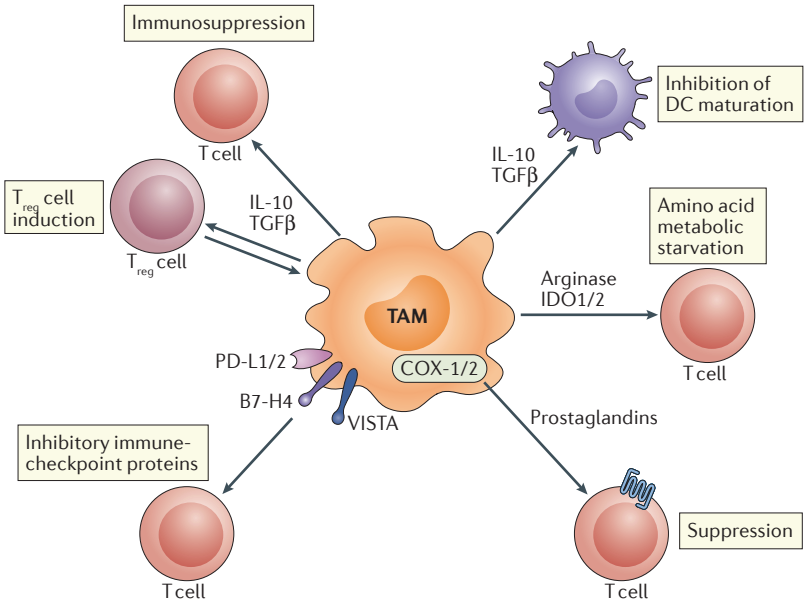

Figure 2 | **Mechanisms of tumour-associated macrophage (TAM)-mediated immune suppression.** TAMs promote the immunosuppressive activity of regulatory T (T_{reg}) cells via bidirectional interactions, which are mediated by immunosuppressive cytokines, including IL-10 and TGFβ. The same TAM-produced mediators inhibit dendritic cell (DC) maturation. The profile of amino acid metabolism in TAMs results in metabolic starvation of T cells via the activity of arginase and/or via production of immunosuppressive metabolites by the indoleamine-pyrrole 2,3-dioxygenase 1/2 (IDO1/2) pathway. Prostaglandins produced by COX-1 and COX-2 expressed in TAMs have immunosuppressive effects, particularly on T cells. TAMs can also express PD-L1 and PD-L2, which trigger the inhibitory PD-1-mediated immune checkpoint in T cells, as well as B7-H4 and VISTA, which might have similar functions. Together, these immunosuppressive activities of TAMs dampen adaptive antitumour immune responses.

The biomarker potential of TAMs

Studies investigating the prognostic significance of TAMs have relied on a variety of methodological approaches, ranging from morphological identification in early efforts[11] to gene-expression profiling[53]. The most extensively used human macrophage marker is CD68, a pan-macrophage marker; however, CD68 can occasionally be expressed in stromal cells as well as in cancer cells themselves; therefore, use of this marker to identify TAMs necessitates the careful evaluation of the data obtained[54]. In many studies, CD163 (which is associated with M2-like polarization of macrophages), CD204 (also known as macrophage scavenger receptor A), and CD206 (expression of which is induced by IL-4) have been used to detect TAMs, with overall results comparable to those obtained using CD68 (REFS 44,45). In addition, a range of other molecules have been used to characterize TAMs, including stabilin-1 (which is a scavenger receptor and adhesion molecule expressed by M2-polarized macrophages and TAMs[55]), chemokines and/or chemokine receptors (such as CCL17)[45], and cytokines and/or cytokine receptors (for example, IL-10 and IL-12)[45]. M1-like macrophages polarized with IFNγ, which have antitumour activity, usually express high levels of HLA-DR[44] and, thus, this marker has been exploited to detect macrophages with an antitumour phenotype; however, HLA-DR is widely expressed in other leukocyte populations.

Different approaches are used for the identification of circulating macrophage precursors, such as monocytes and M-MDSCs, in patients or animals with cancer: these cell types are commonly investigated using multicolour flow cytometry of blood samples — rather than the immunohistochemical methods typically used to detect TAMs in tumour specimens. Monocyte populations detected using flow cytometry are referred to as 'inflammatory' when $CD14^+/CD16^-$, or 'patrolling' when $CD14^{dim}/CD16^+$ (REF. 20). With regard to MDSCs, a consensus definition of M-MDSCs as $CD11b^+/CD14^+/HLA\text{-}DR^{low/-}/CD15^-$ cells has been reached[29]. Macrophages infiltrating mouse and human tumours show considerable diversity within a given cancer, depending on their microanatomical localization[42,55]; as alluded to previously, hypoxia is a major driver of macrophage diversity within tumours[13,56]. Thus, an inherent limitation of available data on the predictive value of TAMs is that the intratumour diversity of these cells has not usually been accounted for.

For many types of solid tumours, a high degree of macrophage infiltration has long been associated with a poor patient prognosis[57]. These observations provided a foundation for the now widely accepted view that TAMs promote tumour progression, as discussed above. In patients with breast carcinoma, macrophage infiltration has been associated with tumour grade, a lack of hormone-receptor expression, the basal-like disease subtype, and an unfavourable outcome[26]. TAM density is also positively correlated with more advanced-stage disease at diagnosis in patients with breast or bladder cancer[46,58], whereas a negative correlation has been reported in patients with ovarian or gastric cancer[59,60]. The overall negative prognostic significance of TAM infiltration across all solid tumour types studied to date has been confirmed in a meta-analysis by Zhang *et al.*[61] that included all of the available data.

In apparent contrast with these findings, in patients with certain tumour types (non-small-cell lung cancer[62], prostate cancer[63], and colorectal cancer (CRC)[64]), a high degree of macrophage infiltration (typically defined as densities above the median, or in the highest quartile in each cohort) has been associated with a favourable prognosis. In CRC, this observation was confirmed in the meta-analysis conducted by Zhang *et al.*[61], and also in an analysis of 209 patients with CRC treated at our institution (Malesci, A. *et al.*, unpublished data). Interestingly, a high density (above the median value) of neutrophil infiltration into CRCs has also been associated with favourable patient survival[65]. In these settings, the favourable prognostic significance of macrophage infiltration is probably related to the positive effects of TAMs on response to chemotherapy.

Despite evidence of intratumoural TAM proliferation observed in some mouse models[25], whether or not TAMs proliferate *in situ* within human tumours remains less clear. In one study, however, $PCNA^+$ cells were identified as proliferating macrophages in human breast carcinoma specimens. The presence of these proliferating TAMs was associated with hormone-receptor-negative disease, a basal-like subtype, and worse outcome than

that of patients without evidence of TAM proliferation[26]. These findings warrant investigation of the presence of PCNA$^+$ TAMs within other tumour types and, if detected, assessment of their prognostic significance.

In patients with classic Hodgkin lymphoma (CHL), a gene signature of TAMs and a high number of CD68$^+$ cells in the tumour have been associated with shortened survival durations after treatment with chemotherapy regimens, compared with that of patients without these characteristics; therefore, TAMs have been proposed as a biomarker for risk stratification[66] (TABLE 1). High CD68 or CD163 expression have subsequently been confirmed to be independent predictors of unfavourable survival in the multicentre, randomized, controlled, E2496 Intergroup trial[67], thus reinforcing the prognostic significance of TAMs in chemotherapy-treated patients with locally extensive and advanced-stage CHL.

