The tumour microenvironment (TME) provides an essential ecological niche for cancer initiation and progression. Inflammatory cells and mediators are key universal components of the TME, and tumour-associated macrophages (TAMs) have served as a paradigm for the connection between inflammation and cancer.

The construction and orchestration of an inflammatory TME can be driven by genetic events that cause cell transformation and progression (the so-called intrinsic pathway) and by inflammatory conditions that predispose to neoplasia (the extrinsic pathway) such as inflammatory bowel disease. There is considerable diversity in the inflammatory components of the TME in cancers from different tissues. However, infiltration of myelomonocytic cells, specifically monocytes, macrophages and dendritic cells, represents a common denominator of cancers, irrespective of their origin and localization.

Myelomonocytic cells Haematopoietic cells, including monocytes, macrophages and monocyte-derived dendritic cells.

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Among myelomonocytic cells, macrophages are double-edged swords with dual potential in cancer, a reflection of their plasticity in response to environmental cues. Macrophages have the potential to kill tumour cells, mediate antibody-dependent cellular cytotoxicity and phagocytosis, elicit vascular damage and tumour necrosis, and activate innate or adaptive lymphoid cell-mediated mechanisms of tumour resistance. By contrast, in most established tumours, macrophages contribute to cancer progression and metastasis by various mechanisms, including promotion of cancer cell survival and proliferation, angiogenesis, and suppression of innate and adaptive immune responses (Fig. 1). Macrophages have an important role in the anti-tumour activity of chemotherapy, radiotherapy and monoclonal antibodies (mAbs) by mediating tumoricidal activity and eliciting adaptive immune responses. Moreover, they are an important target of current checkpoint blockade immunotherapy by expressing inhibitory counter-receptors (such as PDL1 and PDL2), thus suppressing adaptive immune responses.

Strategies specifically aimed at targeting macrophages, including mononuclear phagocytes engineered for cell therapy such as chimeric antigen receptor macrophages (CAR-M), have entered clinical evaluation. Therapeutic approaches undergoing clinical assessment cover a broad range of strategies, ranging from targeting recruitment and differentiation to functional reprogramming by engaging activating or inhibitory (checkpoint) receptors, the latter with encouraging results.

Here, we will review strategies to exploit macrophages as therapeutic tools and targets in cancer therapy, including the role of TAMs in conventional anticancer...
therapies and checkpoint blockade, strategies to reshape and activate TAMs, metabolic approaches, and macrophage cell therapies. Previous reviews on the basic immunobiology of TAMs will provide a framework for this essay.

TAM diversity and prognosis

It has long been held that TAMs originate from bone marrow (BM)-derived monocytic precursors, which replenish the tumour compartment and, in a number of mouse models, the majority of TAMs indeed derive from monocytic precursors (BOX 1). However, the discovery that, under homeostatic conditions, tissue-resident macrophages (TRMs) originate from embryonic precursors that seed organs in embryonic life has raised the issue that TRMs, in addition to BM-derived precursors, might sustain TAM levels (Fig. 1). In a mouse model of pancreatic cancer, TRMs proliferated locally and acquired a pro-fibrotic transcriptional profile, promoting a desmoplastic reaction, typical of pancreatic ductal adenocarcinoma. In addition, in murine ovarian cancer, a population of self-renewing CD163+ Tim4+ macrophages that reside in the omentum promoted metastatic spread by generating a protecting niche for cancer stem cells.

Brain macrophages, also known as microglia, were the first TRM population shown to derive from embryonic precursors; they expand during development and maintain themselves until adulthood. However, in inflammatory conditions associated with disruption of the blood–brain barrier, BM-derived progenitors can supplement the microglial population (BOX 1). In murine glioma, although both brain microglia and infiltrating peripheral macrophages can promote glioma cell proliferation and migration, TAMs mostly derive from resident microglia. Microglia, and not BM-derived macrophages, promote murine glioblastoma via mTOR-mediated immunosuppression. Furthermore, immunoediting induced by the immune response, and specifically by macrophage-derived IFNγ, results in stable epigenetic changes in transcriptional circuits in glioblastoma cells. These include the activation of critical myeloid-affiliated transcription factors and other immune-related pathways, which leads to increased recruitment of TAMs and the establishment of a pro-tumorigenic microenvironment.

Fig. 1 | Pro-tumoural functions and immunosuppressive activity of TAMs. The pro-tumour functions of tumour-associated macrophages (TAMs) are diverse and act at different phases of tumour development. TAMs release nitric oxide (NO) and reactive oxygen intermediates (ROI), which cause DNA damage and genetic instability during the initiation phase. TAMs produce epidermal growth factor (EGF) and several mediators such as IL-6, hepatocyte growth factor (HGF) and GPNMB, which support cancer stem cell expansion. At later stages, TAMs contribute to metastatic spread by releasing IL-1 and transforming growth factor-β (TGFβ), which are also involved — together with several proteases — in extracellular matrix (ECM) remodelling and pathological fibrosis. TAMs are a critical source of angiogenic factors: vascular endothelial growth factor (VEGF) and pro-angiogenic chemokines. TAMs are drivers of immunosuppression in the tumour microenvironment. Secretion of IL-10, TGFβ, prostaglandins and indoleamine 2,3-dioxygenase (IDO) promote the expansion of regulatory T (Treg) cells, inappropriate skewing of dendritic cells towards an immature and tolerogenic state, and T cell metabolic starvation. Immunosuppressive TAMs are characterized by a high expression of immune-checkpoint molecules (PDL1, PDL2, B7-H4) causing T cell exhaustion. EMT, epithelial–mesenchymal transition; ILC3, type 3 innate lymphoid cell; T<sub>reg</sub>, T helper 17.
Until a few years ago, tissue-resident macrophages (TRMs) were thought to originate exclusively from blood monocytes derived from bone marrow haematopoietic stem cells that were constantly recruited to tissues and differentiated into tissue-specific macrophage populations. Fate mapping studies in mice have completely changed the view on the ontogeny of TRMs. In homeostatic conditions, circulating monocytes contribute to tissue macrophages only in specific tissues, such as dermis, intestine and heart, and this contribution increases with age. In other tissues, such as skin, brain, liver or lung, macrophage populations derive from embryonic precursors originating in the yolk sac and fetal liver, and infiltrate these tissues during embryogenesis, then replicating locally throughout adulthood. Thus, Langerhans cells in skin, microglia in brain, Kupffer cells in liver, and alveolar macrophages in lung maintain themselves independently of adult bone marrow cells. Other tissues contain macrophages of both origins, embryonic and bone marrow derived, the frequency of which depends on the tissue and pathological condition. In homeostatic conditions, and particularly upon infection or inflammation, adult bone marrow-derived cells are recruited in the tissue and replace embryonic macrophages, undergoing gene reprogramming and acquiring similarities to their counterpart of embryonic origin. This reprogramming is mostly instructed by the local environment. Indeed, tissue-specific macrophage populations are characterized by distinct transcriptional signatures and epigenetic marks, which are dictated by specific tissue factors.

Macrophages are highly plastic cells undergoing diverse forms of functional activation in response to different signals. Historically and schematically, macrophages have been divided into two forms of macrophage activation, often referred to as M1 (or ‘classical’) and M2 (or ‘alternative’). M1 macrophages may be induced in vitro by bacterial products and interferons produced during type 1 immune responses driven by type 1 T helper and innate lymphoid cells, whereas M2 macrophages are induced by cytokines produced during type 2 immune responses driven by type 2 T helper and innate lymphoid cells, such as IL-4 and IL-13 (REF. 21). M1 polarization is associated with macrophage-dependent tissue damage and tumour cell killing, whereas M2 polarization promotes tissue repair and remodelling and resistance to parasites. In vivo, several inflammatory conditions or tissue damage, dynamic changes in macrophage activation are observed, with classically activated M1 cells implicated in initiating and sustaining inflammation and M2 or M2-like cells associated with the resolution of or smouldering chronic inflammation. However, in general, mixed phenotypes or populations with different phenotypes coexist, indicating that the M1/M2 classification is too simplistic for this transcriptionally dynamic cell type.

In the context of cancer, signals orchestrating this plasticity vary considerably between different tumours, or different parts and stages of the same tumour, resulting in varied tumour-associated macrophage (TAM) phenotypes. Tumour cell-derived cytokines, such as IL-10 and colony-stimulating factor 1 (CSF1), and chemokines, such as CCL2, CCL18, CCL17 and CXCL4, are key promoters of the functional plasticity of TAMs in a pro-tumour mode. ‘M2-like’ polarization of TAMs refers to functional programmes associated with tumour promotion and suppression of effective adaptive immunity. A new emerging factor affecting macrophage polarization is the neurotransmitter γ-aminobutyric acid (GABA), unexpectedly secreted by activated B lymphocytes. GABA can promote monocyte differentiation into anti-inflammatory macrophages that produce IL-10 and inhibit cytotoxic CD8+ T cells. As discussed in this article, in general, single-cell analysis of TAMs in mouse and human tumours has revealed a complex scenario of mononuclear phagocyte clusters well beyond the original simplistic M1/M2 classification (see REFS. 6,7). The M1-like and M2-like terms retain communication value as long as the limitation of this classification is considered and, herein, they are used to reflect the usage in the papers quoted.

