Therapeutic targeting of tumour myeloid cells

Simon T Barry[1,†], Dmitry Gabrilovich[2], Owen J Sansom[3,4], Andrew D Campbell[3], Jennifer P Morton[3,4]

[1]Bioscience, Early Oncology, AstraZeneca, Cambridge UK

[2]Bioscience, Early Oncology, AstraZeneca, Gaithersburg, USA

[3]Cancer Research UK Beatson Institute, Glasgow UK

[4] School of Cancer Sciences, University of Glasgow, Glasgow, UK

†Email: simon.t.barry@astrazeneca.com

Abstract

Myeloid cells are pivotal within the immunosuppressive tumour microenvironment. The accumulation of tumour-modified myeloid cells derived from monocytes or neutrophils — termed 'myeloid-derived suppressor cells' — and tumour-associated macrophages is associated with poor outcome and resistance to treatments such as chemotherapy and immune checkpoint inhibitors. Unfortunately, there has been little success in large-scale clinical trials of myeloid cell modulators, and only a few distinct strategies have been used to target suppressive myeloid cells clinically so far. Preclinical and translational studies have now elucidated specific functions for different myeloid cell subpopulations within the tumour microenvironment, revealing context-specific roles of different myeloid cell populations in disease progression and influencing response to therapy. To improve the success of myeloid cell-targeted therapies, it will be important to target tumour types and patient subsets in which myeloid cells represent the dominant driver of therapy resistance, as well as to determine the most efficacious treatment regimens and combination partners. This Review discusses what we can learn from work with the first generation of myeloid modulators and highlights recent developments in modelling context-specific roles for different myeloid cell subtypes, which can ultimately inform how to drive more successful clinical trials.

Introduction

Cross-talk between tumour cells and cells of the tumour microenvironment (TME) plays a critical role in tumour progression and influences response to treatment[1]. The TME is complex and heterogeneity is observed between and within tumour types, as well as within individual tumours; however, it generally consists of cancer associated fibroblasts (CAFs), cell matrix components such as collagen and fibronectin, tumour vasculature and immune cells, including lymphocytes (T cells, B cells) and a range of different myeloid cells. These myeloid cells include classically activated neutrophils and tumour-associated macrophages (TAMs) that broadly show anti-tumour activity, but also pathologically activated, immunosuppressive subsets including polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), monocytic myeloid-derived suppressor cells (M-MDSCs) and immune suppressive, tumour-promoting TAMs derived from M-MDSCs. These subsets are commonly defined by the expression of

specific cell surface markers[2-7] (**Supplementary Table 1)** [8,9]. The range of phenotypes that can be adopted by these cells and their diverse origins have led to a diversification of the description of myeloid cell populations and the use of different terms to describe similar cell types, which we discuss in **Box 1**.

Translational studies have shown that tumour myeloid cells influence tumour cell function and resistance to chemotherapy and immune checkpoint inhibitors (ICI)[4,10-12]. Preclinical efficacy studies of myeloid modulators in simple subcutaneous mouse syngeneic tumour models have shown that they can reduce the immunosuppressive effect of the TME and enhance chemotherapy or immunotherapy responses[13-16] (**Fig. 1**). Clinical trials of the first generation drugs targeting tumour myeloid cell subsets are ongoing. The most extensively studied myeloid-targeting drugs are modulators of colony stimulating factor 1 receptor (CSF1R), C-C chemokine receptor type 2 (CCR2), C-X-C chemokine receptor type 2 (CXCR2) and phosphoinositide 3-kinase (PI3K). Despite interesting results from single-arm Phase I and small Phase II clinical trials, Phase II and Phase III trials in broad patient populations have not shown compelling efficacy and treatment has been associated with toxicity challenges. This suggests that, as with many cancer therapies, patient selection biomarkers will be required for therapeutic success.

There are a number of recent Reviews that have addressed the general area of myeloid cell biology, and mechanisms regulating myeloid cell function[6,17]. Building on these, we will discuss the challenges associated with developing myeloid therapies, and specifically, what can be learned from the first generation of myeloid inhibitors (**Fig. 2**) to refine our approach to trials testing new myeloid cell modulating mechanisms. We will discuss findings from tumour models that represent specific tumour types such as pancreatic, colorectal or breast cancer to explore how myeloid-cell-driven resistance complements with other tumour cell and TME features to influence therapeutic response. Dendritic cells (DC) are also considered to be a myeloid cell subset[18], but will not be addressed in detail in this review as the focus is primarily the role that macrophage and neutrophil derived cells play in regulating tumour progression and therapeutic response. Finally, we discuss how these features could refine our future approaches to clinical trials.

Targeting macrophages or M-MDSCs

In tumours, higher numbers of TAMs and M-MDSCs, commonly identified by simple histological stains for markers such as CD68, CD86, CD163 or CD206 (among others) are associated with poor prognosis and response to chemotherapy or immunotherapy in many tumour types[19-23]. While giving some insight into cell content, and potentially subsets of macrophages or M-MDSC present, these markers are not able to robustly differentiate functionally different subsets of cells. How these cells are defined and referred to has changed over time and differs between studies. Often the terms macrophages, TAMs and M-MDSCs are used interchangeably depending on the markers and techniques used to identify the cells, making it challenging to compare between studies and resulting in some apparent inconsistency in nomenclature. Specific subsets of macrophages, TAMs or M-MDSC can by identified by co-staining for multiple cell lineage markers. However, in many preclinical studies only a few markers may be used in analysis which does not distinguish between TAMS or M-MDSCs, or functional state. In this case a mixed cell population may be referred to simply as TAMs or suppressive macrophages. For the purpose of this Review, we have used more general terminology (e.g. TAM or M-MDSC versus neutrophil or PMN-MDSC) to refer to different myeloid cell types to help summarise the learning from each study.

Multiple preclinical studies indicate that reducing TAM or M-MDSC content in the tumour could have clinical benefit[13,14,16,24]. Macrophage recruitment to tissues is regulated by specific receptors expressed by monocytes or macrophages, such as CSF1R and CCR2. Early preclinical studies using receptor-blocking antibodies and small molecule inhibitors targeting CSF1R — a tyrosine kinase growth factor receptor expressed by macrophages that is activated by the ligands CSF1 and IL34 — demonstrated modest anti-tumour efficacy in subcutaneous mouse syngeneic tumour models[13,14,16] and inhibiting CSF1R commonly (but not uniformly) resulted in the depletion of mature macrophages and M-MDSCs from both tumour and normal tissue in both mice and humans[13]. Further, ablation of *Ccr2* — a G-protein coupled receptor expressed on macrophages activated by MCP1/CCL2, CCL8 and CCL13 — was found to reduce tumour burden in an intrasplenic syngeneic transplant mouse model that recapitulates liver metastasis[24], possibly because CCR2 inhibition prevents recruitment of M-MDSC and monocyte populations to the tumour. Some tissue-resident macrophages (TRM), in both

mouse and human, are CCR2 negative [25] so these cells, and TRM-derived TAMs, will not be reduced by CCR2 inhibition (**Fig. 2**).

Further preclinical and translational insights into macrophage influence on tumour growth and response to chemotherapy or ICIs have emerged in different tumour types, including those of the colon, pancreas and liver, as discussed below.