Previously, tumours with a high TAM (CD68$^+$ cell) content had also been associated with unfavourable outcomes in patients with follicular lymphoma treated with multiagent chemotherapy[68,69]. This prognostic association is reversed, however, in patients treated with the R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen[70]. Results of an independent study confirmed that high TAM (CD163$^+$ cell) numbers were predictive of a favourable outcome in patients with follicular lymphoma who received R-CHOP immunochemotherapy,

Table 1 | **High density of TAMs as a predictor of patient outcomes after chemotherapy for neoplasia**

Study (year of publication)	Tumour type	Therapy	TAM markers	High TAM density	Outcome prediction
Farinha *et al.*[68] (2005)	Follicular lymphoma	BP–VACOP followed by involved region radiation	CD68	>15 macrophages/HPF (×1,000)	Negatively correlated with DFS and OS (≤0.001)
Taskinen *et al.*[70] (2007)	Follicular lymphoma	CHOP	CD68	Highest decile	Negative correlated with DFS (P = 0.026)
		CHOP + rituximab	CD68	Highest tertile	Positively correlated with DFS (P = 0.006)
Steidl *et al.*[66] (2010)	Classic Hodgkin lymphoma	ABVD*	CD68	>25–50% positive cells	Negatively correlated with DSS (P = 0.003)
E2496 Intergroup trial; Tan *et al.*[67] (2012)	Classic Hodgkin lymphoma (locally advanced and advanced stage)	ABVD	CD68	>12.7% of positive pixels	Negatively correlated with DFS and OS (both P<0.01)
			CD163	>16.8% of positive pixels	Negatively correlated with DFS (P = 0.03) and OS (P = 0.04)
		Stanford V	CD68	>12.7% of positive pixels	Negatively correlated with DFS (P<0.01) and OS (P = 0.02)
			CD163	>16.8% of positive pixels	Negatively correlated with DFS and OS (both P<0.01)
Kridel *et al.*[71] (2015)	Follicular lymphoma	CVP + rituximab	CD163	>3.97% of positive pixels	Negatively correlated with DFS (P = 0.004)
		CHOP + rituximab	CD163	>0.16% of positive pixels	Positively correlated with DFS (P = 0.01 training, P = 0.03 validation)
Algars *et al.*[55] (2012)	Colorectal cancer (stage III)	Unspecified‡	CLEVER-1/ stabilin-1§	>10 peritumoral/0.0625 mm^2 (×400)	Positively correlated with DSS (P = 0.05)
			CD68	>30 peritumoral/0.0625 mm^2 (×400)	Positively correlated with DSS on univariate analysis, but not significantly (P = 0.09)
Di Caro *et al.*[45] (2016)	Pancreatic cancer	No adjuvant chemotherapy	CD68	Highest quartile	Negatively correlated with DFS (P = 0.02)
		Gemcitabine-based adjuvant chemotherapy	CD68	Highest quartile	Positively correlated with DSS (P = 0.05)
Malesci *et al.* (unpublished data)	Colorectal cancer (stage III)	No adjuvant chemotherapy	CD68	>8% of cells in immune-reactive area (tumour border)	None
		5-FU-based adjuvant chemotherapy	CD68	>8% of cells in immune-reactive area (tumour border)	Positively correlated with DFS (P<0.001)

5-FU, 5-fluorouracil; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; BP–VACOP, bleomycin, cisplatin, etoposide, doxorubicin, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CVP, cyclophosphamide, vincristine, and prednisone; CVPP, cyclophosphamide, vinblastine, procarbazine, and prednisone; DFS, disease-free survival; DSS, disease-specific survival; GDP, gemcitabine, dexamethasone, and cisplatin; HPF, high-power field; OS, overall survival; TAM, tumour-associated macrophage. *Or ABVD-like, with or without radiation therapy. Second-line therapy comprised autologous stem-cell transplantation, CVPP or GDP chemotherapy, and/or field radiation. ‡5-FU and folinic acid plus oxaliplatin (FOLFOX) adjuvant chemotherapy is the standard-of-care for stage III colorectal cancer. §CLEVER-1 and stabilin-1 were used as a marker of alternatively activated M2 macrophages.

but, conversely, were associated with an adverse outcome in patients treated with rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP)[71]. Doxorubicin is the drug that differentiates the R-CHOP regimen from the R-CVP regimen; therefore, the parallelism between these clinical observations and early preclinical data supporting a role of TAMs in determining the antitumour efficacy of doxorubicin in a mouse lymphoma model is striking[72]. These findings not only confirm that TAM numbers are predictive of outcome in patients with follicular lymphoma, but also underscore that their prognostic value is dependent on treatment. Nowadays, unlike in the past, patients with neoplastic conditions typically receive pharmacological anticancer treatments, thus the prognostic value of a variable is meaningless unless related to the administered treatment (that is, the variable acquires predictive rather than purely prognostic value). Considering that patients with lymphoma usually receive multiple lines of treatment, one might conclude that TAMs can serve as predictive biomarkers in this setting, and are positively or negatively correlated with outcome depending on the type of chemotherapy used.

Data from patients with solid tumours on the predictive potential of TAMs are limited. Most studies assessing the prognostic value of TAMs do not report the adjuvant therapy regimens used, even when such treatments are considered an international standard of care[55,73,74]. The only published comparison of the association of TAMs with outcome in patients who received chemotherapy after solid tumour resection versus those who did not was focused on pancreatic cancer, and indicated a dual effect of these cells depending on whether adjuvant chemotherapy was used[45]. Specifically, a high TAM density seemed to be a critical determinant of responsiveness to adjuvant gemcitabine treatment, which negated the negative prognosis associated with high TAM numbers in patients who did not receive adjuvant chemotherapy[45]. In parallel, high TAM densities have been associated with favourable outcomes in patients with stage III CRC who received adjuvant 5-fluorouracil-based chemotherapy, but not in untreated patients (Malesci, A. *et al.*, unpublished data). These studies support the role of TAMs as predictive factors of responsiveness to postsurgical chemotherapy, rather than only as prognostic indicators.

Effects of TAMs on treatment responses

Yin–yang effects of chemo/radiotherapy. Chemotherapy can affect macrophage function directly or indirectly, with the latter route primarily involving their modulation of adaptive immune responses, and thus ultimately influencing the outcome of therapy (FIG. 2). Data from a number of studies highlight that different chemotherapeutic agents have varying interactions with immunity. Indeed, an interaction between the antitumour actions of chemotherapeutic agents, such as actinomycin D, and human and murine monocytes/macrophages, was reported more than 30 years ago, as a phenomenon termed 'drug-dependent cellular cytotoxicity' (REF. 75). Moreover, data from an even earlier study revealed that immunity plays a key part in determining the ultimate efficacy of doxorubicin[72]. More-recent reports[76,77] have demonstrated that, in response to selected chemotherapeutic agents, particularly doxorubicin, tumour cells undergo immunogenic cell death — that is, they express alarm signals that trigger effective adaptive immune responses. For instance, in a mouse model, exposure to doxorubicin causes tumour cells to release ATP, which leads to recruitment of mononuclear phagocytes[78]; under these conditions the infiltrating myeloid cells differentiate into antigen-presenting cells that trigger effective adaptive immune responses[78].

The host microbiota has emerged as an important determinant of the efficacy of chemotherapy and immunotherapy in mouse models[79–81]. For example, priming of myeloid cells by microbiota components is essential for the antineoplastic efficacy of platinum combined with adjuvant CpG oligonucleotides in mouse tumour models[79]. In the same vein, the antineoplastic activity of anthracyclines is compromised in mice with genetic inactivation of the formyl peptide receptor 1 (FPR1), a sensor of microbial components and tissue damage that is expressed in myeloid cells[82]. Of note, the presence of a loss-of-function *FPR1* allele has been associated with unfavourable survival in patients with breast carcinoma or CRC after adjuvant chemotherapy[82]. Indeed, data from preclinical models suggest that myeloid cells determine the role of immunity in the antitumour activity of selected chemotherapeutic agents[77], which can be leveraged to increase the efficacy of immune-checkpoint inhibition[83]. In mouse models, repolarization of macrophages has also been reported in the context of targeted therapy, such as treatment of KIT-positive gastrointestinal stromal tumours (GISTs) with imatinib[84], and treatment of hepatocellular carcinoma with sorafenib[85].