Using a rigorous genetic approach, Casanova-Acebes et al. shed new light on the origin of dynamics of TAMs in a mouse model of lung adenocarcinoma. They used lineage tracing to genetically fate-map monocyte-derived macrophages and self-maintained TRMs combined with single-cell RNA sequencing (scRNA-seq) and found that tumour-infiltrating macrophage populations differ in origin and have a distinct temporal and spatial distribution in the TME. TRMs were the main source of TAMs and provided a nurturing niche in the very early phases of progression. Later, monocyte-derived macrophages were recruited at the tumour site and propelled neoplastic development. Similar findings were reported in mouse and human glioblastoma. In apparent contrast to these studies, in lung cancer models, both TRM and monocyte-derived TAMs contributed to generating an immunosuppressive TME in early stages by sustaining regulatory T cells. Perhaps unexpectedly, macrophages in early-stage human lung cancer did not exert immunosuppressive functions.

There are many substantial differences between mouse and human mononuclear phagocytes (including their proliferative capacity and their response to classic polarization signals such as those triggered by IFNγ, bacterial lipopolysaccharides and IL-4), which differ in the degree of plasticity that allows in vivo plasticity to vary considerably between different tumours, or different parts and stages of the same tumour, resulting in varied tumour-associated macrophage (TAM) phenotypes. Tumour cell-derived cytokines, such as IL-10 and colony-stimulating factor 1 (CSF1), and chemokines, such as CCL2, CCL18, CCL17 and CXCL4, are key promoters of the functional plasticity of TAMs in a pro-tumour mode. ‘M2-like’ polarization of TAMs refers to functional programmes associated with tumour promotion and suppression of effective adaptive immunity. A new emerging factor affecting macrophage polarization is the neurotransmitter γ-aminobutyric acid (GABA), unexpectedly secreted by activated B lymphocytes. GABA can promote monocyte differentiation into anti-inflammatory macrophages that produce IL-10 and inhibit cytotoxic CD8+ T cells. As discussed in this article, in general, single-cell analysis of TAMs in mouse and human tumours has revealed a complex scenario of mononuclear phagocyte clusters well beyond the original simplistic M1/M2 classification (see REFS. 6,7). The M1-like and M2-like terms retain communication value as long as the limitation of this classification is considered and, herein, they are used to reflect the usage in the papers quoted.
Macrophage colony-stimulating factor 1 (CSF1), chemokines such as CCL2, cytokines such as IL-4 and IL-1, immune complexes recognized by receptors against the Fc portion of immunoglobulin G (FcγRI), and complement (FIG. 1). In addition, histamine released in allergic reactions and from tumour cells activates macrophages, promoting an M2-like immunosuppressive phenotype in mice and humans, leading to the suppression of CD8^+ T cell functions through the immune checkpoint V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA) and conferring resistance to immunotherapy. The function of TAMs can also be affected by the interaction with the microbiota or in response to the dysbiosis associated with cancer development. Bacterial components enriched in tumour tissues can increase the recruitment of suppressive myeloid cells and their pro-tumour polarization. Similarly, antibiotic treatment can have dual effects on tumour growth and a critical interaction of the microbiome with anticancer strategies has been documented.

Analysis of TAMs at a whole-population level has shown an M2-like phenotype in murine and human tumours (see BOX 1 for a definition of M1 and M2 phenotypes and their limitations). However, dissection of TAMs, using conventional and scRNA-seq or mass cytometry by time-of-flight (CyTOF) approaches, has revealed the existence of diverse macrophage cell clusters with distinct transcriptomic and proteomic profiles, including macrophages with potential anti-tumour activity. In the context of lung cancer, single-cell transcriptomics revealed conserved myeloid (BOX 2) populations across patients as well as in mice. In addition, analyses of scRNA-seq combined with cellular indexing of transcriptomes and epigenomes by sequencing (CITE-seq), which allow transcriptomic and multiplexed single-cell surface protein dissection, showed that the predominant populations enriched in human non-small-cell lung cancer (NSCLC) were monocyte-derived macrophages, whereas alveolar and interstitial macrophages were respectively depleted and stable. In colorectal cancer (CRC), scRNA-seq analyses highlighted the presence of conserved subsets of myeloid cells across human and mouse species, which showed distinct functional signatures (inflammatory or angiogenic), and differential sensitivity to myeloid-targeted therapeutic approaches such as treatment with the antibodies anti-CSF1R and CD40 agonists, which target macrophages and conventional dendritic cells, respectively. Multicellular interaction networks, analysed by spatial profiling, underlie immunological and tumorigenic processes in human CRC. These include a myeloid-rich inflammatory hub at the luminal margin of primary tumours associated with tissue damage, and an immune hub within tumours deficient in mismatch repair pathway, which are enriched with activated T cells and myeloid cells expressing T cell-attracting chemokines. ScRNA-seq analysis of human brain tumours and metastases revealed disease-specific leukocyte enrichment, with pronounced differences in the abundance of microglia, infiltrating monocyte-derived macrophages and other leukocytes between primary and metastatic tumours, indicating that tumours arising within the brain shape their TME differently compared with cancers that metastasize from extracranial sites.

Consistently with a prevailing pro-tumour orientation of TAMs, high macrophage infiltration is associated with poor prognosis in most human tumours, whereas lymphoid infiltration is associated with better prognosis. In liver metastasis from CRC, TAMs with different morphologies and molecular fingerprints coexist and correlate with clinicopathological variables, whereby large TAMs were associated with a poor survival rate compared with small TAMs. In breast cancer, crown-like structures, composed of macrophages surrounding dying adipocytes in a crown-like pattern, support tumour progression and have prognostic potential.

A better understanding of pathways responsible for recruitment and functional skewing of TAMs has provided a basis for the development of macrophage-centred therapeutic strategies. The negative prognostic significance of macrophage infiltration, as assessed by conventional immunohistology, molecular signatures or single-cell analysis, has given impetus for the clinical assessment of macrophage-targeting strategies. However, the redundancy of regulatory pathways and the diversity and plasticity of mononuclear phagocytes in situ represent stumbling blocks and may account for failures as described below.
Macrophages have an important, dual role in the activity of different anticancer modalities, including chemotherapy, radiotherapy, anti-angiogenic and hormonal therapies, and immune-checkpoint blockade (ICB) immunotherapy. Some selected chemo-therapeutic agents (such as doxorubicin) induce the release of tumour antigens and adjuvant molecules, a process known as immunogenic cell death, engaging macrophages in a fruitful cancer-immunity cycle. Other cytoreductive therapies target macrophages by depleting them. Trabectedin is a registered compound extracted from a marine organism and approved as a single agent for the treatment of soft tissue sarcomas and ovarian cancer. Trabectedin and its analogue lurbinectedin — recently approved for the treatment of NSCLC — selectively deplete monocytes and TAMs in patients and mice through the tumour necrosis factor receptor superfamily member 10 (TNFRSF10, also known as TRAIL)-dependent apoptosis pathway. TAM depletion is essential for the full antitumour activity of trabectedin.

Selected anticancer drugs can revert TAM polarization, resulting in increased response to the treatment such as gemcitabine in pancreatic cancer, 5-fluorouracil in CRC and platinum-based neoadjuvant chemotherapy in high-grade ovarian cancer. For drugs that induce DNA damage via the generation of reactive oxygen species (ROS), such as platinum-based chemotherapies, the gut microbiome can prime intratumour mononuclear phagocytes to produce ROS, positively modulating the efficacy of these agents. In the case of radiotherapy,
Coelomic cavities
They include pleural, peritoneal and pericardial cavities that originate during embryogenesis in the gastrulation phase. These cavities are lined by specialized mesodermal cells called mesothelium.

opposing effects of commensal bacteria and fungi were reported following radiation, with depletion of fungi enhancing responsiveness to radiation in mouse models of breast cancer and melanoma⁵⁴.

In this context, macrophages act by contributing to the activation of adaptive immune responses by anti-cancer treatments⁵⁹,⁶¹, by cooperating in tumour cell killing⁶⁰ and by being a target themselves⁶⁰,⁶¹. In a seemingly opposite direction, TAMs limit the effectiveness of selected chemotherapeutic agents, driving detrimental reactive responses to tissue damage cues and rapidly reprogramming towards a pro-remodelling state⁶²,⁶³. Glioblastoma-associated microglia can activate the STAT3–MYC signalling axis in tumour cells, promoting resistance to temozolomide⁷⁰. Antibodies against CSF1 and CSF1R are used to target macrophages by inhibiting their recruitment and depleting and re-educating them (see below, in the Reshaping TAMs section). Accordingly, this strategy may synergize with chemotherapy as shown in breast cancer preclinical models⁶⁸–⁷¹ and is being evaluated in clinical trials⁷²,⁷³.