Colorectal cancer

Studies in murine colon cancer models showed that CCR2 and CSF1R signalling sustain tumour myeloid cell populations[26]. Deletion of *Ccr2*, or blockade of the CCR2 ligand, C-C motif chemokine 2 (CCL2), with a neutralising antibody decreased monocyte/macrophage infiltration in to the tumour. This was associated with a reduction in colitis severity and tumour progression, suggesting CCR2 inhibition exerts an influence on both the tumour and associated tissue inflammation[27]. In the APC^{Min} intestinal tumourigenesis model, in which mice develop multiple intestinal adenomas, tumour-promoting macrophages accumulate at early stages of tumour development and although ablating *Ccr2* expression had little effect on tumour incidence, CSF1R blockade with a murine receptor blocking antibody reduced TAM abundance and tumour incidence[26] suggesting subtle differences in the regulation of myeloid cell subsets by CCR2 vs CSF1R signalling and their contribution to tumour progression.

Pancreatic cancer

CSF1R inhibitors have shown activity in pancreatic tumour models. The CSF1R inhibitor pexidartinib (also known as PLX3397) reduced tumour growth when given alone or in combination with chemotherapy or immunotherapy in several murine KRAS-driven orthotopic tumour models with cells derived from mouse pancreatic tumours[14]. Treatment with the CSF1R inhibitor, AZD7507, also delayed the progression of primary pancreatic ductal adenocarcinoma in the KPC (*LSL-Kras*^{G12D}:*LSL-p53*$^{R172H/+}$:*Pdx1-Cre*) mouse model. A reduction in tumour macrophages and increased infiltration of $CD8^+$ T-cells were associated with anti-tumour benefit[28]. Similarly, CCR2 inhibition with PF-04136309 combined with chemotherapy improved survival in a murine pancreatic cancer model orthotopically transplanted with KPC tumour cells[29].

The above studies suggest a potential role for TAMs or M-MDSCs in subsets of both colon and pancreatic cancer, two challenging-to-treat diseases.

Glioblastoma

The brain tumour microenvironment is enriched in TAMs, M-MDSCs, and specialized resident macrophages known as microglia. High TAM content in human tumours associates with tumour progression and therapeutic resistance[30]. Secretion of the cytokine CCL20 and the tumour necrosis factor receptor superfamily member osteoprotegerin (OPG) by tumour cells increases resident macrophage CCL2 expression, which recruits monocytes and M-MDSCs through signalling via CCR2 and CCR4 receptors on their surface[31].

Microglia or macrophages drive GBM progression in the brain. The small molecule CSF1R inhibitor BLZ945 was shown to reduce the growth of orthotopic tumours; however, it was not effective against subcutaneous glioblastoma tumours[32], indicating that importance of the CSF1R dependent TAMs for tumour progression is context specific. Moreover in these glioblastoma models tumour control was followed by rapid tumour rebound owing to the secretion of insulin-like growth factor 1 (IGF-1) by treatment-resistant macrophages[33]. Combining macrophage targeting strategies with other treatments has shown increased therapeutic benefit; in orthotopic GBM models, pexidartinib enhanced the anti-tumour activity of inhibitors of platelet derived growth factor receptor[34] and radiotherapy[35]. TAMs or M-MDSCs also suppress T-cell function in the glioma TME and a CCR2 inhibitor, CCX872, enhanced immune checkpoint inhibition (ICI) efficacy in this largely immunotherapy-resistant tumour[36].

These studies highlight how inhibiting specific macrophage functions in combination regimens can reduce GBM progression; however, treatment-resistant macrophages, M-MDSC and tumour cells using myeloid suppressor cell features can facilitate immune evasion[37]Pyonteck, 2013 #22}. The reprogramming of TAMs and M-MDSC was in contrast to other settings where suppressive TAMs or M-MDSCs were reduced by CSF1R inhibition[14,26-29].

Breast cancer

Certain subsets of breast tumours have a high content of macrophages or myeloid cells in general, which impacts tumour growth and metastasis[38,39]. In mouse models of inflammatory

breast cancer, tumour cell expression of the CCR2 ligand, CCL2, drives tumour aggressiveness[40] and macrophages recruited by CCL2 facilitate lung metastasis[41]. *Ccr2* ablation reduced bone deposits in a murine model of metastatic breast cancer[42]. Further, CCR2 inhibition with RS504393 reduced tumour TAM content and enhanced the efficacy of immune checkpoint blockade in primary breast cancer and lung metastases in mouse models[43], while macrophages recruited to tumours through CCR2 signalling drove chemoresistance in mammary tumours in the MMTV-PyMT mouse model[44].

In common with other tumour types, CSF1R signalling also controls TAM function in breast tumour models. CSF1R-regulated TAMs drive paclitaxel resistance in the mammary tumours of MMTV mice carrying the activated c-neu oncogene, which is reversed by pexidartinib[11]. Targeting tumour macrophages with murine CSF1R-blocking antibodies increased the activity of platinum chemotherapy in the *Cdh1*:*p53* breast cancer model, inducing tumour interferon signalling and immune cell engagement[45]. Overall, targeting TAMs or MDSCs in breast cancers could improve standard of care treatments.

Interestingly changes in macrophage function in BRCA1-mutant breast cancer models limit the tumour cell response to PARP inhibitors. PARP inhibition in macrophages increased lipid metabolism resulting in a suppressive macrophage phenotype that resulted in resistance of tumour cells to PARP inhibition. However, treatment with murine CSF1R-blocking antibodies to deplete macrophages prevented resistance[46]. The capacity of PARP inhibitors to directly influence myeloid cell function is interesting and the drug-target-mediated adaptive responses of myeloid cells are not commonly considered.

Contexts with negative treatment outcomes

Although the studies discussed above highlight the potential benefit of targeting TAMs or M-MDSCs in specific disease settings, macrophage targeting does not always result in anti-tumour activity in treatment contexts where normal macrophage function might contribute to efficacy. For example, in syngeneic ovarian tumours, macrophages were shown to accumulate following chemotherapy and combining the CSF1R inhibitor, AZD7507, with chemotherapy reduced both efficacy and the anti-tumour immune response over chemotherapy alone[47]. This positive role for macrophages in the chemotherapy response contrasts with findings in pancreatic ductal adenocarcinoma and colorectal cancer[14,29,45]

discussed earlier, where TAMs or M-MDSC drive resistance to chemotherapy, highlighting that it cannot be assumed that macrophages in tumours universally drive therapy resistance. Similarly in mouse models of pancreatic ductal adenocarcinoma, normal macrophage function is required for an effective response to mesothelin-targeted chimeric antigen receptor T-cell (CAR-T) therapy; responding tumours had higher macrophage content and donor $CD8^+$ T-cells become dysfunctional upon macrophage depletion[48]. This is not the case for all CAR-T studies, as in human transplant models, folate receptor-positive macrophages limit the efficacy of mesothelin-targeting CAR-T therapy [49,50]. Defining the positive and negative roles of macrophages in mediating the response to a given therapy will be important to help refine treatment regimens.

Contexts involving macrophage reprogramming

Considering the response of TAMs and M-MDSCs to specific myeloid modulators in different settings is important. For example, in the RCAS-TVA glioma model, the CSF1R inhibitor BLZ945 did not deplete tumour macrophages and instead reduced M2-like transcriptional programmes commonly associated with suppressive macrophages[32]. Pexidartinib showed a similar effect in xenograft mouse models of human liver cancer[51].