Trabectedin, which is approved by the EMA for the treatment of soft-tissue sarcomas and ovarian cancer, and by the FDA for sarcoma therapy, is a DNA-binding agent that causes DNA damage and cell-cycle arrest in tumour cells; however, this 'conventional' effect accounts for only part of the drug's complex mechanism of action. By interacting with DNA-binding proteins, trabectedin affects the transcription of selected genes, including some that encode inflammatory cytokines and chemokines, as well as angiogenic factors[86]. Clinical observations of delayed but prolonged responses after trabectedin treatment are inconsistent with the antitumour activity of this agent being mediated only by effects on cancer cells. This experience has prompted analyses of the effects of this drug on immunity[87,88]. Trabectedin has been found to trigger activation of caspase 8, the key effector molecule of the extrinsic apoptotic pathway (programmed cell death activated via cell-surface receptors), selectively in monocytes, inducing their apoptotic death. In preclinical models, depletion of macrophage numbers has been demonstrated to be a key mechanism mediating the antitumour activity of this compound[87]. Furthermore, reduced TAM infiltration and decreased angiogenesis have both been observed in tumour samples from patients with sarcoma treated using trabectedin, compared with

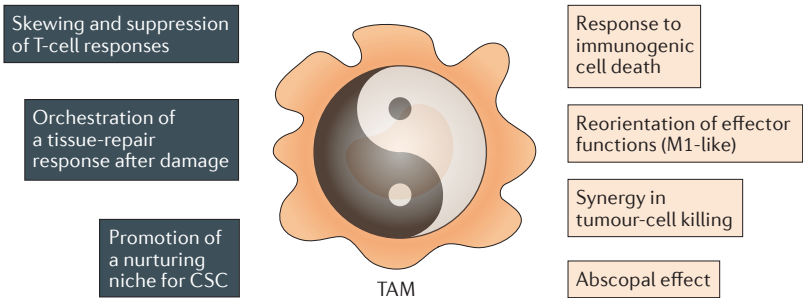

Figure 3 | **The yin and yang of tumour-associated macrophages (TAMs) in response to chemotherapy and radiotherapy.** Macrophages have bimodal, yin and yang roles in orchestrating immune responses, and can either hamper (left-hand side), or foster (right-hand side) the effectiveness of conventional anticancer strategies. Yin: cytotoxic agents enhance tumour infiltration by immunosuppressive macrophages, which subsequently activate chemoprotective T cells and tame adaptive antitumour immune responses; chemotherapy-induced and/or radiotherapy-induced tissue damage triggers the recruitment of immunosuppressive myeloid cells, which orchestrate a misdirected tissue-repair response, promoting tumour growth and revascularization; macrophages, an essential component of the tissue stem-cell niche, can protect cancer stem cells (CSCs) from cytotoxicity. Yang: certain chemotherapeutic agents (such as doxorubicin) increase the immunogenicity of malignant cells by inducing immunogenic cell death, resulting in stimulation of myeloid cells to differentiate into antigen-presenting cells and thus triggering effective adaptive immune responses; anticancer agents, including gemcitabine, can directly skew macrophage effector functions towards an antitumour mode and increase their cytotoxicity, resulting in a favourable synergism; low-dose γ-irradiation can instill macrophages with an antitumour phenotype, thus promoting tumour regression at sites distant from the irradiated lesions, a so-called 'abscopal effect'.

pretreatment biopsy samples[87]. Thus, preclinical and clinical evidence suggests that a reduction in macrophage numbers is a key component of the antitumour activity of trabectedin.

Macrophages do not, however, have a universally positive effect on responsiveness to chemotherapy. M2 and M2-like polarized macrophages orchestrate tissue repair. Consistent with this general property of cells of the monocyte–macrophage lineage, evidence indicates that, under selected conditions, TAMs can limit the effectiveness of cytotoxic agents (FIG. 3). These agents include platinum-containing compounds, paclitaxel, and also doxorubicin[33,89–93]. In mouse tumour-transplantation models, M2-like macrophages were found to accumulate in perivascular areas of the tumour after chemotherapy, and promoted tumour revascularization and relapse[94]; recruitment of these cells was found to be CXCR4–CXCL12 dependent[94].

The apparent discrepancy in the role of TAMs in mediating the response to doxorubicin[72,89–93] probably reflects differences in the mouse tumour models used (for instance, in terms of immunogenicity). As discussed, however, a high degree of TAM infiltration is associated with favourable prognosis in patients with lymphomas treated with doxorubicin-containing regimens[71], mirroring preclinical data[72]. The positive interaction between chemotherapy and macrophage-mediated host defense is also reflected in the prognostic and/or predictive significance of TAMs in pancreatic cancer[45] and CRC (Malesci, A. *et al.*, unpublished data). Mirroring these clinical associations, gemcitabine was found to synergize with macrophages in tumour-cell killing *in vitro*[45].

Two general mechanisms emerge as responsible for the antagonistic effects of TAMs on chemotherapy outcomes. In mouse models, chemotherapy-induced tissue damage has been demonstrated to trigger recruitment of immunosuppressive myeloid cell[33,89], or to elicit a protumorigenic type 17 T-helper (T_H17)-cell-skewed immune response promoted by IL-1 (REF. 91). An alternative pathway is centred on cancer stem cells (CSCs): TAMs have been reported to protect mouse CSCs from cytotoxicity[92,93]. Indeed, macrophages are an essential component of the stem-cell niche in a variety of tissues. Thus, the dark side of the interaction of chemotherapy with TAM is a reflection of the fundamental properties of macrophages — that is, their roles in the tissue stem-cell niche, in taming adaptive immunity, and in orchestrating repair responses.

Similar to the effects of chemotherapy, the effects of radiotherapy on myeloid cells can have dual implications for patient outcomes. In mouse models, the influx of monocytes into tumours following radiotherapy drives a profibrotic tissue response and might promote tumour recurrence[8,95]. Conversely, in patients, tumour regression at sites distant from the irradiated lesions — termed the 'abscopal' effect[96] — could plausibly be explained by activation of host anticancer immunity. In a mouse model, neoadjuvant low-dose γ-irradiation was found to set macrophage functions to an antitumour mode characterized by a lack of both immunosuppressive and proangiogenic activity, and the production of T-cell-attracting chemokines[97]. Therefore, TAMs can either reduce or amplify the magnitude of the antitumour effect of radiotherapy depending on context.

Influence on hormonal therapy. Evidence suggests that two classic pathways that promote tumour development and growth, inflammation, and hormone signalling via sex steroids, are linked[1]. In prostate cancer cells, IL-1β produced by macrophages triggers a switch in the activity of selective androgen receptor modulators (SARMs), from inhibitory to stimulatory[98]. In prostate cancer specimens from patients who had been treated with androgen-blockade therapy, a high TAM density (above the observed median value) was associated with an increased recurrence rate[99]. Of note, androgen-blockade therapy induced production of macrophage-attracting cytokines, particularly CSF-1, by tumour cells in a preclinical prostate-cancer model[100]. In this model, inhibition of the tyrosine kinase activity of the CSF-1 receptor (CSF-1R) had synergistic antitumour activity with androgen blockade[100]. Thus, targeting TAMs might amplify the susceptibility of prostate cancer cells to hormonal interventions.

Roles in antiangiogenic therapy. Strategies targeting VEGF signalling are part of the current therapeutic armamentarium in oncology. In addition to eliciting angiogenesis, VEGF is a potent attractant of monocytes, acting via VEGFR-1 (REFS 101,102). Interestingly, in a mouse mammary carcinoma model, Qian *et al.*[102] found that expression of VEGFR-1 is upregulated in metastasis-associated macrophages. VEGF has long been

known to be chemotactic for monocytes in this model; however, this cytokine did not drive the accumulation of macrophages within metastases. Instead, VEGF signalling was found to activate an autocrine CSF-1-mediated feedback pathway in metastasis-associated macrophages that underlies their tumour-promoting activity.