Tumour-infiltrating leukocytes are key players in the antitumour activity of selected mAbs that act by triggering immune cells — TAMs in particular — that express FcyR to perform tumour cell killing and phagocytosis⁷³–⁷⁵. Such therapeutic antibodies are currently in use in the clinical setting and include rituximab (targeting CD20 [REF. ⁷⁶]), trastuzumab (an antibody against HER2), cetuximab (which targets epidermal growth factor receptor (EGFR))⁷⁷ and daratumumab (which targets CD38 on myeloma cells). Functional polymorphisms in human FcyRIIA that affect the killing capability of macrophages correlate with rates of response to mAbs in patients with lymphoma treated with rituximab⁷⁸, patients with breast cancer treated with trastuzumab⁷⁹ and patients with metastatic CRC treated with cetuximab⁸⁰.

The density of TAMs frequently correlates with the density of vessels in tumour tissues⁸¹ as TAMs both secrete and actively respond to angiogenic growth factors, primarily vascular endothelial growth factor (VEGF)⁸²,⁸³. Accordingly, the activity of anti-angiogenic therapy is modulated by TAMs. VEGF antagonists induce vascular normalization⁸⁴ and concomitantly remodel the TAM phenotype⁴,⁸¹, whereas myeloid cells mediate resistance to anti-angiogenic therapies via compensatory pathways such as cathepsin B and angiopoietin 2 (ANG2). This evidence has provided a basis to test bispecific ANG2–VEGF antibodies, which have shown promising results in preclinical models of glioblastoma⁴,⁸².

Similarly, hormonal therapy is influenced by inflammatory pathways orchestrated by myeloid cells. Inflammatory cytokines, such as IL-1 and IL-6, can activate oestrogen receptor signalling on tumour cells⁸³,⁸⁴, linking inflammation to tumour growth and endocrine resistance. In prostate cancer, TAMs express the androgen receptor, which drives their pro-tumour activation⁸⁵. Anti-androgen treatment reduced the number of TAMs in human prostate cancer⁸⁶ whereas, in a preclinical model, blockade of myeloid cell-derived IL-23, another key inflammatory cytokine released by myeloid-derived suppressor cells (MDSCs), improved response to androgen-deprivation therapy⁸⁷.

The results presented above suggest that myelomonocytic cells have an important influence on the activity of cancer chemotherapy, radiotherapy⁸⁸, anti-angiogenic agents and hormonal therapy. Their role is complex and dual, serving as amplifiers or inhibitors of antineoplastic activity. Although progress has been made in dissecting the yin-yang of macrophages in conventional antitumour treatment modalities, the actual translation of deeper knowledge into more effective treatments remains a challenge.

**TAMs and ICB immunotherapy**

Unleashing T cell-mediated type 1 immune responses represents the cornerstone of ICB immunotherapy. Myelomonocytic cells, major orchestrators of immunosuppressive circuits, are an important determinant of response to ICB and are critically involved in resistance and eligibility for this treatment⁴,⁶.

Myelomonocytic cells are part of tumour-extrinsic pathways of primary and adaptive resistance to ICB⁹⁰ by expressing several immunosuppressive molecules, including checkpoint ligands, such as PD-L1, PD-L2, CD80 (also known as B7-1) and CD86 (also known as B7-2), and poliovirus receptor (PVR; also known as CD155 and one of the TIGIT ligands)⁹¹–⁹³. PDL1 assessment by immunohistochemistry is approved as a companion diagnostic for anti-PD1 therapy in NSCLC and other tumours⁹⁴–⁹⁶, but its predictive capability may vary according to the cell type considered, such as tumour or immune cells⁹⁷,⁹⁸. In preclinical models, PD-L1 expression on tumour-infiltrating immune cells but not on tumour cells was associated with response to anti-PD1 or anti-PDL1 antibodies. Unexpectedly, macrophages were reported to express PD1, which negatively correlates with their phagocytic activity towards tumour cells⁹⁹–¹⁰¹. Interestingly, myelomonocytic cells express other counter-receptors that interact with negative regulators expressed by T cells and natural killer (NK) cells. VISTA is expressed by macrophages and interacts with P-selectin glycoprotein ligand 1 (PSGL1)⁹⁸, functioning as a T cell checkpoint inhibitory ligand. In human monocytes, mAbs against VISTA elicited transcriptional and functional changes, including increased antigen presentation, activation and migration⁹⁹.

Tissue context is important in dictating the role of macrophages in ICB. For instance, in triple-negative breast cancer, distinct myeloid cell profiles, including neutrophils and macrophages, mediated resistance to ICB¹⁰² and hepatic macrophages in liver metastases negatively regulated responsiveness to systemic immunotherapy by eliminating T cells¹⁰³. The pleural and peritoneal cavity represent sites of tumour progression in an immunosuppressive microenvironment. In mouse models and in patients, TIM4+ macrophages in coelomic cavities suppress CD8+ T cell responses and inhibit ICB¹⁰⁴,¹⁰⁵ and blocking TIM4 with antibodies enhanced the efficacy of ICB at these sites. In human renal cell carcinoma (RCC), a predominance of M2-like macrophages was associated with resistance to ICB¹⁰⁶,¹⁰⁷. Response to immunotherapy...
can be modulated by the composition of the microbiome as shown for anti-CTLA4 and anti-PD1, in which abundance and diversity of the gut bacteria shape tumour myeloid infiltration.

Depletion of macrophages can potentiate various immunotherapeutic strategies, including vaccination and checkpoint inhibitors. Several clinical trials combining checkpoint inhibitors and anti-CSF1R antibodies or other TAM-centred therapeutic strategies are ongoing (Table 1).

Reshaping TAMs

Functional reprogramming of TAMs can be achieved with different strategies (Fig. 2).

Targeting TAM recruitment and accumulation. It has long been known that chemokines and CSF1 have a major role in the recruitment of monocytes in tumours and in shaping their function in the TME. Monocyte attractants, such as CCL2 and its cognate receptor CCR2, have been targeted using mAb and receptor antagonists in solid tumours and haematological malignancies. However, despite a wealth of data in preclinical models, chemokine targeting strategies have not yielded positive clinical results alone or in combination and have been discontinued (Table 1). Therapeutic strategies targeting CSF1 and CSF1R have also used mAbs or receptor antagonists. Several studies in preclinical models proved that CSF1R inhibition reduced the density of TAMs and tumour growth, and increased the sensitivity to chemotherapy. Although the small-molecule inhibitor BLZ945 did not deplete TAMs, these underwent a reprogramming towards an antitumour phenotype; upon CSF1R blockade, macrophages showed lower expression of pro-tumorigenic genes and upregulation of genes associated with antigen presentation and lymphocyte activation. BLZ945 reshaped the immune microenvironment inducing positive crosstalk among TAMs, IFNγ-producing NK and T cells, and IL-12-producing dendritic cells, thus sustaining a virally productive TAMs; in murine tumour models, these CAR T cells mediated selective elimination of FRβ+ TAMs, which resulted in the recruitment of pro-inflammatory monocytes and tumour-specific CD8+ T cells and restrained tumour growth.

Another limitation of strategies targeting macrophage recruitment and survival rests in the diversity of the mononuclear phagocyte populations in tumours. In a mouse melanoma model, global targeting of the total TAM population was not as effective as depletion of the CD163+ cells endowed with immunosuppression activity. Hence, strategies for specific TAM subsets are warranted.

Complement. Complement has emerged as an important component of tumour-promoting inflammation in murine and human cancers. The complement system consists of a cascade of sequential proteolytic reactions activated by three different pathways: the classical pathway, which is initiated by binding of C1q to immune complexes; the lectin pathway, which is initiated by the interaction of mannose-binding lectin or ficolins with pathogen-associated molecular patterns or aberrant carbohydrate structures on damaged cells; and the alternative pathway, which is directly activated by damaged cells or microbes. The activation of the central components C3 and C5 results in the release of their bioactive fragments (anaphylatoxins) and in the assembly of the cytolytic membrane attack complex. Unconventional complement activation may also occur owing to the proteolytic activity of coagulation and fibrinolytic enzymes.

In transplanted tumour models, the release of these anaphylatoxins, C5a, in particular, was associated with the recruitment of MDSCs into the tumour, thus promoting immunosuppression. In mouse tumour models of sarcomagenesis induced by 3-methylcholanthrene and in two transplanted sarcoma models, the lectin pathway-dependent C3a–C3AR axis played a dominant role in TAM recruitment and functional skewing, driving immunosuppression and tumour promotion.

In squamous carcinogenesis, urokine (uPA)– macrophages regulated C3-independent release of C5a during premalignant progression, which in turn promoted the pro-tumorigenic properties of C5aR1+ mast cells and macrophages, including suppression of T cell cytotoxicity. Both in mice and humans, factor H deficiency has been recently associated with increased susceptibility to hepatocellular carcinoma; the deficiency of this negative regulator of the alternative pathway was associated with spontaneous deposition of neutrophils, which may play an immunosuppressive part, and the apparent redundancy of the chemokine system with multiple ligands and receptors acting on monocytes.