The different response of different tumour types to macrophage-targeted therapy could be caused by differences in the ratio of TAMs derived from infiltrating monocytes and TRMs (**Box 1**)[52]. Indeed, the majority of TAMs in glioma are derived from resident microglia — a tissue resident macrophage cell — and not infiltrating monocytes[53]. TRMs are reported to contribute to the pool of TAMs in lung cancer[54]; expand during tumour development in the KPC pancreatic cancer model[55]; and TRMs derived from the omentum promote the metastatic spread of ovarian cancer[56]. TAMs or M-MDSCs can also be replaced by cells of a different origin over time: in a breast cancer model, resident macrophages were gradually replaced by monocyte-derived TAMs or M-MDSCs during tumour progression[57]. However, understanding how TRM and circulating monocyte-derived TAMs influence treatment response is difficult to study owing to a lack of consensus markers for TRMs.

Mechanisms controlling TAM or M-MDSC function following CSF1R inhibition have emerged that highlight TAM and M-MDSC plasticity. In a mouse model of breast cancer brain metastasis, chronic treatment with BLZ945 induced the upregulation of granulocyte-

macrophage colony-stimulating factor (GM-CSF), which drives macrophage reprogramming to a resistance phenotype through STAT5[58]. Tumour cell expression of GM-CSF also drives STAT3-dependent accumulation of suppressive macrophages or M-MDSCs in estrogen-receptor-positive breast cancer[59] and in orthotopic hepatocellular cancer[51] and pancreatic tumour mouse models[60]. Therefore, targeting more than one signalling mechanism controlling TAM or M-MDSC function may be required to sustain long term reduction of TAMs or M-MDSC, or perhaps adaptive resistance could be prevented by intermittent treatment with, for example, CSF1R inhibitors, attenuating upregulation of alternative regulatory mechanisms.

It should be noted that other cell types can also substitute for TAMs and M-MDSCs to drive immune-suppression. PMN-MDSCs[61] and FOXP3-positive T regulatory (T_{reg}) cells[62] accumulate following chronic CSF1R inhibition with JNJ-40346527 and pexidartinib respectively, and suppress cytotoxic T cell function, which highlights that targeting TAMs or M-MDSC alone may not be sufficient for an efficacious response.

Macrophages and monocytes play an essential role in normal tissue function and as a result, chronic inhibition of macrophage function can also drive toxicity. For example, CSF1R maintains hepatic Kupffer cells **[G]** and depletion of these cells leads to liver toxicity in humans as measured by changes in liver enzymes in the blood[63]. Unfortunately toxicities such as this are not easily modelled in the mouse, therefore it is important not to rely purely on mouse modelling to understand the longer term consequences of treatment on likely tolerability, and insights can only be confidently derived from clinical trials. Moreover, many preclinical studies use relatively short dosing durations that do not model the longer-term impact of TAM or M-MDSC inhibition.

Clinical trials targeting CSF1R

More than 30 Phase I/II trials of small molecule inhibitors or blocking antibodies of CSF1R inhibitors and small molecule CCR2 inhibitors have been initiated (**Table 1**; **Supplementary Table 2**). Antibodies targeting CSF1R or its ligand CSF1 that have been trialled include emactuzumab (RG7155)[64], AMG 820[65], IMC-CS4 (LY3022855)[66], cabiralizumab (BMS-936558/FPA-008)[67], which target CSF1R, and MCS110, which neutralises CSF1[68]. Trialled small

molecule inhibitors of CSF1R include ARRY-382/PF-07265804[69], BLZ945[70], pexidartinib[71], and JNJ-40346527[72].

CSF1R inhibitors, including pexidartinib[73] and emactuzumab[74], have clinical activity in tenosynovial giant cell tumours **[G]** (TGCT), a rare benign tumour driven by the over-expression of CSF1, and hence dependent on CSF1R-CSF1 signalling. However, many of the trials in other tumour indications were initiated with minimal supporting preclinical data[13,14,16,24] and have shown modest clinical efficacy, even when combined with chemotherapy or immune checkpoint blockade. While the lack of compelling clinical activity in solid tumour settings is frustrating, it should be noted that the studies were often performed in broad patient populations, in diseases that are hard to treat, often recruited patients late in their treatment journey — at which point achieving significant clinical benefit can be challenging — and, in a number of cases, the studies were performed in mixed tumour settings primarily designed to establish preliminary safety and efficacy (**Table 1**; **Supplementary Table 2**). Further, although these studies do broadly show that CSF1R inhibitors reduce circulating monocyte numbers, increase expression of CSF1R ligands such as CSF1 (a common feedback response to inhibiting growth factor receptors), and change the TME with down regulation of TAMs and increases in T cells, consistent with target engagement[65,66], it is possible that the degree of macrophage or M-MDSC modulation achieved in solid tumours is generally not sufficient to deliver robust efficacy. More mechanistic biomarker data is required to understand the impact of treatment in the TME in a more quantitative manner and determine whether the clinically tolerated doses give sufficient target engagement. At the time of initiating these trials, it was also not clear how to define patient populations, combination partners or line of treatment that would be most appropriate for myeloid therapies [75-77]. Given that many approaches sought to enhance the efficacy of existing agents, without patient selection the combination would have to be active in a broad patient group for efficacy to be evident in these smaller trials.

The clinical studies also commonly employ chronic dosing strategies, where the drug is given continuously, and only interrupted to alleviate toxicity. Chronic dosing of CSF1R inhibitors has been limited by toxicity, including hepatic toxicity following depletion of Kupffer cells — as measured by increased levels of alanine transaminase and aspartate aminotransferase in the blood [63] — and peri-orbital oedema[74,78], resulting in dose reduction or treatment

discontinuation. It is also possible that chronic dosing may not be the optimal treatment strategy as it could also attenuate sustained immune responses, for example by reducing function of normal macrophages which help sustain T cell responses.

Other challenges associated with interpreting results from early small-molecule CSF1R inhibitor development are exemplified by pexidartinib, which has been tested extensively as a monotherapy and in combination with immune checkpoint blockade, radiotherapy and chemotherapy. Pexidartinib is a potent inhibitor of CSF1R and also inhibits FLT-3, the receptor for the Fms-like tyrosine kinase 3 ligand cytokine, and the protooncogene c-Kit at clinically relevant doses[71]. As such pexidartinib inhibits a number of different kinases at clinical exposures, rather than selectively targeting CSF1R. Indeed, specific clinical trials have been initiated to explore the monotherapy activity of pexidartinib in haematological disease and glioblastoma dependent on FLT-3 and c-Kit signalling (**Supplementary Table 2**). Predicting the effects of inhibitors that target multiple kinases such as pexidartinib can be challenging. c-Kit inhibition could give additional benefit by reducing mast cell numbers in the tumour and other tissues[79], whereas inhibiting FLT-3[80] might impact long-term immune responses by limiting dendritic cell function, which is required for robust responses to immune checkpoint blockade[80,81]. Indeed, exogenous FLT-3 ligand enhances dendritic cell function and vaccine response in pancreatic ductal adenocarcinoma[82]. Therefore, positive short term effects of pexidartinib might be transient as chronic inhibition of CSF1R and FLT-3 could inhibit the macrophage or dendritic cell functions required for a sustained response. More selective inhibitors such as BLZ945, DCC3014, and antibodies targeting CSF1R or CSF-1 are in clinical trials, and it would be informative to understand the differential impact these agents have on the TME compared with agents such as pexidartinib.