Indeed, macrophages, including TAMs, are a major source of angiogenic growth factors, including VEGF, that act on vascular and lymphatic vessels[13]. Correspondingly, TAM density and vascular density are generally closely correlated in human tumours[1,46,47]. Moreover, resistance of tumours to current anti-VEGF therapies is frequently associated with high levels of myeloid-cell infiltration[56]. Preclinical evidence suggests that hypoxia resulting from destruction of the vascular bed, as a consequence of antiangiogenic treatment, triggers compensatory recruitment of myeloid cells that promote angiogenesis via alternative mechanisms to those targeted therapeutically, such as via production of thymidine phosphorylase, Bv8, and cathepsin B[8,13,47,103].

Angiopoietin-2 (ANG-2) is a regulator of vessel-wall integrity that is functionally linked to angiogenesis and TAM function. In addition to providing an escape pathway from VEGF inhibition, ANG-2 can activate a pro-angiogenic phenotype in macrophages[104]. Macrophage infiltration into human glioblastomas is correlated with a poor prognosis, and this tumour type is resistant to anti-VEGF therapy. In preclinical models of this disease, a bispecific anti-ANG-2/VEGF antibody has appreciable antitumour activity and reprogrammes TAMs from a protumour M2 phenotype to an antitumour M1 phenotype[105,106]. Thus, targeting TAMs might complement current antiangiogenic therapies and improve the effectiveness of this treatment.

Influence on immune-checkpoint blockade. Immunotherapy using immune-checkpoint inhibitors is becoming an established part of the therapeutic armamentarium for an increasing number of cancers[107]. Clinically validated targets include CTLA-4, PD-1, and PD-L1, and others are undergoing clinical evaluation. Myelomonocytic cells are a key component of the immunosuppressive pathways targeted by immune-checkpoint inhibitors, and might, therefore, offer tools to predict or increase the activity of such treatments. For example, macrophages can express the PD-1 ligands PD-L1 and PD-L2, as well as the CTLA-4 ligands B7-1 (CD80) and B7-2 (CD86), and the related protein B7-H4 (which interacts with an as yet unidentified immune-checkpoint receptor on T cells). PD-L1 and PD-L2 are upregulated on the surface of macrophages in response to various stimuli, including cytokines and hypoxia[108,109]. TAMs present in a variety of human tumour types, such as hepatocellular carcinoma[110,111], glioblastoma[112], and pancreatic cancer[113], express high levels of PD-L1 and/or B7-H4. How, and to what extent, the expression of PD-L1 (and/or PD-L2) and B7-H4 on macrophages contributes to the immunosuppressive function of this cell type has not been fully elucidated. Nevertheless, the expression of these triggers of inhibitory immune checkpoints by TAMs needs to be carefully assessed as a predictor of response to therapy, particularly to immune-checkpoint blockade[114,115].

Analysis of the mode of action of CTLA-4-blocking monoclonal antibodies (mAbs) has revealed an unexpected role of TAMs. In preclinical models, FcγR-expressing macrophages eliminated CTLA-4-positive, mAb-coated T_{reg} cells from tumours via antibody-dependent cellular cytotoxicity (ADCC)[116,117]; the TAM-mediated removal of T_{reg} cells unleashed effective antitumour immunity. The role of macrophage-mediated ADCC in the activity of the anti-CTLA-4 mAb ipilimumab has been examined in 15 patients with melanoma who responded to this agent and 14 matched 'non-responders' (REF. 118); responders had higher numbers of peripheral blood $CD16^+$ monocytes and, upon treatment, a higher $CD68^+/CD163^+$ macrophage ratio (used as a correlate of M1 skewing) at tumour sites. Moreover, responses to ipilimumab were associated with a decrease in T_{reg}-cell densities within the tumour at 4 weeks after treatment[118].

In general, however, macrophages contribute to the immunosuppression observed in the tumour microenvironment through multiple mechanisms (FIG. 2); therefore, targeting of TAMs might complement the action of immune-checkpoint inhibitors by removing additional inhibitory factors that might continue to restrain the action of T cells in spite of checkpoint blockade. Indeed, in a model of pancreatic cancer, inhibition of CSF-1-signalling synergized with checkpoint-blockade immunotherapy[89,119]. Combination therapies with mechanisms of action based on this principle are undergoing early clinical evaluation (TABLE 2).

Contributions to antibody therapies. Phagocytosis is a fundamental mechanism of innate resistance against microorganisms and of effete (aged and/or damaged) cell disposal (specifically termed efferocytosis). Historically, many researchers hypothesized that phagocytosis is not an important process underlying the antitumour activity of activated macrophages, and that mechanisms of extracellular killing are more relevant to macrophage-mediated killing of cancer cells[10]. Evidence has now challenged this long-held view, spurring interest in the clinical implications of tumour-cell phagocytosis.

The CD47–SIRPα pathway has an important role in regulating phagocytosis, which in turn is a key factor in tissue homeostasis. Both SIRPα and CD47 are members of the immunoglobulin superfamily, and are expressed on macrophages and candidate target cells, respectively[120–122]. Upon binding to CD47, SIRP-α acts as a docking protein for the SHP-1 and SHP-2 phosphatases, which dampen intracellular signalling, and thereby negatively regulates phagocytosis. Thus, CD47 acts physiologically as a 'don't eat me' signal. Of note, CD47 is frequently overexpressed by cancer cells[120,121,123,124]. Masking of CD47 on cancer cells using mAbs or an engineered soluble SIRPα–Fc construct can trigger antibody-dependent cellular phagocytosis (ADCP) of tumour cells *in vitro,* and anti-CD47 mAbs have antitumour activity in diverse mouse models[120,121,123–125]. Such agents have now entered clinical testing (TABLE 2).

Table 2 | **Clinical trials of agents targeted at macrophages in tumours**

Compound (sponsor)	Clinical phase (status)	Tumour type	Combination partner(s)	ClinicalTrial.gov reference
CSF-1R inhibitors				
Pexidartinib (PLX3397; Plexxikon)	Phase I/II (ongoing)	Sarcoma, nerve-sheath tumours	Sirolimus	NCT02584647
	Phase II (ongoing)	Melanoma	NA	NCT02071940
	Phase I (ongoing)	Prostate cancer	Radiotherapy and ADT	NCT02472275
	Phase I/II (ongoing)	Solid tumours	Pembrolizumab (anti-PD-1 antibody)	NCT02452424
	Phase I (ongoing)	Advanced-stage pancreatic cancer or CRC	Durvalumab (anti-PD-L1 antibody)	NCT02777710
	Phase III (ongoing)[160]	PVNS or GCT-TS	NA	NCT02371369
	Phase III (ongoing)	Breast cancer	Eribulin	NCT01596751
	Phase Ib/II (ongoing)	Leukaemia, sarcoma, or neurofibroma	NA	NCT02390752
	Phase I/II (ongoing)	Acute myeloid leukaemia	NA	NCT01349049
	Phase I/II (ongoing)	Glioblastoma	Radiotherapy and temozolomide	NCT01790503
	Phase I (ongoing)	Advanced-stage solid tumours	Paclitaxel	NCT01525602
	Phase I/II (ongoing)	Breast cancer	Standard neoadjuvant chemotherapy*	NCT01042379
PLX7486 (Plexxikon)	Phase I (ongoing)	Advanced-stage solid tumours	NA	NCT01804530
Anti-CSF-1R antibodies				
LY3022855 (IMC-CS4; Eli Lilly)	Phase I (ongoing)	Solid tumours	Durvalumab (anti-PD-L1 antibody) and tremelimumab (anti-CTLA-4 antibody)	NCT02718911
	Phase I (ongoing)	Breast or prostate cancer	NA	NCT02265536
	Phase I (ongoing)	Solid tumours	NA	NCT01346358
Emactuzumab (RO5509554/ RG7155; Roche)	Phase I (ongoing)	Solid tumours	Atezolizumab (anti-PD-L1 antibody)	NCT02323191
	Phase I (ongoing)[158,159]	Solid tumours	± Paclitaxel	NCT01494688
AMG820 (Amgen)	Phase I/II (ongoing)	Pancreatic cancer, CRC, or non-small-cell lung cancer	Pembrolizumab (anti-PD-1 antibody)	NCT02713529
	Phase I (completed)	Solid tumors	NA	NCT01444404
Anti-CD47 antibodies				
Hu5F9-G4 (Stanford University)	Phase I (ongoing)	Myeloid leukaemia	NA	NCT02678338
CC-90002 (Celgene)	Phase I (ongoing)	Myeloid leukaemia or myelodysplastic syndrome	NA	NCT02641002
	Phase I (ongoing)	Advanced-stage solid or haematological malignancies	NA	NCT02367196
CD47–Fc fusion protein				
TTI-621 (Trillium)	Phase I (ongoing)	Haematological malignancies	NA	NCT02663518
Agonistic anti-CD40 antibodies				
CP-870,893 (Pfizer, UPenn)	Phase I (completed)	Melanoma	NA	NCT02225002
	Phase I (completed)	Solid tumours	Paclitaxel and carboplatin	NCT00607048
	Phase I (completed)[181]	Pancreatic adenocarcinoma	Gemcitabine	NCT01456585
RO7009789 (Roche)	Phase I (ongoing)	Solid tumours	Vanucizumab (anti-ANG-2–VEGF bispecific antibody)	NCT02665416
	Phase I (ongoing)	Pancreatic adenocarcinoma	Nab-paclitaxel and gemcitabine	NCT02588443
Anti-IL-1α antibody				
Xilonix (XBiotech)	Phase III (ongoing)	CRC	NA	NCT01767857
Anti-CCL2 antibodies				
Carlumab (CNTO 888; Centocor)	Phase II (completed)[147]	Prostate cancer	NA	NCT00992186
	Phase I (completed)[144,149]	Solid tumours	Gemcitabine, paclitaxel and carboplatin	NCT01204996