Rodriguez-Garcia et al. have proposed an original approach to reduce the number of TAMs in tumours by exploiting the cytotoxic activity of CAR T cells against macrophages. The authors identified a subset of TAMs with immunosuppressive activity that expresses the folate receptor-β (FRβ) in addition to other M2 markers (CD204, CD206 and CD163). Engineered T cells with a CAR directed to FRβ specifically targeted immunosuppressive TAMs; in murine tumour models, these CAR T cells mediated selective elimination of FRβ+ TAMs, which resulted in the recruitment of pro-inflammatory monocytes and tumour-specific CD8+ T cells and restrained tumour growth.
| Compound (sponsor) | Clinical phase (status) | Tumour type | Combination partners | NCT identifier |
|--------------------|-------------------------|-------------|----------------------|----------------|
| **Chemokine inhibitors** | | | | |
| Carlumab (anti-CCL2 antibodies; Centocor) | Phase II (completed) | Prostate cancer | NA | NCT00992186 |
| BMS-813160 (CCR2/CCR5 antagonist; Bristol Myers Squibb) | Phase II (ongoing) | Renal carcinoma | Nivolumab plus ipilimumab | NCT02996610 |
| | Phase I/II (ongoing) | Pancreatic cancer, CRC, NSCLC | Nivolumab, Nab-paclitaxel | NCT03184870 |
| | Phase II (ongoing) | Hepatocellular carcinoma | Nivolumab | NCT04123379 |
| PF-4136309 (CCR2 antagonist; Pfizer) | Phase II (completed) | PDAC | Nab-paclitaxel, gencitabine | NCT01413022 |
| CCR5 antagonist (Pfizer) | Phase I (completed) | CRC | Pembrolizumab | NCT03274804 |
| | Phase I (ongoing) | Pancreatic cancer, CRC | Nivolumab plus ipilimumab | NCT04721301 |
| **CSF1R inhibitors** | | | | |
| PLX3397 (Plexxikon) | Phase I/II (ongoing) | Sarcoma, nerve-sheath tumours | Sirolimus | NCT02584647 |
| | | Advanced melanoma and solid tumours | Pembrolizumab | NCT02452424 |
| | | Breast cancer | Eribulin | NCT01596751 |
| | Phase I (completed) | Glioblastoma | Radiotherapy, temozolomide | NCT01790503 |
| BLZ945 (Novartis) | Phase I/II (ongoing) | Solid tumours | PDR001 (anti-PD1) | NCT02829723 |
| **Anti-CSF1R antibodies** | | | | |
| LY3022855 (IMC-CS4; Eli Lilly) | Phase I/II (ongoing) | Melanoma | MEK/BRAF inhibitors | NCT03101254 |
| Emactuzumab (RO5509554/RG7155; Roche) | Phase II (ongoing) | Gynaecological neoplasms and ovarian cancer | Bevacizumab, paclitaxel | NCT02923739 |
| | Phase I/II (ongoing) | PDAC | Nab-paclitaxel, gencitabine | NCT03193190 |
| | Phase I (completed) | Solid tumours | Paclitaxel | NCT01494688 |
| | Phase I (completed) | Solid tumours | Atezolizumab | NCT02323191 |
| | Phase I (completed) | Solid tumours | RO7009789 (agonist anti-CD40) | NCT02760797 |
| AMG820 (Amgen) | Phase I/II (completed) | Pancreatic cancer, CRC, NSCLC | Pembrolizumab | NCT02713529 |
| ARRAY-382 (Pfizer) | Phase I/II (completed) | Solid tumours | Pembrolizumab | NCT02880371 |
| Cabiralizumab (Bristol Myers Squibb) | Phase I/II (ongoing) | TNBC | Paclitaxel, carboplatin, nivolumab | NCT04331067 |
| **Anti-CD47/SIRPs antibodies** | | | | |
| HuSF9-C4 (Stanford University) | Phase I/II (completed) | Solid tumours | Cetuximab | NCT02953782 |
| | Phase I/II (ongoing) | Urothelial and bladder cancer | Multiple immunotherapy | NCT03869190 |
| BI 754091 (OSE Immunotherapeutics) | Phase I (ongoing) | Solid tumours | BI 754091 (anti-PD1) | NCT03990233 |
| **CD47–Fc fusion protein** | | | | |
| TTI-621 (Trillium) | Phase I (ongoing) | Haematological malignancies | Nivolumab, rituximab | NCT02663518 |
| | Phase I (ongoing) | Haematological malignancies | Chemotherapy | NCT03530683 |
| ALX148 (ALX Oncology) | Phase II (ongoing) | HNSCC | Pembrolizumab | NCT04675294 |
| | Phase II (ongoing) | HNSCC | Chemotherapy, pembrolizumab | NCT04675333 |
| **Agonist anti-CD40 antibodies** | | | | |
| CP-870,893 (Pfizer; UPenn) | Phase I (completed) | Melanoma | NA | NCT02225002 |
| | Phase I (completed) | Solid tumours | Paclitaxel, carboplatin | NCT00607048 |
| | Phase I (completed) | Pancreatic cancer | Gemcitabine | NCT01456585 |
| SEA-CD40 (Seagen) | Phase I (ongoing) | Solid and haematological tumours | Pembrolizumab, gencitabine, Nab-paclitaxel | NCT02376699 |
of complement activation fragments throughout the sinusoids, chronic activation of inflammatory signalling pathways, and increased hepatic infiltration of macrophages and T lymphocytes.124.

In patients, the prognostic potential of complement molecules, in particular C4d, C5a and C5aR1, has been demonstrated in different types of cancer.120 For instance, the complement activation fragment C4d

| Compound (sponsor) | Clinical phase (status) | Tumour type | Combination partners | NCT identifier |
|-------------------|-------------------------|-------------|----------------------|---------------|
| Agonist anti-CD40 antibodies (cont.) | | | | |
| APX005M (Apexigen) | Phase I (ongoing) | Melanoma, renal carcinoma | Nivolumab, ipilimumab | NCT04495257 |
| | Phase I (ongoing) | Melanoma | Pembrolizumab | NCT02706353 |
| | Phase II (ongoing) | Oesophageal cancer | Radiation, paclitaxel, carboplatin | NCT03165994 |
| | Phase I/II (ongoing) | Pancreatic cancer | Nab-paclitaxel, gemcitabine, nivolumab | NCT03214250 |
| RO7009789 (Roche) | Phase I (completed) | Solid tumours | Vanucizumab (anti-Ang2–VEGF bispecific antibody) | NCT02665416 |
| | Phase I (completed) | Pancreatic cancer | Nab-paclitaxel and gemcitabine | NCT02588443 |
| CDX-1140 (Roswell Park Cancer Institute) | Phase I (ongoing) | Breast cancer | Radiation, biological therapy, poly-ICLC | NCT04616248 |
| NG-350A adenoviral vector (PsiOxus Therapeutics Ltd) | Phase I (ongoing) | Solid tumours | Immune-checkpoint blockade immunotherapy | NCT05165433 |

**TLR agonists**

| TLR3 agonists | | | | |
| Hiltonol (Oncovir, Inc.) | Phase II (completed) | Solid tumours | Autologous dendritic cells | NCT01734564 |
| Poly-ICLC | Phase II (ongoing) | Solid tumours | Durvalumab, tremelimumab | NCT02643303 |
| BO-112 (Nanoplexed TLR3 agonist; Highlight Therapeutics) | Phase II (ongoing) | CRC, gastric cancer | Pembrolizumab | NCT04508140 |
| SHV2150 | Phase I/II (ongoing) | Solid tumours | Anti-PD1 and/or anti-CD47 | NCT04588324 |
| TransCon (TLR7/8 agonist; Ascendis Pharma) | Phase I/II (ongoing) | Solid tumours | Pembrolizumab | NCT04799054 |
| BDC-1001 (TLR7/8 agonist plus trastuzumab Bolt Biother) | Phase I/II (ongoing) | HER2+ solid tumours | Pembrolizumab | NCT04278144 |
| CMP-001 (TLR9 agonist; Checkmate Pharmaceuticals) | Phase I (terminated) | Melanoma | Pembrolizumab | NCT03084640 |
| Phase II (ongoing) | Melanoma | Nivolumab | NCT04401995 |

**TREM2 inhibitor**

| PY314 (anti-TREM2 antibodies; Pionyr Immunotherapeutics) | Phase I (ongoing) | Solid tumours | Pembrolizumab | NCT04691375 |

**Clever 1 inhibitor**

| FP-1305 (anti-Clever 1 antibodies; Faron Pharmaceuticals) | Phase I (ongoing) | Solid tumours | NA | NCT03733990 |

**Complement inhibitors**

| IPHS401 (anti-C5aR antibodies; Innate Pharma) | Phase I (ongoing) | Solid tumours | Durvalumab | NCT03665129 |

**Macrophage cell therapy**

| CT-0508 (adenovirus-transduced macrophages with anti-HER2-CAR; Carisma Therapeutics) | Phase I (ongoing) | HER2+ solid tumours | NA | NCT04660929 |
| TEMFERON (autologous HSPC-derived myeloid cells expressing IFNa2; Genenta Science) | Phase I/II (ongoing) | Glioblastoma | NA | NCT03866109 |

CRC, colorectal cancer; CSF1R, colony-stimulating factor 1 receptor; HSPCs, haematopoietic stem and progenitor cells; NA, not applicable; NSCLC, non-small-cell lung cancer; HNSCC, head and neck squamous cell carcinoma; NCT, National clinical trial; PDAC, pancreatic ductal adenocarcinoma; SIRPα, signal regulatory protein-α; TLR, toll-like receptor; TNBC, triple-negative breast cancer; TREM2, triggering receptor expressed on myeloid cells 2.
Inflammasome and IL-1. Although IL-1 can trigger protective immune responses, the predominant function of this pathway is to promote carcinogenesis and metastasis. IL-1 promotes cancer-associated immunosuppression through complex mechanisms, including driving emergency haematopoiesis and the generation and recruitment of MDSCs as well as promoting TAM immunosuppressive and tumour-promoting functions. Accordingly, in a model of RCC, the combination treatment with anti-IL-1β and anti-PD1 or the multi-targeted tyrosine kinase inhibitor cabozantinib led to a synergistic antitumour effect, a pronounced decrease of MDSCs and skewing of TAMs. Incidental findings from CANTOS — a large, phase III randomized, double-blind, placebo-controlled trial of canakinumab, an IL-1β blocking antibody, involving 10,061 patients with a history of myocardial infarction with atherosclerosis — demonstrated that canakinumab was associated with a dramatic (>60%) reduction of incidence and mortality from lung cancer. Based on preclinical studies, it is conceivable that inhibition of MDSCs and unleashing of innate and adaptive effective antitumour responses contribute to cancer prevention by canakinumab as observed in the CANTOS trial.