The magnitude of the reduction in TAMs or M-MDSCs in the TME achieved or required clinically, or whether inhibition is equally effective in different tumour types or normal tissues, is hampered by a lack of data. A monotherapy trial of the CSF1R inhibitor, LY3022855, in metastatic breast cancer and castration-resistant prostate cancer showed macrophage depletion was associated with a modest increase in T cell activation in the peripheral blood[66], but other biomarker data is limited. Insights into dose response and deeper phenotyping of tumours treated with CSF1R inhibitors over time would show the impact on suppressive

macrophage function acutely and chronically, and whether treatment impacts macrophage functions associated with the therapeutic response.

It is important to be cautious when using preclinical data with one agent to position another without further validation, or to interpret model-specific observations as indicators of potential in a broad range of tumour types. For example, the combination of BLZ945 with radiation was demonstrated to be efficacious in a disease model representing a specific subtype of glioblastoma[32]. However, the clinical study was performed with pexidartinib and not BLZ945, and did not show a positive efficacy signal. This could be taken as a failure to translate from mouse to humans, however, firstly, the pexidartinib study was performed in an unselected population that did not recapitulate the patient subtype indicated by pre-clinical data, and secondly, BLZ945 and pexidartinib have different selectivity profiles and may therefore have differential impact in patients.

Ongoing clinical studies such as those testing the CSF1R-blocking antibody cabiralizumab and the CSF1-neutralizing antibody lacnotuzumab (MCS-110) in specific combinations and settings supported by preclinical data might yield more promising results than previous trials (**Table 1; Supplementary Table 2**). Ongoing trials with lacnotuzumab focus on combinations with ICIs. Although targeting soluble cytokines can be challenging because expression of the cytokine is commonly upregulated in response to the reduction in free cytokine following therapeutic treatment, targeting CSF1 may be better tolerated than targeting CSF1R with less acute impact on cells dependent on CSF1R for survival e.g. Kupffer cells. However compensation through the alternate CSF1R activator, IL34, might impact its effectiveness. Comparing these results with CSF1R inhibitors such as the CSF1R blocking antibody cabiralizumab or the small molecules AMG382, DCC3104 or BLZ945 which are currently in clinical trials will be informative.

Clinical trials targeting CCR2

CCR2 inhibition is less widely explored than CSF1R inhibition (**Table 1**; **Supplementary Table 2**). The most promising clinical data for CCR2 inhibition is in pancreatic cancer. CCR2 inhibition with the small molecule inhibitor PF-04136309 (also known as PF-6309) combined with FOLFIRINOX chemotherapy[83] showed an improvement in efficacy over FOLFIRINOX alone, although this improvement was not statistically significant. PF-04136309 also showed a

modest impact on circulating monocytes and an increased in T cell number in the TME, albeit in a small number of patients. Although PF-04136309 has been discontinued, a comprehensive pancreatic cancer program has been initiated using the CCR2/CCR5 inhibitor BMS8131360[84] as CCR5 influences macrophage recruitment to tissues[85]. Clinical trials testing combinations of this inhibitor with both chemotherapy and ICIs, in settings guided by preclinical studies, are ongoing[24,29,43,83]. Further studies are looking into development of image-based patient selection strategies for tumours enriched in CCR2-positive macrophages[86], which will aid patient selection.

Assessing whether therapeutic target-negative myeloid cells, or adaptive changes in the TME can compensate as resistance mechanisms in these clinical trials will be important. Interestingly both CCR2-positive and CCR2-negative monocyte-derived macrophage populations have been identified, therefore CCR2 inhibitors might be limited as a result compensation by TAMs or M-MDSCs lacking CCR2, but regulated by other mechanisms[87]. Supporting this, work profiling the myeloid architecture of human CRC has identified different macrophage subsets, with analysis of a small number of tumours suggesting residual macrophages following treatment with anti-CSF1R antibodies retain pro-tumour or immune suppressive properties[88]. It will also be important to consider carefully whether the preclinical models provide insights that are more reflective of established disease, early disease, or preventative settings.

Targeting neutrophils or PMN-MDSC

Neutrophils and myeloid-derived suppressor cells derived from neutrophil progenitors, known as PMN-MDSC, regulate tumour progression and the therapeutic response[5] (**Fig. 1**). Targeting neutrophils is more challenging than TAMs or M-MDSCs owing to a lack of tractable targets; however, inhibiting CXCR2 — a G-protein coupled receptor that is activated by a number of ligands including CXCL1,2,3,5,7 and 8 in humans and KC and MIP-2 in mice — has been used to prevent neutrophil recruitment to tissues[89,90]. Although CXCR2 is expressed on other cell types such as endothelial cells and epithelial cells, inhibitors of CXCR2 predominantly impact neutrophils and PMN-MDSC recruitment in the majority of tumour models (**Fig. 2**).

Pancreas

In the KPC model of pancreatic ductal adenocarcinoma, targeting neutrophils by deletion of *Cxcr2*, or with inhibitors such as AZD5069 or a pepducin antagonist of CXCR2, alone and in combination with the chemotherapy gemcitabine reduced metastasis and stromal density[91]. *Cxcr2* ablation reduced progression of murine orthotopic KRAS driven pancreatic tumours[92]. Similar to macrophages or M-MDSCs, neutrophils or PMN-MDSCs mediate resistance to immunotherapy in mouse models of pancreatic tumours[93] and in advanced liver metastases in the KPC model[94]. Neutrophil mediated T-cell suppression could be mediated by IL17-dependent neutrophil extracellular traps; in KPC tumours, neutralizing IL17 reduces tumour progression[94] and in orthotopic pancreatic tumours, IL17 blockade enhances the efficacy of PD-1 blocking antibodies[95]. In all these studies, T-cell infiltration is increased following neutrophil depletion.

Colorectal and liver cancer

Reducing neutrophils by *Cxcr2* ablation or treatment with a pepducin CXCR2 antagonist also reduced tumour progression in murine tumour models drive by loss of the *Apc* tumour suppressor gene (*Apcmin/+*) or loss of both *Apc* and the tumour suppressor phosphatase and tensin homolog **[G]** (*Pten)* (*Apcfl/+:Ptenfl/fl*)[96]. In murine colorectal tumour models driven by activated *Kras*, *Apc* mutation and *Trp53* loss, anti-PD1-inhibitor resistance is mediated by CXCR2-dependent PMN-MDSCs[97] and CXCR2-dependent myeloid cells can also facilitate metastasis of colorectal cancers to the liver[98]. In the highly aggressive, metastatic KPN mouse model of CRC (*Kras*^{G12D}:*p53*$^{-/-}$:*Notch1*^{ICD}), epithelial TGFβ2 and CXCL5 — the CXCR2 ligand — were shown to recruit tumour neutrophils, promoting metastasis and differentiation of the primary tumour to the human consensus molecular subtype (CMS) 4 subtype. Conversely, CXCR2 inhibition with AZD5069 or depletion of neutrophils with a murine neutrophil depleting antibody reduced metastasis, and increased tumour T cell content[99]. The importance of neutrophils and PMN-MDSCs in the liver TME is reinforced by the finding that neutrophils contribute to the progression of hepatocellular carcinoma in both chemical-induced and diet-induced models[100-103], with CXCR2 inhibition enhancing the response to immune checkpoint blockade in mouse orthotopic and autochthonous cancer models [104].