Table 2 (cont.) | **Clinical trials of agents targeted at macrophages in tumours**

Compound (sponsor)	Clinical phase (status)	Tumour type	Combination partner(s)	ClinicalTrial.gov reference
CCR2 antagonist				
PF-04136309 (Pfizer)	Phase I (completed)[150]	Advanced-stage pancreatic adenocarcinoma	FOLFIRINOX	NCT01413022
Bruton kinase inhibitor				
Ibrutinib	Phase II/III (ongoing)	Pancreatic adenocarcinoma	Gemcitabine and nab-paclitaxel	NCT02436668
Modified vitamin-D-binding protein (macrophage-activating factor)				
EF-022 (Efranat)	Phase I (ongoing)	Solid tumours	NA	NCT02052492

Data were obtained from http://clinicaltrials.gov. ADT, androgen-deprivation therapy; ANG-2, angiopoietin-2; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; FOLFIRINOX, 5-fluorouracil, folinic acid, irinotecan, and oxaliplatin; GCT-TS, giant-cell tumour of the tendon sheath; NA, not applicable; PD-1, programmed cell-death protein 1; PD-L1, programmed cell death 1 ligand 1; PVNS, pigmented villonodular synovitis; UPenn, University of Pennsylvania; VEGF, vascular endothelial growth factor. *In this trial, I-SPY 2, various experimental agents are being added to standard neoadjuvant therapy (with paclitaxel, doxorubicin, cyclophosphamide and trastuzumab (anti-HER2 antibody), depending on molecular subtype), including: pexidartinib; trebananib (AMG 386; angiopoietin-1/2-neutralizing 'peptibody') with or with trastuzumab; ganitumab (AMG 479; anti-IGF-1R) plus metformin; MK-2206 (AKT inhibitor) with or without trastuzumab; T-DM1 (trastuzumab emtansine) and pertuzumab (anti-HER2 antibody); pertuzumab and trastuzumab; ganetespib (heat shock protein 90 inhibitor); veliparib (ABT-888; poly[ADP-ribose] polymerase inhibitor); neratinib (HER2 tyrosine kinase inhibitor); and pembrolizumab (anti-PD-1 antibody).

Importantly, CD47-targeting agents have proven synergy with diverse antitumour mAbs, including anti-CD20 and anti-HER2 antibodies[116,117,119–121,126]. These findings are consistent with the ability of SIRPα to downregulate FcγR signalling in macrophages[120,121].

Interestingly, anti-CD47 mAb treatment was found to prime adaptive antitumour immune responses in mouse models, either directly or via activation of accessory cell function[127,128]. Indeed, the ultimate efficacy of CD47 blockade in the B16F10 mouse melanoma model requires activation of an adaptive immune response, as demonstrated by the need for additional PD-L1 blockade in order to achieve durable antitumour immunity[129]. Of note, CD47 has been found to be highly expressed on pancreatic CSCs, and targeting of this protein not only promoted phagocytosis of these cells by macrophages, but also directly induced cancer cell apoptosis in the absence of macrophages. Moreover, although antagonism of CD47 did not have relevant antitumour activity in a patient-derived xenograft model, such treatment resulted in synergistic antitumour activity when combined with chemotherapy[130].

For four decades, macrophages have been known to kill tumour cells extracellularly via ADCC[131]. In fact, ADCC, which can be mediated by NK cells in addition to macrophages, is an essential component of the activity of antitumour mAb therapies, including those targeting CTLA-4, CD20, HER2 and EGFR[118,132]. Polymorphisms in the genes encoding FcγRIIIA and FcγRIIA are correlated with responsiveness of patients with lymphoma to rituximab (an anti-CD20 antibody), those with colon cancer to cetuximab (an anti-EGFR antibody), and women with breast carcinoma to trastuzumab (an anti-HER2 antibody)[133–135]. FcγRIIA is only expressed in platelets, macrophages and neutrophils; however, only macrophages are potent effectors of ADCC and, therefore, these data suggest an important role of these immune cells in the clinical activity of mAb-based treatments. In a preclinical lymphoma model, the antitumour activity of rituximab has been shown to be dependent on chemokine-mediated macrophage recruitment and on macrophage effector functions[136]. Interestingly, signals that skew TAM function to an M2-like phenotype (IL-10 and CSF-1), and are potentially present in the tumour microenvironment, have been found to enhance macrophage-mediated phagocytosis of rituximab-opsonized lymphoma cells[137]. Consistent with these observations, although high TAM density has been associated with an unfavourable prognosis in lymphoma[68], high TAM infiltration was associated with a favourable outcome in patients treated with rituximab-containing regimens[70]. Similarly, in a breast cancer model, TAMs promoted tumour growth, but were essential for the therapeutic efficacy of a mAb targeting CD142 (REF. 138), as well as trastuzumab[126]. Thus, findings from preclinical models, mechanistic analyses, and clinical correlative studies indicate that the functional activation of TAMs can promote antibody-dependent macrophage effector functions and might be beneficial to anticancer therapy.

Agents that enhance TAM-mediated ADCC and ADCP could potentially be used in combination approaches with immunogenic chemotherapy, which stimulates macrophage recruitment, activation, and/or effective antitumour immunity. For example, in a mouse model of B-cell acute lymphoblastic leukaemia, cyclophosphamide-mediated tissue damage elicited macrophage recruitment via the release of chemokines and cytokines; addition of the anti-CD52 mAb alemtuzumab to cyclophosphamide chemotherapy synergistically increased cancer cell death and macrophage-mediated elimination of leukaemia cells[139].

Approaches to targeting macrophages

A number of potential strategies to target macrophages in anticancer therapy are being explored (FIG. 4; TABLE 2). In general, macrophage-centred therapeutic approaches are aimed either at inhibiting the localization of these cells at tumour sites and their functions related to the promotion of tumour progression, or at activation of their antitumour activities.