IL-1 can be targeted directly, for instance, through canakinumab, or upstream through inflammasome inhibition. For instance, in a mouse melanoma model, pharmacological inhibition of tumour-derived NLRP3, a sensor component of the inflammasome, limited MDSC-mediated T cell suppression and tumour recognition molecule pentraxin 3 (PTX3) can act as an extrinsic tumour suppressor gene through the regulation of complement-dependent and macrophage-sustained tumour-promoting inflammation in sarcomas.

Preclinical studies provide evidence for the therapeutic potential of C3a and C5a inhibition, for instance, by targeting immunosuppressive MDSCs in cervical cancer and lung metastasis from breast cancer, or macrophages in sarcomas. Of particular interest are studies on the combined blockade of complement and the PD1–PD1 immune-checkpoint axis. In particular, in lung cancer models, the combination of C5a and PD1 blockade markedly reduced tumour growth and metastasis and led to prolonged survival. This effect was associated with increased frequency of CD8+ T cells and reduced frequency of MDSCs within tumours, suggesting the restoration of antitumour immune responses (Fig. 2). Along the same line, the combination of a C3aR antagonist (SB 290157 trifluoroacetate salt) with anti-PD1 treatment resulted in a significant reduction of primary tumour growth and incidence, compared with anti-PD1 or C3aR treatment alone, indicating that complement inhibition increases the clinical benefit of ICB.

Based on the above preclinical and clinical evidence, complement targeting strategies deserve to undergo (or are undergoing) clinical assessment (TABLE 1). We surmise that assessment of the actual involvement of complement pathways in different tumours or tumour subsets should guide clinical assessment of anti-complement strategies.
progression. The antitumour potential of NLRP3 inhibition was further amplified by the combination with anti-PD1 treatment\textsuperscript{159}. These results call for prevention trials with anti-IL-1β and/or inflammasome inhibitors. A pharmacoprevention trial (Canal, 2020-002773-10), using canakinumab (anti-IL-1β) in patients at high risk of developing lung cancer (such as heavy smokers) has been planned but not activated to date owing to the SARS-CoV-2 pandemic. We feel that bold pharmacoprevention approaches targeting tumour-promoting inflammation pathways should be considered in selected patient populations.

**mRNA and microRNA.** In vitro-transcribed mRNA has recently come into focus as a potential new drug class to induce the production of effector molecules or reprogramme cells. mRNA formulated into an injectable nanocarrier has been used to genetically reprogramme TAMs into antitumour effectors. In different mouse cancer models, infusion of nanoparticles formulated with mRNAs encoding the transcription factor interferon regulatory factor 5 (IRF5) in combination with its activating kinase, inhibitor of NF-κB kinase subunit-β (IKKβ), reversed the immunosuppressive TME and reprogrammed TAMs, achieving tumour regression\textsuperscript{139}. MicroRNA (miRNA) are small non-coding RNA molecules with an important role in regulating post-transcriptional gene expression. miRNA have emerged as key modulators of macrophage differentiation and polarization, including TAMs\textsuperscript{140,141}. miRNA-based therapies in the context of cancer have been tested in experimental tumour models with multiple delivery strategies. As an example, miRNA-155 enveloped in lipid-coated phosphonate nanoparticles decorated with mannose can specifically deliver miRNA-155 to TAMs and successfully reprogrammed them towards antitumour effectors (Fig. 2); treated mice showed a significantly reduced tumour burden\textsuperscript{141}. Of interest, conditional deletion in macrophages of Dicer1, encoding miRNA-processing enzyme endoribonuclease DICER, resulted in marked activation of IFN-γ–STAT1 signalling that rewired TAM immunosuppression\textsuperscript{142}. So far, no clinical trials have been initiated using miRNA delivery to reprogramme tumour macrophages.

**Macrophage activation**

The tumoricidal activity of M1-like macrophages (Box 1) is triggered by receptors sensing microbial molecules and cytokines such as IFNγ. In this section, we will focus on three classes of macrophage activators\textsuperscript{143,144} (Fig. 2).

**CD40.** The CD40 receptor is a member of the TNF receptor family expressed on antigen-presenting cells, including macrophages. When engaged by its specific ligand CD40L, expressed on the surface of activated T helper lymphocytes, it triggers the production of TNF, ROS and reactive nitrogen species. These factors mediate the bactericidal and tumoricidal activity of macrophages. The CD40–CD40L axis is a feedforward loop that activates antigen-presenting cells, which further stimulate T cell–mediated immunity (Fig. 2). Several agonistic CD40 mAbs have been generated that mimic the effect of CD40. In preclinical studies, these CD40 agonistic mAbs successfully re-educated immunosuppressive TAMs by switching them into cytotoxic effectors, eventually resulting in immune surveillance and reduced tumour growth\textsuperscript{150}. Upon administration of CD40 agonists, there was an increased influx of CD4 T cells into the TME in response to the chemokine CCL5 produced by CD40-stimulated TAMs\textsuperscript{146}. In addition, the treatment caused a transitory modulation of the macrophage phenotype with increased expression of CD86 and MHC class II, promoting antigen-presenting potential. Agonistic CD40 mAbs are currently under clinical evaluation and may be used in combination with other treatments. Transient inhibition of CSF1R combined with CD40 agonists resulted in rapid reprogramming of TAMs before their depletion\textsuperscript{147}. The safety of the CD40 agonistic antibody APX005M was assessed in a phase I study in patients with metastatic pancreatic cancer in combination with chemotherapy, with or without immunotherapy; treatment-related toxicity was clinically manageable and 14 of the 24 evaluated patients had measurable clinical responses\textsuperscript{148}. These encouraging results warrant further investigation in phase II/III clinical trials (Table 1).

**Toll-like receptors.** Engagement of toll-like receptors (TLRs) activates an immunostimulatory response and this approach has been used to bypass the immunosuppressive activity of macrophages in tumours (Fig. 2). Several agonists of TLRs with immunostimulatory activity have been investigated for the reprogramming of TAMs in different tumour models, showing increased cytotoxic activity against tumour cells and production of immunostimulating cytokines\textsuperscript{149–153}. The first TLR-stimulating agent approved by the FDA was BCG (Bacillus Calmette-Guérin), a live attenuated strain of *Mycobacterium bovis* that stimulates TLR2 and TLR4. BCG is still in use to treat patients with bladder cancer\textsuperscript{150,152}. In mice, monophosphoryl lipid A (MPLA), a TLR4 ligand, injected with IFNγ, reprogrammed TAMs to become tumoricidal\textsuperscript{151}. TLR3, TLR7, TLR8 and TLR9 are localized in endosomal compartments and serve as nucleic acid sensors. Engagement of these receptors activates NF-κB, the master transcription factor of inflammation, and triggers the secretion of immunostimulatory cytokines, including the antitumour cytokine type I interferon. The TLR7 agonist imiquimod is the only one that has been approved by the FDA for the topical treatment of squamous and basal cell carcinoma. Two derivatives of imiquimod, resiquimod or R848 (TLR7 and TLR8 agonist) and motolimod (TLR8 agonist), showed promising immunostimulatory activity in a variety of preclinical models\textsuperscript{154}. Although other cells of the innate immunity can be activated by TLR ligands, in these models, priming of the antitumour immune response was mediated by TAMs\textsuperscript{154,155}. Poly(I:C), a TLR3 agonist, demonstrated significant antitumour responses in experimental settings and its nanocomplex formulation with poly-β-lysine (BO-112) is now being evaluated in clinical trials\textsuperscript{156}. Another analogue, poly-ICLC, complexed with carbosymethylcellulose (Hiltonol), is under investigation in combination with anti-PD1 therapy\textsuperscript{157}. 