Head-and-neck and lung cancers

In murine orthotopic head and neck tumours, neutrophils suppressed the function of adoptively transferred natural killer (NK) cells, an effect that was reversed following the inhibition of CXCR1/2 using the inhibitor SX682[105]. Similarly in lung cancer, increased neutrophil numbers in the tumour are associated with ICI treatment failure in patients, with SX682 reversing resistance in pre-clinical models[106]. Neutrophils or PMN-MDSCs also mediate resistance to tumour targeting therapies; CXCR2-dependent myeloid cells drive resistance of lung cancers to inhibitors of SH2-containing protein tyrosine phosphatase-2 (SHP2). A combination of the SHP2 inhibitor, SHP099, with SX682 enhanced survival in both KRAS-driven and EGFR-driven lung cancer models[107].

Prostate cancers

Prostate cancers are commonly resistant to immune checkpoint blockade[108] and overexpress IL8 and other CXCR2-modulating chemokines[109,110]. High neutrophil-to-lymphocyte ratios and high monocyte levels in the peripheral blood are associated with poor prognosis[111,112]. In a castration-resistant prostate cancer model, depletion of PMN-MDSCs using SX682 or disruption of PMN-MDSCs using neutralizing anti-IL8 antibodies enhanced responses to immune checkpoint blockade[109,113]. In phosphatase and tensin homolog **[G]** (PTEN)-null prostate tumours, macrophage-like myeloid cells that appear distinct from TAMs or M-MDSCs drive resistance to anti-androgen therapy through IL-23 induced tumour senescence, which is reversed by inhibiting IL23 and CXCR2[114,115]. The concept of CXCR2/IL23-dependent myeloid-mediated resistance to anti-androgen therapy is currently being tested in the clinic with small-molecule CXCR2 inhibitors[116].

It is intriguing that the CXCR2-dependent myeloid cell type driving anti-androgen resistance in the context of genetically engineered mouse models of prostate cancer is macrophage like and does not appear to be a neutrophil or PMN-MDSC. Currently the properties of these cells are under-explored but it is possible that the prostate cancer TME has a unique myeloid population compared to other tumours. Indeed macrophage depletion can also influence prostate cancer progression by preventing macrophage derived lipids from sustaining tumour cell survival [107,117]. The subtle differences in the regulation of key functional myeloid cell phenotypes in different tissues such as prostate, versus liver or pancreas, warrant further exploration, are perhaps under-appreciated given the reliance on simple syngeneic models to

define and study different myeloid subsets, and highlight the important of exploring biology in pre-clinical models that reflect specific disease indications.

In summary, reducing tumour neutrophils or PMN-MDSCs in specific disease settings has the potential to influence response to chemotherapy, immunotherapy and targeted therapy. The available data suggest that targeting CXCR2 may have a broader utility in enhancing T-cell function and chemotherapy responses, and in the context of liver tumours might be particularly beneficial.

Targeting neutrophils and PMN-MDSCs clinically

Elevated neutrophils or PMN-MDSCs in the peripheral blood and high neutrophil-to-lymphocyte ratios are common in patients with cancer and associated with poor prognosis[118]. Inhibitors of CXCR2 or its ligands are the most advanced therapies targeting neutrophils or PMN-MDSCs in clinical trials (**Table 1**, **Supplementary Table 2**). Agents targeting the CXCR2 receptor (AZD5069[90,119], SX682[120]) or CXCL2 ligand (BMS-986253, Humax-IL8[121,122]) are in trials in combination with chemotherapy and immune checkpoint blockade [86,116,123-129] (**Table 1**, **Supplementary Table 2**). Although the results of many of these trials have not been published, reparixin — a CXCR1/2 peptidomimetic — was inactive in combination with paclitaxel in triple negative breast cancer (TNBC) [130], and no positive clinical benefit was reported for AZD5069 in head and neck cancers. As discussed previously for CSF1R and CCR2 inhibitors, it is possible the trials targeting neutrophils or PMN-MDSC do not have an optimal design; for example, the AZD5069 trial in head and neck cancer was performed in unselected patients[128] and a trial in pancreatic cancer that showed no benefit enrolled late stage patients where achieving clinical benefit is very challenging[127].

There are some potential challenges to successful clinical application of CXCR2-targeting drugs. As neutrophils play a critical role in primary immune defence, a reduction in peripheral neutrophils is considered a concerning toxicity. CXCR2 regulates the release of neutrophils from the bone marrow[89] and its inhibition reduces circulating neutrophil levels in patients [90], inducing neutropenia **[G]**. In clinical trials of CXCR2-inhibitors in asthma and chronic obstructive pulmonary disease this limited the maximal clinical dose that could be achieved. As with macrophages, the degree of target inhibition and neutrophil or PMN-MDSC reduction required for a therapeutic effect is not clear, nor is it clear whether sustained suppression is

required. Although it is difficult to translate the degree of target engagement and suppression or depletion of the PMN-MDSC achieved in preclinical models (where neutrophil reduction is well-tolerated) to humans, if these cells are highly suppressive, a high level of depletion might be required to drive efficacy. Dose optimization to achieve high levels of target engagement and neutrophil suppression while managing potential side effects will be important to maximise the clinical benefit. It is also important to consider how pathway redundancy may limit efficacy; in humans, both CXCR1 and CXCR2 are stimulated by IL-8 and targeting CXCR2 alone could result in compensation through CXCR1. Conversely, CXCR2 can be stimulated by multiple ligands including IL-8, CXCL1, CXCL2, CXCL3 and CXCL5, among others and therefore the effect of neutralizing IL-8 alone might be compensated for by other ligands. Finally, as discussed earlier for macrophage and M-MDSC modulating therapies, neutrophils are essential for host defence and can play important roles in anti-tumour response directly [131,132], by enhancing recruitment of other immune cells such as NK cells or T-cells[133], and may even cross present antigen[134,135]. Therefore, it is important to also consider that sustained inhibition of neutrophils may also attenuate long term anti-tumour control, or in certain early disease settings, attenuating neutrophil function could be detrimental.

Selecting tumours most likely to be sensitive to neutrophil-mediated therapy resistance is important. Two recent studies in bladder, renal, melanoma and lung cancer cohorts suggested high numbers of neutrophil and levels of IL8 in the peripheral blood are associated with poor response to first-line immune checkpoint blockade [136,137] and thus it would seem reasonable to assess neutrophil-targeting combinations in these biomarker defined subsets.

Combined targeting of different myeloid cells

Targeting granulocytic and mononuclear myeloid cells simultaneously could provide clinical benefit. Combining CXCR2 (SB225002) and CSF1R (JNJ-40346527) inhibition improved anti-tumour effects versus CSF1R inhibitor treatment alone in syngeneic lung cancer and melanoma models[61]. Furthermore, CCR2 inhibition by PF-04136309 or RS504393 in a murine model of pancreatic cancer prevented macrophage recruitment but increased tumour neutrophil content. Combining PF-04136309 with the CXCR2 inhibitor SB225002, or CXCL8 neutralising antibody further increased response to chemotherapy[29]. This demonstrates potential for some functional compensation between myeloid sub-types. In the KPC model, targeting CXCR2 and CSF1R-dependent myeloid cells differentially affected tumour

progression. CSF1R inhibition reduced squamous features and primary tumour growth with increased influx of $CD8^+$ T-cells [28], whereas CXCR2 inhibition reduced liver metastasis but required ICI to increase T-cell infiltration and response[28,91]. This suggests that while TAMs/M-MDSCs and neutrophils/PMN-MDSCs have some functional overlap, they influence different features in a given tumour setting and in primary versus metastatic disease.