Targeting recruitment and localization. As discussed previously, the mediators involved in regulating macrophage recruitment to tumours are diverse and include

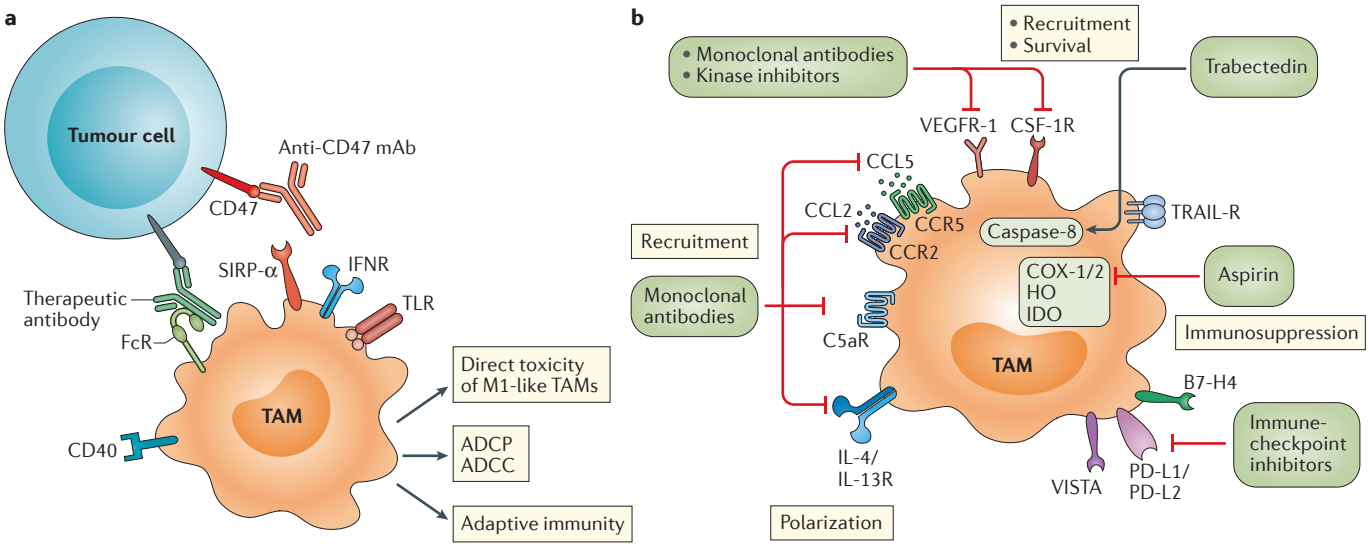

Figure 4 | **Macrophage-targeting antitumour treatment approaches.** Macrophage-centred therapeutic approaches are aimed at either activating the antitumour activity, or inhibiting the recruitment and tumour-promoting functions of this cell type. **a** | The concerted action of microbial moieties (acting via Toll-like receptors; TLRs) and IFNγ induces M1-like functional polarization of macrophages (BOX 1), and can activate these cells to kill tumour cells either directly, or indirectly by promoting adaptive immune responses. Macrophage-mediated antibody-dependent cellular cytotoxicity (ADCC) is often integral to the antitumour effect of therapeutic antibodies. Activation of this process involves recognition of the therapeutic antibodies by Fc receptors (FcRs) expressed on the surface of macrophages. Tumour cells can express CD47, a 'don't eat me' signal that, via interaction with SIRPα prevents phagocytosis by macrophages. Thus, interference with the SIRPα–CD47 pathway — for example, using antagonistic antibodies — can activate macrophage-mediated antibody-dependent cellular phagocytosis (ADCP), which subsequently results in functional skewing of macrophages in an M1 direction that is associated with antitumour activity. In addition, activation of the co-stimulatory receptor CD40 using an anti-CD40 antibody has been demonstrated to re-educate immunosuppressive, tumour-promoting, M2-like macrophages in the tumour microenvironment to an M1-like phenotype, leading to re-establishment of tumour immune surveillance. **b** | Monocyte-attracting molecules, including chemokines (such as CCL2 and CCL5), cytokines (for example, VEGF and CSF-1), and complement mediators (C5a) are involved in the recruitment of tumour-associated macrophage (TAM)-precursors to tumours. Inhibitors of these chemoattractants or their receptors with specific monoclonal antibodies (such as the anti-CCL2 antibody carlumab, or the anti-CSF-1 antibody emactuzumab), or antagonists (for example, the CCR5-antagonist maraviroc) can prevent macrophage accumulation within the tumour microenvironment, thereby reducing tumour growth and dissemination. CSF-1 antagonists also have the potential to inhibit macrophage survival. The DNA-binding agent trabectedin, an approved anticancer therapy, activates caspase-dependent apoptosis selectively in cells of the monocyte lineage that express TRAIL receptors (TRAIL-R), thus causing partial depletion of both circulating monocytes and TAMs. The protective function of nonsteroidal anti-inflammatory drugs (NSAIDS), particularly aspirin, against the development of primary cancer and metastases is related to inhibited synthesis of prostaglandins, which have immunosuppressive properties; TAM contribute to suppression of adaptive immunity via expression of cyclooxygenases (COX-1/2) involved in prostaglandin production, as well as other proteins with immunosuppressive functions, including indoleamine-pyrrole 2,3-dioxygenase (IDO), TGFβ, and IL-10. Moreover, TAMs express ligands, such PD-L1, PD-L2, B7-H4, and VISTA, that trigger inhibitory immune-checkpoint receptors. HO, haeme oxygenase.

chemokines, complement components, CSF-1, and VEGF. Chemokines have long been implicated in macrophage accumulation within tumours[11,15–17,19,27,140]. The fact that multiple chemokines and chemokine receptors are involved in phagocyte chemotaxis ('robustness' and redundancy of the system), and that individual chemokines act on multiple cell types (resulting in less-efficient inhibition of macrophage function, specifically) are challenges to translating chemokine-targeting therapeutic strategies into clinical benefit in patients with inflammatory and neoplastic diseases. Specific inhibition of CCL2 with antibodies has been shown to reduce tumour growth and dissemination in different experimental models of prostate, breast, lung or liver cancer, or melanoma; when administered in combination with chemotherapy, anti-CCL2 antibodies improved the efficacy of treatment[141–145]. In a mouse model of breast cancer, however, withdrawal of anti-CCL2 treatment has also been associated with a rebound effect, with increased mobilization of bone-marrow monocytes as well as infiltration of these cells into tumours, thus accelerating the development of lung metastasis[146].

Nevertheless, antibodies selectively targeting CCL2 have entered phase I and II clinical testing[147–149] (TABLE 2). In a phase I trial, the anti-CCL2 antibody carlumab (CNTO 888) showed preliminary antitumour activity in patients with advanced-stage solid tumours, and was well tolerated[144]. No responses were observed, however, in a phase II study of this agent in patients with castration-resistant prostate cancer[147]. Combinations

of carlumab with conventional chemotherapy have been studied in patients with solid tumours in a phase Ib clinical trial (TABLE 1); good safety profiles were observed, but with no objective tumour responses[149]. The feasibility of combining PF-04136309, a novel oral CCR2 small-molecule antagonist, with conventional chemotherapy in patients with locally advanced pancreatic adenocarcinoma who are not eligible for surgery has been demonstrated in a phase 1b clinical trial[150]. In this study, patients received FOLFIRINOX (5-fluorouracil, folinic acid, irinotecan, and oxaliplatin) alone, or in combination with PF-04136309. Patients in the latter group did not experience worse toxicity than those receiving chemotherapy alone, and 16 of 33 imaging-evaluated patients had partial tumour responses (49%), with 32 patients (97%) having local stable disease (compared with no objective responses, but four stable disease responses (80%), in five evaluable patients treated with chemotherapy only)[150].