[Box 1: TLR agonists]
Most frequently, TLR agonists have been formulated with nanoparticles and administered intratunourally in accessible lesions as their systemic administration may cause inflammatory toxicity[2,138–140]. The TLR9 ligand CpG formulated in a virus-like particle was administered intratumourally together with systemic anti-PD1 mAb pembrolizumab to patients with melanoma who were refractory to PD1 blockade. Promising clinical activity was observed in 25% of patients[84].

With the idea to combine the precision of a therapeutic antibody with the stimulation of innate immunity cells into a single agent, Ackerman et al. recently reported the design of BDC-1001, an immune-stimulating antibody conjugate, engaging TLR7 and TLR8 and conjugated to a tumour-targeting antibody against HER2. In mice, systemic administration of this immune-stimulating antibody conjugate successfully reduced tumour growth and stimulated an antitumour immune response mediated by macrophages and other antigen-presenting cells, without eliciting inflammation[85]. SBT6050 is a TLR8 agonist conjugated to a HER2-directed antibody. Both BDC-1001 and SBT6050 are under evaluation in a first-in-human trial in patients with HER2-positive tumours (TABLE 1).

Several clinical phase I–III trials involving the administration of TLR agonists to patients with cancer are ongoing, either as monotherapy or, most frequently, in combination with targeted therapy or immunotherapy[9,140,144,146,147]. TLR agonists have also been used as adjuvants for anticancer vaccines and in combination with checkpoint inhibitors or adoptive T cell therapy to ‘warm’ immunologically cold tumours[146,148–150]. Overall, these treatments seem well tolerated, and evidence of clinical activity, especially disease stabilization, has been observed also in heavily pretreated patients. Still, none of these novel agents has so far been approved by regulatory agencies for use in patients with cancer[16–20] (TABLE 1).

STING. Stimulator of interferon genes (STING) protein receives input from different cytoplasmic receptors that sense ectopic nucleotides from pathogens as well as from endogenous damaged DNA, leading to the production of type I interferon. Therefore, STING also has a key role in antitumour immune responses[171,172]. This property of STING generated attention and prompted the development of synthetic STING agonists[173] (FIG. 2). In early times before the identification of STING, DMXAA (3,6-dimethylxanthene-4-acetic acid), a vascular disrupting agent based on flavone acetic acid, was known to have anticancer activity mediated by interferon and TNF in experimental tumour models[174]. DMXAA is the best-known STING activator, and stimulation of STING in innate immunity cells (but also in cancer cells) successfully elicited antitumour immunity in several preclinical tumour models and synergized with radio-chemotherapy and immunotherapy[175,176]. Although compounds targeting the STING pathway are not macrophage specific, preclinical studies have demonstrated that activation of antitumour immunity engages infiltrating macrophages and dendritic cells. Because early pharmacokinetic studies showed that most STING agonists have a short half-life, novel compounds with improved half-life have been developed and are moving ahead for evaluation in patients with cancer[175,177,178]. Most frequently, STING agonists have been administered intratumourally; however, an available formulation for oral administration has been tested in mice and resulted in significant tumour regression and synergism with checkpoint blockade immunotherapy[177]. STING is also activated by microbiota-derived agonists, such as c-di-AMP, which regulate interferon production and macrophage polarization[179]. Interestingly, modulation of the microbiota with a high-fibre diet improved the efficacy of ICB[180].

Myeloid checkpoints and regulators

The function of myelomonocytic cells is tightly controlled by a number of negative regulators that directly inhibit or divert their effector functions[15,180,181]. Some of these molecules (such as members of the signal regulatory protein-α (SIRPα), sialic acid–binding immunoglobulin-like lectin (SIGLEC) and leukocyte immunoglobulin-like receptor B (LILRB) families) have been referred to as myeloid checkpoints, similar to regulatory molecules of T cells[182,183]. Other relevant molecules are scavenger receptors, which mediate the clearance of debris and dead cells by macrophages, a function that is pivotal to mitigating inflammation (during the resolution phase) and preventing tissue damage. These pathways, physiologically used to protect from unwanted attack of host tissues, result in a state of immunosuppression and activation of the TAM tissue-trophic functions, which eventually can be hijacked by tumour cells to evade recognition from the immune system and proliferate.

SIRPa and CD47. CD47 is expressed on normal cells and serves as a ‘don’t eat me’ signal, instructing mononuclear phagocytes and neutrophils expressing SIRPα to spare host cells from removal[182,184]. Loss of CD47 is associated with ageing of red blood cells and allows their disposal, an illustration of the importance of this pathway. Tumour cells overexpress CD47 in many cancer types, disguising as healthy cells and avoiding phagocytosis. CD47-targeting approaches include both, antibodies anti-CD47 (Hu5F9-G4 (NCT02216409), SRF231 (NCT035123), IBI188 (NCT03763149)) and anti-SIRPα (BI-765063 (NCT03990233)) (FIG. 3b). Emerging evidence points to combination as the key to success of immune-based strategies. The strength of therapeutics disrupting the CD47–SIRPa axis resides in the possibility to concomitantly render macrophages more phagocytic and increase their antigen load, thus enhancing antigen presentation to T cells[183,185]. Therefore, in a stepwise manner, CD47 approaches may be synergistically effective in combination with T cell checkpoint inhibitors, first improving phagocytosis and antigen presentation and second unleashing a response of activated T cells.

In preclinical models, therapeutic combinations with anti-CD47 were particularly effective in those tumours for which targeted therapies against tumour antigens are already available such as CD20 for lymphoma, HER2 for breast cancer or EGFR for CRC (FIG. 5a). The mechanism of action of tumour-opsonizing mAbs, such as rituximab (anti-CD20), trastuzumab (anti-HER2) or...
cetuximab (anti-EGFR), can be greatly enhanced with a concomitant signal that dampens CD47 and boosts Fc-mediated functions\textsuperscript{186}. The combination of cetuximab with an anti-CD47 antibody markedly increased macrophage antibody-dependent phagocytosis in preclinical models\textsuperscript{187} and is being tested in clinical trials for CRC (NCT02953782). In a phase Ib study, CD47 blockade combined with rituximab showed activity in patients with lymphoma\textsuperscript{15,180}. So far, promising results have been observed for haematological malignancies\textsuperscript{15,180}, but studies on solid tumours are under way and results are awaited. Preclinical results in cell lines of lung\textsuperscript{188}, breast\textsuperscript{189} and other types of cancer show that macrophage-mediated phagocytosis can critically contribute to the therapeutic function of the antibody and trigger adaptive immune responses.

The blockade of CD47, a ubiquitously expressed molecule on normal cells, raised concern about potential on-target toxicity, in particular anaemia and thrombocytopenia, owing to the expression of CD47 on platelets and red blood cells. A possible strategy to overcome this problem is blocking SIRP\textalpha\textsuperscript{186} or using anti-CD47 antibodies with weak killing properties, such as antibodies with an IgG4 tail, which can reduce NK cell engagement; for example, TTI-621 (a SIRP\textalpha decoy receptor fused to an active IgG1 Fc; NCT03530683) or TTI-622 (identical to TTI-621 but with an IgG4 Fc that has a weaker killing function; NCT02890368). ALX148 (NCT04675333) combines a high-affinity CD47 binding domain with an inactive Fc domain. Off-tumour effects can also be reduced by increasing specificity for the tumour target such as with bispecific agents that recognize CD47 and a tumour-associated antigen (FIG. 5c). Such molecules, so far developed for dual recognition of CD47 and CD20 (RTX-CD47\textsuperscript{190}), PDL1\textsuperscript{191}, EGFR\textsuperscript{192} and CD19 (TG-1801, NCT03804996), restrain the phagocytic action of the unleashed macrophage to the tumour cells that express the antigen, sparing the host cells that do not express the tumour antigen, such as platelets and red blood cells (FIG. 5c), showing limited toxicity. More recently, delivery of the anti-CD47 antibody with an oncolytic virus has been tested in preclinical models as a strategy to improve drug availability to the tumour site, reducing its toxicity\textsuperscript{193}.

The SIGLEC family. SIGLEC molecules are membrane proteins that bind sialic acid and engage in cell–cell interactions. These proteins contain tyrosine-based inhibitory receptor motifs (ITIMs) in their cytoplasmic tail, which are typically components of those immune receptors that inhibit and suppress activation signals, thus regulating the functions of several immune cells.

![Fig. 3 Myeloid checkpoints and other inhibitory receptors expressed by macrophages.](image-url)

**a** | Overview of myeloid checkpoints and inhibitory receptors expressed by tumour-associated macrophages (TAMs) and their ligands expressed on tumour cells or cell debris. These include the receptor/ligand pairs signal regulatory protein-\alpha (SIRP\textalpha)–CD47, LILRB1–HLA1, sialic acid-binding immunoglobulin-like lectin 10 (SIGLEC10)–CD24, and PD1–PDL1, which inhibit phagocytosis, and macrophage receptor with collagenous structure (MARCO), CD169 and mannose receptor scavenger receptors. Clever 1, triggering receptor expressed on myeloid cells 2 (TREM2) and P-selectin glycoprotein ligand 1 (PSGL1) are also depicted. Targeting of Clever 1 and TREM2 does not specifically interfere with phagocytosis but with immunosuppressive activation. **b** | In strategies featuring the use of therapeutic antibodies, such as anti-CD20 or anti-epithelial growth factor receptor (EGFR), combinatorial use of anti-CD47 enhances antibody-dependent cellular phagocytosis (ADCP) and increases antigen presentation to T cells. **c** | Bispecific CD47 antibodies are designed to recognize CD47 and tumour-associated antigens (such as CD20 or PDL1), which enhances selective blocking of CD47 on tumour cells, avoiding on-target toxicity due to recognition of CD47 on red blood cells and platelets. ADCC, antibody-dependent cellular cytotoxicity; MHC II, MHC class II.
SIGLEC1 (also known as sialoadhesin or CD169) is expressed by a fraction of macrophages and is upregulated in human cancer, with its expression correlating with worse prognosis. Depletion of CD169+ TAMs was effective in reducing tumour burden and metastasis in mouse models of breast cancer. SIGLEC7 and SIGLEC9 were targeted in a humanized murine model with a significant reduction in tumour burden.