The bias of myeloid cell content can be influenced by the tumour. In a pancreatic genetically engineered mouse (GEM) model driven by inducible oncogenic *Kras*, switching KRAS expression off — mimicking therapeutic intervention — changed the TME from neutrophil-rich to macrophage-rich. This change was driven by increased tumour cell CCL2 expression mediated by HDAC5, increased macrophage recruitment and resistance to KRAS elimination via macrophage TGFβ secretion[138]. This demonstrates the complexity of the cross regulation that can occur in the tumour, but also how the tumour cell status can orchestrate the TME. Finally, in a Flp-recombinase-driven KPC-like model, deletion of mouse *Col1a1* (which encodes collagen 1) from fibroblasts led to the influx of neutrophils and macrophage-like suppressive myeloid cells and increased progression, which could be reversed by the inhibition of both CXCR2 and CCR2[139].

These studies highlight the complexity of the interplay between different elements of the stromal and myeloid compartments. This complexity is further demonstrated by a study using primary PDAC-derived tumour models where pharmacologically normalizing cancer associated fibroblasts (CAFs) towards a less fibrotic, secretory phenotype with decreased stromal ECM deposition reduced the number of TAMs, including those polarized to a suppressive phenotype[140]. Although comprehensive suppression of myeloid recruitment might result in favourable outcomes, there are some concerns with this approach. In humans, comprehensive myeloid cell suppression might not be tolerated and the relative myeloid profiles in different tumour types are not well defined. Insights into the identity of residual myeloid cells that persist after treatment are needed to guide rational combination approaches.

Reprogramming myeloid cells

Targeting PI3Kγ

The differentiation and function of myeloid cells can be manipulated by small-molecule inhibitors (**Fig. 1**), for example those targeting PI3Kγ (for example, IPI549[141] and AZD3458[142]) and STAT3 (AZD9150)[143] (**Fig. 2**). PI3Kγ is an atypical PI3K that is expressed in immune cells, is activated by G-protein coupled receptors (GPCRs)[144] and plays a pivotal role in macrophage differentiation[141] and neutrophil activation[144]. Early preclinical studies showed that *Pik3cg* ablation or PI3Kγ inhibition with IPI549 or AZD3458 enhanced ICI activity in mice with syngeneic subcutaneous tumours, including those derived from B16F10, 4T1, MC38 and LLC cell lines. Further, deletion or inhibition with IPI549 or the PI3Kγ/δ inhibitor TG100-115 reduced tumour progression in the murine PyMT breast tumour model[141] and the KPC mouse model of PDAC, respectively[145]. Efficacy following PI3Kγ inhibition was associated with changes in antigen presentation in macrophages, along with downregulation of IL10 expression and upregulation of IL12 expression [141,142,146].

PI3Kγ inhibitors might have potential in other settings. In an orthotopic glioblastoma model in which glioblastoma were implanted in mice subcutaneously, PI3Kγ controlled tumour-modified microglial cell differentiation and function, and treatment with the PI3Kγ inhibitor IPI549 reversed resistance to temozolomide chemotherapy[147]. A further study showed that *Pik3cg* ablation reduced the progression of colitis-associated colorectal tumours through reduction of myeloid cells in tumours and inflamed tissue [148].

Different PI3Kγ inhibitors can deliver distinct mechanistic effects by targeting PI3K isoforms other than PI3Kγ. For example, the PI3Kγ inhibitor IPI145 (duvelisib), which has additional activity against PI3Kδ[149], regulates T_{reg} cell function[150], with reduction of tumour T_{reg}s leading to increased cytotoxic T-cell function. IPI145 has activity in a number of humanized breast cancer models and syngeneic murine cancer models including those derived from HPV+/- head and neck cancers, 4T1 breast cancer cells and B16F10 melanoma cells[151-153]. T_{reg} cells can drive immune-suppression following suppressive macrophage depletion with the CSF1R inhibitor pexidartinib [62]. Therefore, the additional impact of IPI145 on T_{reg} function, as well as effects on myeloid cells, could give benefit by blocking a potential alternative cell type mediated resistance. Finally, whether PI3Kγ inhibition influences neutrophil or dendritic cell function in the TME is unclear and should be explored further.

Clinical trials with IPI549 in combination with chemotherapy or ICI are underway in mixed tumour settings[86,154-156] (**Table 1**; **Supplementary Table 2**). Promising early studies suggesting a potential clinical benefit of IPI549 when used in combination with paclitaxel in TNBC breast cancer or with ICI in bladder cancer have led to its designation as a breakthrough therapy; however, data from clinical trials have not yet published. It will be important for randomized trials to assess the activity of IPI549 and identify patient subsets that could gain the greatest benefit from their use. Further PI3Kγ inhibitor combinations with ICI and chemotherapy have not yet been explored more broadly in pre-clinical models reflecting specific tumour types.

Targeting JAK–STAT

Suppressive myeloid cell phenotypes can be regulated by transcriptional modulators (**Fig. 3**). IL-6–JAK–STAT signalling controls myeloid cell differentiation and activation and neutralizing IL-6 enhanced the activity of ICI in pancreatic cancer models[157]. However, small molecule JAK inhibitors tested clinically show limited activity in solid tumours. The transcription factor STAT3 can be targeted with antisense oligonucleotides (ASO)[143], which are preferentially taken up by macrophages, T cells and endothelial cells, and treatment with the STAT3-specific ASO AZD9150 has been shown to enhance the efficacy of ICI in several subcutaneous syngeneic tumour models[143], including ICI-resistant tumour models driven by tumour cell deletion of *STK11*[158]. AZD9150 was assessed in two combination trials in head and neck cancer[128] and lung cancer[159,160] that included patients that had previously failed immunotherapy, however, in the head and neck trial there was no compelling clinical activity, while the data from the lung cancer trial is not published (**Table 1**).

Targeting C/EBPα

The expression of the myeloid transcriptional regulator C/EBPα is deregulated in liver, breast, and lung tumours[161]. A small activating RNA that upregulates C/EBPα expression in M-MDSCs and TAMs, known as MTL-CEBPA, has been shown to reverse the suppressive activity of these cells and enhance ICI efficacy in subcutaneous syngeneic tumour models, although the therapeutic benefit has not been explored in models that reflect specific tumour type or genetic segments of disease. MTL-CEBPA treatment did result in tumour regression in 27% of patients with viral-aetiology hepatocellular carcinoma (HCC)[162-164]. Combinations of MTL-CEBPA with ICI or the broad-range protein kinase inhibitor sorafenib are currently being

trialled in HCC[165,166] and biomarker studies from these trials will be important to link the preclinical findings to the human setting.

Epigenetic regulators such as histone deacetylases can modify macrophage phenotypes and inhibitors of HDAC function are being investigated in combination with ICI[167 168]. However, these agents are not specific to myeloid cells, modifying the phenotype of many cells, and have been associated with clinical toxicity.