The chemokine CCL5 has been reported to be responsible for the functional skewing of TAMs toward a protumour phenotype[151]. At tumour sites CCL5 is usually produced by cancer cells and macrophages, but in this study[151], $CD4^+$ and $CD8^+$ lymphocytes were the major sources of CCL5 within CRC liver metastases. Maraviroc, an antagonist of CCR5, the cognate receptor for CCL5, is approved as a treatment for patients with AIDS. In a small cohort of patients with advanced-stage CRC, treatment with maraviroc has been associated with biological and clinical responses[151]. Thus, targeting of the CCL5–CCR5 axis deserves further investigation, given that activation of this pathway is well established, for example, in the pathogenesis of breast cancer[140].

Inhibiting the CSF-1-CSF-1R axis. CSF-1R is exclusively expressed by cells of the monocytic lineage and, therefore, is an obvious target to enable interference with TAMs directly, or indirectly via effects on TAM-precursor cells. Indeed, CSF-1 is the major growth and differentiation factor for cells of the monocyte–macrophage lineage, and is abundantly expressed by several tumour types[1,6]. Thus, the CSF-1–CSF-1R axis has been extensively investigated in tumour models and is paradigmatic of the TAM–cancer cell interaction[6,152–154]. High CSF-1 or CSF-1R expression levels in the tumour or peritumoural tissue have been associated with poor patient survival in different malignancies, such as CHL, breast cancer, and hepatocellular carcinoma[155,156].

As a receptor tyrosine kinase, CSF-1R is an attractive therapeutic target (FIG. 4), and a number of small-molecule and antibody antagonists have been developed and tested in preclinical models[33,89,154,157,158]. For example, the humanized mAb emactuzumab (RG7155) binds to CSF-1R and prevents receptor dimerization, thereby abrogating binding to dimeric CSF-1 and activation of signalling[158]. In one study, treatment of mice with emactuzumab reduced TAM densities (owing to both reduced numbers of TAMs and circulating monocytes) and increased the $CD8^+$:$CD4^+$ T-cell ratio in tumour samples, compared with those seen in mice treated with a control antibody[158]; the same effects were observed in a comparison of pre-treatment and post-treatment biopsy samples from patients with various solid malignancies, who were included in a phase I clinical trial performed as part of the same study[158] (TABLE 2). Notably, particularly promising signs of clinical activity were seen in patients with diffuse-type tenosynovial giant-cell tumour, a rare neoplasia characterized by overexpression of CSF-1R[158]. Thus, a dose-escalation and dose-expansion study was performed in patients with this disease[159], with 26 of 28 patients (93%) achieving objective responses; no dose-limiting toxicities were observed, although common adverse events included facial oedema, asthenia, and pruritus[159]. Pexidartinib (PLX3397), a small-molecule CSF-1R inhibitor that can be administered orally, also induced clinical regression in patients with tenosynovial giant-cell tumours[160] (TABLE 2), thus confirming the validity of TAMs as a therapeutic target in this disease.

Pexidartinib is able to penetrate the blood–brain barrier and has been tested in a phase II study in patients with recurrent glioblastoma[161]. The drug was well tolerated and, as proof-of-principle of its activity, circulating $CD14^{dim}$/$CD16^+$ monocyte numbers were reduced after treatment; however, no objective responses were reported and the primary end point of 6-month progression-free survival was met only in a minority (8.6%) of the 37 patients treated[161]. Clearly, the potential of these inhibitors needs to be maximized via use of rational combination therapy approaches.

Radiotherapy is known to increase the extent of macrophage infiltration into irradiated tissues, and this response can be detrimental to the therapeutic response[37,162]. In preclinical mouse xenograft models of intracranial human glioblastoma, radiotherapy has been demonstrated to increase CSF-1 expression and the degree of tumour infiltration by myeloid cells[163]. In this model, treatment with pexidartinib potentiated the therapeutic effects of radiotherapy, suggesting that the effectiveness of radiotherapy in patients with glioblastoma can be improved by combination with CSF-1R inhibition[163]. In a syngeneic mouse model of $BRAF^{V600E}$-driven melanoma, pexidartinib also improved the antitumour efficacy of adoptive cell-transfer immunotherapy by inhibiting the intratumoural accumulation of immunosuppressive macrophages[164].

Another small-molecule CSF-1R inhibitor, BZL945, has been shown to attenuate the progression of glioma and improve survival in preclinical models[33]. Interestingly, CSF-1R blockade with this agent did not result in depletion of TAM numbers, but instead contributed — together with glioma-derived factors (GM-CSF and IFNγ) — to 're-education' of macrophages from a protumour M2-like phenotype to an antitumour M1-like effector cell type[33]. Of note, an analysis of glioblastomas recurring in mice after CSF-1R inhibition with BZL945 has revealed an interplay between cancer cells and microenvironmental factors. Specifically, IL-4 reprogrammes macrophages to the M2-like phenotype and, via STAT6 and NFAT signalling, results in production of IGF1; the IGF1 secreted

by these cells signals via IGF-1R on tumour cells, causing activation of the PI3K pathway[165]. Accordingly, combined inhibition of IGF-R1 or PI3K in tumour cells and CSF-1R in macrophages resulted in prolongation of survival durations in this mouse model[165].

In a mouse model of ovarian cancer, the CSF-1R inhibitor GW2580 decreased the numbers of tumour-infiltrating M2-like macrophages when administered at the late stages of disease (after peritoneal dissemination)[166]. This treatment approach dramatically reduced ascites volume, and induced normalization of the disorganized peritoneal vasculature[166]. These preclinical findings indicate that some therapeutic biological effects could be expected even in patients with advanced-stage tumours. GW2580 treatment also enhanced the activity of gemcitabine in a transgenic model of gemcitabine-resistant pancreatic ductal adenocarcinoma[167]. Mechanistically, GW2580 reduced tumour infiltration by macrophages that contribute to chemoresistance in this model by upregulating expression of the gemcitabine-metabolizing enzyme cytidine deaminase in the cancer cells[167]. These findings suggest that treatment targeting macrophages could be a complementary strategy to enhance the efficacy of conventional chemotherapy. Along these lines, in a transgenic mouse model of mammary adenocarcinoma, paclitaxel-based chemotherapy resulted in upregulated production of CSF-1, IL-34 (another cytokine that signals via CSF-1R), and the chemokine CCL8; blockade of the CSF-1–CSF-1R axis, using either an anti-CSF-1 antibody or a CSF-1R inhibitor, enhanced the therapeutic efficacy of paclitaxel, inhibited metastasis, and increased $CD8^+$ T-cell infiltration into tumours[89]. Inhibition of the CSF-1–CSF-1R axis might also increase the effectiveness of other systemic therapies. For example, in a mouse model of prostate cancer, androgen-blockade therapy induced cancer cells to express CSF-1 and other cytokines that caused increased infiltration of TAMs[100]. Correspondingly, addition of CSF-1R inhibitors, such as GW2580 or pexidartinib, to androgen-blockade therapy resulted in more durable therapeutic responses compared with hormonal therapy alone[100]. Collectively, preclinical data strongly suggest that targeting the CSF-1–CSF-1R axis has the potential to complement conventional therapeutic strategies.

Evidence has been provided of the specific factors that are important for the survival and expansion of myeloid cells in tumours. For example, the nuclear receptor RORγ is expressed in myeloid cells and drives cancer-related myelopoiesis in response to colony-stimulating factors[168]. Importantly, ablation of RORγ expression in the myeloid compartment restrains tumour development and impairs the generation of suppressive MDSCs, while also promoting M1-polarization of TAMs, which is associated with antitumour activity[168]. Thus, RORγ is a potential molecular target of myeloid-cell-centred anticancer therapy.