The sialglycoprotein signal transducer CD24 has been evaluated as an anti-phagocytic molecule expressed by multiple cancer cells and holds promise as an additional checkpoint with therapeutic potential. Binding of CD24 to SIGLEC10, which is overexpressed by TAMs, inhibited phagocytosis of tumour cells and interference of this axis with a mAb against SIGLEC10 rescued the macrophage capability to limit tumour growth in preclinical models of ovarian cancer.

The LILRB family. Downregulation of MHC class I molecules is probably one of the best-known mechanisms of evasion used by cancer cells to circumvent recognition by T cells. However, tumour cells can exploit MHC class I as a mechanism of evasion from phagocytes by interacting with LILRB family members. LILRB1 is an MHC-binding protein widely expressed on immune cells and enriched on TAMs; it contains an ITIM motif and transduces an inhibitory signal. LILRB expression was associated with the inhibition of phagocytosis of cancer cells. In fact, its role as a myeloid checkpoint was discovered by analysing cancer cell lines resistant to the anti-CD47 antibody. The expression of MHC class I by tumour cells correlated with the degree of their resistance to anti-CD47, and phagocytosis induced by the anti-CD47 antibody was restored by LILRB1-blocking antibody.

Macrophages also express LILRB2, whose blocking enhanced their pro-inflammatory activation and phagocytic activity. Despite the similarity between the two members of the LILRB family, whether the checkpoint activity of LILRB2 is accounted for blocking phagocytosis or for a general modulation of macrophage activation remains to be elucidated. In terms of the development of new macrophage-based therapeutics, efforts are ongoing to identify binding partners to LILRB molecules, which are expected to provide control over the capability of the tumour to evade phagocytosis. MK-4830, a human mAb directed against LILRB2, was tested in advanced-stage solid tumours in a phase I dose-escalating study (NCT03564691) as monotherapy or in combination with pembrolizumab. Initial results showed durable responses, supporting further development. Immune correlates of response included expression of pro-inflammatory cytokines, such as GM-CSF and TNF, and an enhanced cytotoxic T lymphocyte-mediated antitumour immune response.

LILRB4 was found on a variety of intratumour immune cells in murine tumour models and human cancers, most prominently on TAMs, where it strongly suppressed tumour immunity. Its blockade reshaped tumour-infiltrating T cells and modulated phenotypes of TAMs towards a less suppressive phenotype.

Scavenger receptors. Several types of scavenger receptor are abundantly expressed in TAMs, and their targeting is emerging as an option to potentiate a pro-inflammatory switch (Fig. 2).

Clinical evidence points to a significant association between macrophages expressing the scavenging receptor CD163 and tumour progression in several cancers, including pancreatic cancer and melanoma. CD163 enables macrophages to remove damaged erythrocytes by binding to haptoglobin-haemoglobin complexes formed upon intravascular haemolysis and its expression is traditionally associated with M2-like macrophages. Notwithstanding, the exact mechanisms of its tumour-promoting functions are unclear. Depletion of CD163+ macrophages, through a genetic and a nanoparticle-based approach resulted in tumour regression in a mouse model of melanoma resistant to anti-PD1 therapy. This strategy induced a complete re-education of the TME, featuring infiltration of cytotoxic T cells and inflammatory monocytes, ultimately restoring response to anti-PD1 treatment.

Macrophage mannose receptor 1 (MR1, also known as CD206) is a macrophage scavenger receptor that binds several endogenous ligands in addition to pathogen moieties such as tumour mucins. Engagement of the mannose receptor (MR) on macrophages, either by mucins or agonist anti-MR mAbs, induced an immunosuppressive phenotype with increased IL-10 production. This immunoregulatory function of the MR1 was confirmed in models of intestinal inflammatory conditions, in which MR1-deficient mice had more-severe colitis. Jaynes et al. identified the RP-182 peptide, which binds to the MR1 and induces a change in configuration. When administered to tumour-bearing mice, the synthetic peptide RP-182 partially depleted CD206+ macrophages and reprogrammed the remaining TAMs into antitumour M1-like effectors with increased inflammatory cytokine production and the ability to phagocytose cancer cells. In murine cancer models, RP-182 suppressed tumour growth, extended survival and synergized with combined immunotherapy.

The macrophage receptor with collagenous structure (MARCO) is highly expressed on TAMs. When antibodies blocking this receptor were tested in preclinical tumour models, they did not reduce the number of TAMs but they induced an antitumour immune response through reprogramming of TAMs into pro-inflammatory effectors. In NSCLC, macrophages polarized to express MARCO showed an immunosuppressive phenotype. Silencing of cancer cell-derived IL-37, an anti-inflammatory cytokine of the IL-1 family, or blocking of its receptor, which signals through IL-1R8, rescued the MARCO-associated immunosuppressive phenotype. IL-18 also negatively regulates IL-18 signalling and acts as a new NK cell checkpoint restraining NK cell antitumour and anti-viral potential. Activation of NK cell killing through the TRAIL pathway has been recently identified as one of the mechanisms of action of anti-MARCO mAbs. Indeed, treatment with anti-MARCO mAbs in a mouse melanoma model did not engage CD8+ T cells and was mediated only by NK cells. In glioblastoma,
MARCO\textsuperscript{149} TAMs significantly accelerated tumour engraftment and growth in vivo\textsuperscript{151}.

The receptor Clever 1, also known as Stabilin 1, was originally described as an adhesion and scavenger receptor expressed by a variety of cells, including circulating monocytes, lymphatic and sinusoid endothelial cells, and immunosuppressive M2-like macrophages\textsuperscript{122}. In homeostatic conditions, Clever 1 binds several ligands, primarily lipoproteins and carbohydrates, mediating endocytosis of scavenged material and its delivery to the endosomal compartment, ultimately resulting in suppression of macrophages and impaired activation of T helper 1 lymphocytes. Antibody blockade of Clever 1 in preclinical studies caused a phenotypic switch in TAMs from immunosuppressive to pro-inflammatory, activation of T cell responses and delayed tumour growth\textsuperscript{212,213}. These results provided a roadmap for a phase I trial to test the safety and preliminary efficacy of FP-1305, a humanized anti-Clever 1 antibody administered to heavily pretreated patients with metastatic solid tumours, with encouraging results\textsuperscript{17} (TABLE 1). To avoid potential side effects due to the expression of Clever 1 on lymphatic vessels, the antibody has been optimized to escape Fcy-mediated cytotoxicity and complement-mediated functions. Notwithstanding, blocking of Clever 1 on the lymphatic endothelium may have an impact on tumour metastases. Mechanically, inhibition of Clever 1 resulted in a pro-inflammatory switch of CD14\textsuperscript{+} blood monocytes, enhanced the capability of macrophages to cross-present scavenged antigens, and activated peripheral T cells\textsuperscript{17}.

**PD1.** PD1 expression by TAMs inhibits phagocytosis and tumour immunity\textsuperscript{49}, twisting the traditional view of the PD1--PD-L1 axis as a specific T cell checkpoint. PD-L1 expression on cancer cells may thus concomitantly enable evasion from T cell cytotoxicity and macrophage-mediated phagocytosis, suggesting that blockade of this axis might unleash antitumour immunity by both adaptive and innate mechanisms. The mechanism of phagocytosis inhibition triggered by the engagement of PD1 on macrophages has not yet been elucidated nor have the signals inducing PD1 upregulation. SIRPα, LILRB1 and PD1 all contain an ITIM domain, which could be instrumental for the downstream signals inhibiting phagocytosis, but a formal demonstration has not been provided. On this basis, studies aimed at monitoring response in patients with cancer undergoing checkpoint inhibitor treatment should consider the myeloid compartment as a potential target and as a predictive biomarker.

**TREM2.** Triggering receptor expressed on myeloid cells 2 (TREM2) is expressed by macrophages of several tissues and is upregulated on TAMs in human and mouse tumours\textsuperscript{214,215}, in which targeting of TREM2 macrophages restricted tumour growth and sensitized the response to anti-PD1 therapy\textsuperscript{216}. TREM2 scavenges large molecules such as phospholipids, lipoproteins and apoptotic cells\textsuperscript{217}. PY314, a humanized mAb targeting TREM2 macrophages, is currently undergoing evaluation in a phase I clinical trial in advanced solid tumours that are refractory to previous treatments (NCT04691375) (TABLE 1).