Activating macrophage function

Macrophages can be activated by targeting cell surface proteins (**Fig. 3**). Using agonistic anti-CD40 antibodies to stimulate CD40 — a receptor on the surface of T-cells and B-cells that facilitates effective macrophage antigen presentation — promotes macrophage activation and antigen presentation *in vitro*[169]. This approach has not been explored extensively in pre-clinical *in vivo* models, although the combination of murine anti-CD40 antibodies and chemotherapy improved survival in KPC PDAC-bearing mice[170,171]. Promising data in Phase I failed to translate into a clinical signal in a controlled Phase II trial in unselected patients[172]. However, mechanistic translational studies identified subsets of patients with positive immune changes in the TME following treatment including changes in tumour macrophage content and increased CD4+ T-cell infiltration[172].

The macrophage mannose receptor (CD206) and the macrophage receptor with collagenous structure (MARCO) are also potential therapeutic targets. CD206 blockade enhanced anti-tumour immune response in syngeneic models and the mouse KPC pancreatic tumour model[173]. In separate studies MARCO inhibition activated NK and T-cells *in vitro*, and modified the TME in mouse syngeneic melanoma and lung tumour models[174,175].

Theoretically, macrophage phagocytic function can be modulated by targeting interactions between CD47 and SIRP1α[176]. CD47 is a widely expressed cell-surface protein that is overexpressed in cancer cells and acts as a “don’t eat me” signal through binding SIRP1α on the surface of macrophages. Antibodies targeting SIRP1α[177] or CD47 [178] have potential in haematological disease, but it is unclear if they are effective in solid tumour models despite these mechanisms being explored in solid tumour settings clinically. Likewise, LILRB1, a macrophage cell surface receptor that inhibits normal macrophage function when activated,

can be inhibited with antibodies to prime macrophage activation[179] although again the effectiveness of this approach has not been widely explored in solid tumour models.

Finally, stimulating CD11b/CD18 integrins — which control the recruitment and function of both monocyte-derived and neutrophil-derived myeloid cells in tissues — with small molecule mimetics of the integrin ligand binding motif, such as GB1275, modifies TAM activation and polarization, enhancing ICI efficacy in syngeneic murine models of pancreatic and lung cancer[180,181]. Clinical trials with GB1275 are ongoing. It will be interesting to examine the efficacy of these inhibitors in murine models of specific tumour types such as pancreatic, colorectal or breast cancer where myeloid cells drive tumour progression and resistance, as well as explore their impact on myeloid cells other than macrophages.

Harnessing innate immune pathways

The stimulation of Toll-like receptors (TLRs) on the surface of macrophages and dendritic cells by pathogen-associated molecular patterns (PAMPs) triggers the activation of these cells and the production of inflammatory chemokines and cytokines. It has been shown that TLR3 and TLR9 agonists repolarise TAMs and inhibit tumour growth [182-184] and the TLR7/8 agonist resiquimod (R848) reduced the growth of pancreatic KPC tumours[185]. In another study resiquimod was formulated as a nanoparticle to specifically target macrophages, and polarised TAMs to an anti-tumour phenotype and reduced tumour growth in syngeneic mouse tumour models[186].

Macrophages sense tumour cell-derived cytosolic DNA through the cGAS–cGAMP–STING pathway, stimulating type-I IFN production, the maturation of DCs and subsequent T-cell priming[187,188]. In a *Brca1*-deficient mouse model of breast cancer, a STING **[G]** agonist mediated macrophage reprogramming and reversed the resistance of the cancer to PARP inhibitors[189].

Currently, targeting STING or TLR requires intra-tumoural injection of therapeutic agents owing to their high toxicity when used systemically. New drugs targeting STING that can be given orally have been developed and have demonstrated anti-tumour activity in preclinical models, although it is challenging to assess toxicity in mice[190,191]. It should be noted that innate immune pathways such as STING are common to many cell types and therefore agonists of these pathways will target many different cells. It is therefore challenging to interpret the

effects of targeting these pathways in macrophages unless the agent is delivered directly to the cell either by formulation as a nanoparticle that is taken up as a result of phagocytosis, or by targeting to macrophage, TAM or M-MDSC via antibody-drug conjugates to cell surface receptors such as CD206 or CD163. Targeted delivery may however improve clinical utility.

Targeting immuno-metabolism pathways

Altered tumour cell metabolism and TME-derived metabolites suppress or modify immune cell function[192] (**Fig. 3**), and lipid biosynthesis pathways have emerged as important modulators of myeloid cells.

Prostaglandins **[G]** such as prostaglandin E2 (PGE2) released by tumour cells and cells in the TME modulate the function of macrophages, TAMs and M-MDSCs through the EP4 receptor[193,194]. The EP4 antagonists MF-766[195] and E7046[196] reverse myeloid cell mediated immune-suppression in standard subcutaneous syngeneic tumour cell line transplant models. The EP4 antagonist TPST-1495 is currently being tested clinically in combination with pembrolizumab[197], although no data have been published yet. In neutrophils, inhibiting lipid uptake by inhibiting the fatty acid transporter FATP2 reduces suppressive activity of neutrophils, providing a novel way to specifically target suppressive neutrophils and PMN-MDSCs[198].

Arginine, adenosine, glutamine, tryptophan, kynurenine, and lactate have all been implicated in TAM reprogramming[199]. Arginase-1, which metabolizes arginine to ornithine and urea, is upregulated in immune-suppressive macrophages and M-MDSCs in mice. Arginase inhibitors such as CB1158 (INCB001158) give anti-tumour activity in subcutaneous syngeneic mouse tumour models by reducing the depletion of arginine and sustaining T cell function[163,164]. A novel inhibitor of arginase-1 known as compound 9 promoted an anti-tumour immune response in KRAS-driven lung GEM tumours showing that inhibiting arginase-1 may have potential in lung tumours, can be effective in a setting other than simple subcutaneous tumour models [200]. To date, however, there is no compelling clinical signal for arginase-1 inhibitors, and translating results from mice to human could be difficult as arginase-1 is expressed in macrophage-like suppressor cells in mice, where in humans it is largely expressed in neutrophils[201].

Blocking adenosine receptor A2 or reducing adenosine generation by blocking CD39 or CD73[202] inhibited the growth of mouse syngeneic tumour allografts when combined with ICI [203-205]. Inhibitors of adenosine receptors are being tested clinically but no positive data has been published.

Finally, indoleamine 2,3-dioxygenase (IDO) — which converts tryptophan into the suppressive metabolite kynurenine — is elevated in TAMs and M-MDSCs[206]. Encouraging Phase II results with IDO inhibitors in combination with ICI[207,208] failed to translate to Phase III trials[209,210] because of metabolic adaptation and upregulation of nicotinamide adenine dinucleotide (NAD) in tumour cells which is also immunosuppressive[211]. Although the influence of metabolites on both tumour and immune cell function is well established, targeting individual metabolic pathways might not give durable clinical effects because of redundancy and feedback adaption.

Tissue-specific myeloid cell roles

Different myeloid cells are responsible for establishing the metastatic niche in different organs. In mouse models, neutrophils have been shown to play a dominant role in establishing liver metastasis and resistance to chemotherapy and ICI[91,98,99,212]. In orthotopic metastatic breast tumours, macrophages drive primary disease progression and neutrophils promote metastasis to the lung[213], in part through the protease cathepsin C[214]. CXCR2-dependent myeloid cells facilitate tumour outgrowth in syngeneic breast and melanoma lung-seeding models, with myeloid-cell-specific CXCR2 ablation or the CXCR2 inhibitor SX682 reducing metastasis and increasing T-cell numbers in residual tumours. Similarly, in a KPC pancreatic tumour-derived liver metastasis model, modified neutrophils negative for the P2X (purinergic) receptor 1 (P2RX1) facilitated metastasis[215].