Bisphosphonates. Bisphosphonates have cytotoxic effects on myeloid cells and are used for the treatment of osteoporosis and prevention of complications associated with bone metastases. Drugs of this class inhibit the activity of farnesyldiphosphonate synthase, a key enzyme responsible for cholesterol synthesis and protein prenylation, and have high affinities for bone hydroxyapatite; accordingly, they are predominantly internalized by and result in apoptosis of bone macrophages (osteoclasts)[169,170]. Nevertheless, tissue macrophages other than those in bone, including TAMs, have been reported to be affected by bisphosphonates[171], in particular, by clodronate delivered in a liposomal formulation[172,173]. Currently, bisphosphonates are used clinically in the treatment of breast cancer and other solid malignancies, in combination with chemotherapy or hormonal therapy. In postmenopausal women with breast cancer, disease recurrence and overall mortality have been substantially reduced using this approach, compared with the outcomes of hormonal therapy or chemotherapy alone[174]. Moreover, clodronate has been reported to reduce the incidence of new metastases both in bone and visceral tissues in patients with breast cancer, an observation that points to actions unrelated to the bone metastatic niche[175]. In patients with hormone-therapy-naive prostate cancer with bone metastases, treatment with zoledronic acid has been associated with reduced rates of skeletal-related events and improved progression-free survival durations[174]. The relative importance of targeting macrophages, particularly those in the bone metastatic niche, versus modifying bone resistance to osteolysis in the clinical activity of bisphosphonates remains to be assessed.

Trabectedin. As discussed, trabectedin, which was originally developed as an antiproliferative agent, can partially deplete circulating monocyte and TAM populations[87,88]. The studies that revealed these effects stemmed from the clinical observation of delayed, persistent responses to trabectedin in patients with cancer. The monocyte depletion induced by this agent includes the monocytic component of MDSCs (M-MDSCs)[87]. Trabectedin has been shown to activate a TRAIL-dependent pathway of apoptosis[87] (FIG. 4); monocytes are exquisitely sensitive to TRAIL because, unlike other leukocyte subsets (particularly neutrophils), they express very low levels of TRAIL decoy receptors[176]. In mouse tumour models and in human sarcoma specimens, trabectedin-induced reductions in TAM density are associated with decreased angiogenesis[87]. These observations raise the question as to whether the combination of trabectedin with antiangiogenic agents and/or immune-checkpoint inhibitors might increase the effectiveness of treatment.

Functional activation of TAMs. Microbial preparations and microorganism-derived molecules (such as bacterial muramyl-dipeptide) prime macrophages for tumour cytotoxicity and have undergone clinical testing in this context[177]. Intravesical Bacillus Calmette-Guérin (BCG) is the only remainder from the bacterial era of immunotherapy, and is used in the treatment of recurrent bladder carcinoma. In addition to, or in concert with microbial moieties, such as lipopolysaccharide,

IFNγ is a classic inducer of macrophage M1 polarization and killing of tumour cells[10]. The therapeutic utility of this cytokine has been investigated in patients with ovarian cancer; with the aim of avoiding unwarranted systemic macrophage activation, IFNγ was administered intraperitoneally, first in women with advanced-stage ovarian cancer, and subsequently to those with minimal residual disease[178,179]. Intraperitoneal IFNγ resulted in activation of tumour cytotoxicity and clinical responses[178,179]. Whether the potential of IFNγ immunotherapy has been fully exploited under these conditions remains unclear.

A more specific, although unexpected, approach to targeting macrophages was discovered after administration of an agonistic anti-CD40 antibody to mice with pancreatic cancer[180]. In this setting, alternatively activated, M2-like macrophages in the tumour microenvironment were re-educated towards an M1-like phenotype, and acquired antigen-presenting capabilities, leading to re-establishment of tumour immune surveillance and short-term reductions in tumour volumes[180]. This preclinical evidence spurred phase I clinical trials to test a fully humanized antibody CD40 agonist (CP-870,893) in combination with chemotherapy[181] (TABLE 2). In patients with advanced-stage pancreatic cancer, combined treatment with CP-870,893 and gemcitabine was well tolerated, with four of 22 patients achieving partial responses. The investigators noted that a decrease in 2-[^{18}F]fluoro-2-deoxy-D-glucose uptake on PET–CT imaging of hepatic lesions was correlated with improved survival in patients receiving the combination therapy[181].

Repolarization of proangiogenic and immunosuppressive M2-like macrophages towards the antitumour M1-like phenotype has also been achieved in mice via expression of antiangiogenic and immunomodulatory protein histidine-rich glycoprotein (HRG)[182]. This effect of HRG was dependent on downregulation of placenta growth factor (PlGF) in macrophages, supporting the evaluation of PlGF-blockade-based strategies as anticancer therapies[182]. In addition, a modified form of vitamin-D-binding protein, EF-022, is undergoing early clinical evaluation (TABLE 2), based on evidence of effects on macrophage activation[183].

ADCC and ADCP are amenable to therapeutic strategies capitalizing on the effector function of TAMs. However, enhancement of macrophage-dependent ADCP via interference with the inhibitory CD47–SIRPα pathway might involve mechanisms that lie beyond pure activation of TAM effector function. In a mouse xenograft model of glioblastoma, in which the M1:M2 TAM ratio has prognostic significance, ADCP elicited by anti-CD47 antibody therapy resulted in functional skewing of mouse macrophages towards an M1 phenotype, thus contributing to antitumour immune responses[129].

In a preclinical model of pancreatic cancer, crosstalk between B cells and FcRγ$^+$ TAMs has been shown to promote M2-like macrophage programming via BTK–PI3Kγ signalling, and was implicated in tumour progression; administration of a PI3Kγ inhibitor or the BTK inhibitor ibrutinib reset macrophages toward a M1-like phenotype that promoted CD8$^+$ T-cell cytotoxicity and curbed pancreatic ductal adenocarcinoma growth[184]. This strategy is currently under clinical evaluation in combination with gemcitabine–nab-paclitaxel chemotherapy in patients with pancreatic cancer[119] (TABLE 2).

The use of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, is associated with protection against the occurrence of many tumour types, as well as against the development of metastasis in patients with cancer[185–187]. Prostaglandin E_2 (PGE_2) has well-established immunosuppressive effects (FIG. 2), for instance, on dendritic cells, and is known to promote the development of MDSCs[188]. Intriguingly, PGE_2 has been shown to cause transactivation of CSF-1R via Src family kinase signalling in mouse macrophage cell lines, thus enhancing their migratory capacity (and acting synergistically with CSF-1 in inducing macrophage migration)[189]. Prostaglandins (such as PGE_2) have also been reported to be involved in the M2-like polarization of macrophages, in part, through activation of the cAMP pathway[190,191]. Thus, it is tempting to speculate that targeting the tumour-associated myeloid cells plays a major part in the protective function of NSAIDs against both primary cancer and metastasis.

Conclusions

Cells of the monocyte–macrophage lineage are an essential inflammatory component of the ecological tumour niche, and have key roles in regulating disease progression. Progress has been made in defining the molecular landscapes and mechanisms of macrophage differentiation and diversity in tissues, including tumours[21,24,192]. Macrophage diversity relates to the presence of TAMs with different functional profiles within tumours, which is often dictated by hypoxia. Current general paradigms relating to TAMs reflect assessments of these heterogeneous cells at the total population level. Deconvoluting TAM heterogeneity at the single-cell level and integrating such information into functional studies are important challenges that must be addressed in the future, in order to provide new insights into cancer-related inflammation.

Macrophages can exert dual influences on the effects of conventional cytoreductive therapies and radiotherapy. Moreover, TAMs contribute to creating an immunosuppressive tumour microenvironment through multiple routes, including triggering of inhibitory immune checkpoints in T cells. Determining whether TAMs are predictive biomarkers that guide the use of cytoreductive therapies and immunotherapy, and thereby contribute to personalized patient care, will be important.

Macrophage-centred therapeutic approaches are entering the clinical arena (TABLE 2). These strategies include blockade of the tumour-promoting activities of TAMs, and exploitation of macrophage antitumour effector functions (including ADCC, ADCP, and M1-like phenotypes). Macrophage-targeting strategies can *per se* result in therapeutic benefits; however, our tenet is that macrophage-directed therapeutics are best used to complement conventional cytoreductive therapies, angiogenesis inhibitors, and immunotherapy.

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Competing interests statement
The authors declare no competing interests.