**PSGL1.** PSGL1 is widely expressed in cells of haematopoietic origin, strongly upregulated by M2 polarizing signals in macrophages and expressed at high levels in TAMs. Its ligands include VISTA and selectins\textsuperscript{80}. An anti-PSGL1 mAb repolarized human M2 macrophages to an M1-like phenotype, induced a hot inflammatory functional pattern in ex vivo human tumour tissue cultures, and had antitumour activity in humanized mouse models\textsuperscript{134}. Thus, PSGL1 can represent a valuable target to re-educate TAMs\textsuperscript{118}.

**Targeting TAM metabolism**

A network of transcription factors, epigenetic modifications and mRNAs underlies the plasticity and polarized activation of macrophages\textsuperscript{4,15,217}. Downstream rewiring of macrophage function involves profound changes in amino acid, lipid and iron metabolism\textsuperscript{210-212}. This complex cascade provides potential targets to reprogramme macrophage function. Epigenetic regulation by inhibition of class IIa histone deacetylases (HDACs) is a promising approach to harness the antitumour potential of macrophages. TMP195 was identified as a selective class IIa HDAC inhibitor that was able to modify the transcription profile of macrophages. In preclinical breast cancer models, TMP195 resulted in a macrophage-mediated reduction of tumour growth\textsuperscript{212}. Tefinostat (CHR-2845) is an HDAC inhibitor that is cleaved to an active acid by non-specific esterase liver carboxylesterase 1 (CES1), the expression of which is limited to cells of monocytic lineage and some hepatocytes, allowing selective accumulation of active drug within monocytic cells. Because of this feature, it has been successfully used in a phase I clinical trial in patients with advanced haematological malignancies such as myelodysplastic syndromes and chronic myeloid leukaemia (NCT00820508)\textsuperscript{123}. In principle, CES1 may provide a tool to develop macrophage-selective targeting of drugs.

The extreme metabolic conditions generated in the TME in terms of hypoxia and nutrient deprivation are causatively linked to the recruitment of macrophages that are, most commonly, functionally impaired\textsuperscript{218-220}.

Lactic acid, produced by tumour cells as a by-product of glycolysis, functionally polarizes macrophages towards an M2-like phenotype characterized by higher expression of arginase 1\textsuperscript{124}, suggesting that strategies blocking glycolysis may positively reprogramme TAMs. However, the same glycolytic pathway is fundamental for macrophage activity against tumour cells and glucose supply is needed for ROS production and phagocytosis\textsuperscript{226}, raising questions as to the utility of blocking glycolytic pathways. So far, most of the studies aimed at targeting glycolysis to revert macrophage polarization have relied upon glycolytic inhibitors, such as 2-deoxy-d-glucose (2-DG)\textsuperscript{225}, which are far from specific. In this line, the respiratory complex I inhibitor metformin, an antidiabetic agent, is being investigated in several clinical trials enrolling patients with different tumour types. In preclinical models, metformin was able to remodel the TME, reducing the density of TAMs and increasing their phagocytic function\textsuperscript{226}.

Despite the clear causative association between a hyperglycolitic environment and macrophage...
Macrophage-based cell therapies may overcome this limitation, given the constant trafficking of mononuclear phagocytes into tumours. Macrophage-based cell therapy strategies have been based either on the capacity of monocytes to act as Trojan horses, delivering cytokines or nanoparticles to the TME, or on arming mononuclear phagocytes with engineered receptors. In a proof-of-concept study, monocytes replenished with drug-loaded nanoparticles and intravenously injected into tumour-bearing mice were able to reach the tumour site with superior efficiency than free nanoparticles. The possibility of using macrophages to bring IFNα to the tumour site and consequently activate an immune response was investigated by De Palma et al., who transduced the Ifna1 gene in haematopoietic progenitors under the Tie2 promoter. Tie2-expressing monocytes, having a high tumour-homing ability, successfully migrated to tumours and delivered IFNα in the TME, triggering the activation of immune cells and inhibiting tumour growth and angiogenesis. A clinical trial in glioblastoma patients is ongoing (Table 1). Similarly, soft particles called ‘backpacks’, containing the cytokine IFNα on their internal side, were stuck on the macrophage surface. The study demonstrated that backpack-loaded macrophages acquire an M1 phenotype and, when injected intratumourally, maintain this phenotype without being affected by the immunosuppressive TME. Tumour growth and metastatic burden were significantly reduced in mice treated with macrophages carrying IFNα backpacks. In a murine sarcoma model, premetastatic niches were characterized by an immune suppression gene signature centred on myeloid cells. Myeloid cells were genetically engineered to express IL-12. Upon adoptive transfer, IL-12 expressing bone marrow-derived myeloid cells elicited a type 1 immune response in the lungs and reduced metastasis and primary tumour growth.

The difficulty in transducing human macrophages has been a hurdle in developing mononuclear phagocyte-based cellular therapy, a stumbling block addressed by the development of different technological platforms. Human CAR-M armed with receptors recognizing carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), CD19, CD22, HER2 and CD51,245–247 have been developed to attack haematopoietic and solid tumours (Fig. 4); various signalling platforms have been used (see Related link). CAR-M cells mediate phagocytosis and express M1 functions in a stable way and traffic to primary and metastatic tumours. Clinical trials are currently under way or planned to assess the potential of CAR-M in different tumours (Table 1).

Conclusion

Macrophages are a universal component of the TME and engage in a complex interaction with cancer cells, stroma and immunocompetent cells. The recent discovery that glioblastoma cells acquire the expression of myeloid genetic programmes speaks to the fundamental role of macrophages in carcinogenesis and cancer progression.

Macrophages play a dual part in the effectiveness of current treatment modalities from chemotherapy to ICB immunotherapy. In many human tumours, TAMs are the main drivers of checkpoint blockade in T cells.
and mediate resistance to immunotherapy. Despite their crucial role in the origin, progression and treatment of cancer, direct targeting of myelomonocytic cells has so far failed to have a major therapeutic impact, although ongoing trials targeting TAMs in combination with other treatment modalities may change the overall picture248 (Table 1). Stumbling blocks in the therapeutic translation of TAM immunobiology include the diversity and plasticity of mononuclear phagocytes in tumours. The dissection of TAM diversity at the single-cell level and their ontogenetic relationship may provide new views on strategies to selectively deplete tumour-promoting versus tumour-inhibiting subsets. It is our tenet that spatial and single-cell analysis will require crystallization of working hypotheses amenable to diagnostic and therapeutic testing in a clinical context.

Although macrophage infiltration is a common denominator of different tumours with shared properties, there is evidence for substantial differences in TAM phenotype and role in tumours arising in or disseminating to different organs. For instance, while TAM infiltration is associated with poor prognosis in most tumours, there are notable exceptions such as primary CRC249. Moreover, as discussed above, the tissue-intrinsic properties at the primary site or in metastatic lesions imprint the characteristics of the inflammatory microenvironment to some extent. Therefore, exploitation of TAMs may have to consider the tissue contexture of primary and secondary localization of tumours.

Macrophage function is regulated by surface receptors, some of which inhibit effector function or skew it in an inappropriate direction. These regulators of myelomonocytic cell function belong to different molecular classes and are obvious therapeutic targets. Early clinical trials of mAbs targeting the CD47–SIRP1α axis, LILRB2 and Clever 1 for the first time gave positive signals15–17. These positive responses, though still at an early stage, may herald the dawn of myeloid checkpoint immunotherapy. Based on the current understanding of the immunobiology of TAMs, it is our tenet that these approaches are unlikely to represent stand-alone strategies and their potential rests in complementing cytoreductive therapy and ICB immunotherapy.

In most, if not all, human tumours, TAMs are replenished by the circulating myeloid precursor pool. This long-held view provided the basis to develop CAR-M strategies. Poor recruitment at tumour sites has been a limiting factor for the application of CAR T cells to solid tumours. The ‘fatal attraction’ of monocytes to tumour tissues may provide a tool to circumvent this stumbling block in the development of cell therapy for solid tumours. However, several open questions remain for the exploitation of the cancer-homing propensity of monocytes. In addition to the choice of receptor and signalling
components to arm monocytes, there is still limited or no understanding of the replenishment rate of the intratumoural pool by circulating precursors, the length of survival in the tumour context, the retention of appropriate functional orientation, or the spatial localization within the tumour tissues. Clinical trials may represent a unique opportunity to address these key questions of paramount importance for the development of CAR-M therapies.

Prevention of cancer using retinoic acid or drugs targeting hormones (pharmacoprevention) has failed. Macrophages and their mediators are companions and propellers of carcinogenesis. This fundamental consideration and the results of the CANTOS trial discussed above raise the issue of revisiting pharmacoprevention from the point of view of tumour-promoting inflammation. A trial using an anti-IL-1β mAb in participants at high risk of developing lung cancer (Canal trial) is being planned. Orally active inflammmasome inhibitors, if proven safe and effective in conventional inflammatory conditions, could be ideally suited for pharmacoprevention efforts. The immunobiology of cancer-related inflammation and TAMS calls for giving careful consideration to prevention strategies in selected high-risk patients based on the premises discussed here.

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