How neutrophils enhance metastasis is poorly understood. In addition to their immunosuppressive function, DNA extruded in suppressive neutrophil extracellular NETs might aid metastasis[216-219], potentially through the receptor tyrosine kinase DDR1 expressed on tumour cells[219] or tumour-cell-expressed transmembrane protein CCDC25[218].

Following chemotherapy, tumours can recruit macrophages, which then facilitate metastasis[220]. Macrophage-mediated metastasis could be targeted by reprogramming macrophages; in a model of lung-metastatic rhabdomyosarcoma, macrophages genetically

engineering to constitutively express IL12, a proinflammatory anti-tumour immunomodulatory cytokine that activates T-cells, natural killer (NK) cells and reprogrammes or reduces suppressive myeloid cells in the TME, reduced metastasis and disrupted fibrosis and tumour cell infiltration into the lung[221].

Translating from mouse to human

An important question commonly asked when using insights into myeloid cell therapies developed in murine tumour models to define a potential clinical trial is the equivalence of human and mouse myeloid cells. Comparative profiling studies have started to give useful insights into this question. Profiling of human and mouse lung tumours showed a high degree of overlap between phenotypically similar human and mouse macrophage populations present in tumours, suggesting a similar role in early stage lung cancer in mice and humans[87]. Broad comparison of tumour-infiltrating myeloid cell populations including neutrophils, macrophages, monocytes, and DCs from human and mouse lung tumours indicated that between the two species these myeloid cells have similar features based on cell surface markers, but more importantly, specific gene expression signatures developed through single cell sequencing, although gene expression profiling suggested human macrophage populations were more complex, with evidence of more subtypes[87,222]. Other studies analysing neutrophil-like myeloid cells in different tissue sites in humans and mice suggested neutrophils show a diverse range of differentiation and functional phenotypes depending on the tissue they are recruited to[223]. Human and mouse populations of neutrophils and PMN-MDSCs appeared similar, although gene expression analysis predicted more diverse and complex subsets in humans[223].

A second important question when translating from mouse to humans is how functionally different subsets of cells are between species. Human PMN-MDSC gene signatures that mirror those seen in mice were associated with poor outcome to ICI treatment in humans [224,225], giving some confidence in the relevance of murine data. One study comparing the anti-tumour effects of neutrophil released factors showed a key difference between human and murine myeloid subsets; only human neutrophils released a catalytically active form of neutrophil elastase, which was capable of killing cancer cell types while sparing non-cancer cells[131]. Human neutrophils might therefore possess a greater capacity for tumour cell killing and the chronic suppression of all neutrophil-like cells might not be desired in the human context.

Profiling tumours for patient selection

Gaining insights into the function of specific myeloid cells in different human cancers is essential to aid patient selection. The comprehensive multi-omics analysis of 130 pancreatic tumours using cytometry time-of-flight (CyTOF) mass spectrometry and single-cell sequencing revealed tumours enriched for myeloid cells over T-cells have a worse outcome independent of other features[226], suggesting myeloid therapies may benefit tumours with low T-cells and high metastasis risk. A smaller analysis of brain tumours revealed two distinct macrophage subtypes that are differentially regulated by treatment[227], which could provide the basis of a patient selection strategy in glioblastoma. Such selection strategies could be developed by using histological markers that assess macrophage content or differentiation state, but validated using more in depth profiling approaches. Alternatively in the absence of a prospective biomarker test they could be used to segment an unselected cohort of patients after treatment to determine if greater benefit is observed in a subset of patients positive for specific macrophage biomarkers.

When designing a clinical trial tumour stage will be important as tumour myeloid cell content and dependency can change over time. In human and mouse lung tumours, tissue resident macrophages appear to contribute to tumour progression or immune-evasion in early disease and possibly as the metastatic niche establishes. However, as the tumour progresses, tissue resident macrophages are restricted to the tumour periphery and monocyte-derived macrophages accumulate in the established TME[87]. Targeted reduction of tissue resident macrophages might therefore target early disease, whereas reducing recruited monocyte-derived macrophages or M-MDSCs might affect disease at a later stage.

Large-scale profiling in clinical studies can offer important insights into modifiers of therapeutic response. In clinical studies, peripheral blood biomarker data is commonly used to infer the impact of a treatment on the tumour, although this data should be used with caution as tumour myeloid cells differ phenotypically to those in the peripheral blood[87,222]. Two clinical studies have linked myeloid cells and ICI response; in independent Phase III trials in renal and bladder cancer, high serum IL8 and peripheral blood mononuclear cells associated with resistance to ICIs, even in patients with high tumour T-cell content[136,137]. A second

retrospective study of lung and renal cancer Phase III trials also identified that IL8 and intra-tumoural neutrophils associate with poor outcome to therapy[137]. Interestingly, poor response to VEGF inhibition — a core treatment for renal cancer — is associated with signatures indicating a low angiogenic phenotype, implying poor tumour angiogenesis, but high infiltration of macrophages in the tumour[228]. These studies indicate specific settings where neutrophil-targeting or macrophage-targeting therapies could be used to test a therapeutic resistance hypotheses; for example, subsets of renal cancer patients with high serum IL-8 or peripheral myeloid cells — in combination with VEGF treatment or ICI — might show an improved response to treatment with neutrophil-modulating or macrophage-modulating drugs.

Profiling studies can yield novel therapeutic targets. In renal cancer, high tumour content of macrophages positive for TREM2, APOE and C1Q is associated with recurrence[229]. TREM2 is a potential target for renal cancer as macrophage-specific deletion of TREM2 promotes anti-tumour immune responses[230] and TREM2-targeted antibodies enhanced anti-PD1 efficacy in syngeneic renal cancer models[231].

Using profiling insights to inform clinical development will be essential to improve success. However, describing phenotypically distinct cells could add a layer of complexity that might be unhelpful in some cases. Understanding which subtypes are functionally diverse, whether subsets performing similar functions in the tumour are regulated by a common mechanism and if therapeutic intervention can reverse the suppressive myeloid cell population is critical.

ICI resistance: myeloid cells in context

As discussed in this Review, there is a wealth of evidence that depleting myeloid cells or modifying their phenotype reduces immunosuppression and enhances ICI efficacy. However, myeloid cells are part of a broader immune resistance landscape (**Fig. 3**). Within the TME, the distribution of T cells, CAFs, T_{regs}[62], immunometabolites[232] and suppressive cytokines such as TGFβ[233,234] also mediate resistance to ICI. Moreover, immunosuppressive metabolites impact many cell types. Kynurenine, lactate and adenosine inhibit T cells, NK cells, DCs and enhance T_{regs} and the activity of MDSCs[192]. Adenosine production from extracellular ATP is mediated by CD39 and CD73, and exhausted T cells themselves express high amounts of CD39 that might contribute to the immunosuppressive environment [235]. Preclinical work targeting CD39 or

CD73 has shown enhanced $CD8^+$ T-cell proliferation, a reduction in T_{regs} in the tumour and improved response to ICI[202,236]. CAFs facilitate immune escape by providing a physical barrier and through the secretion of immunosuppressive cytokines. TGFβ-driven CAF signalling correlates with poor response to ICI, and several studies have reported synergy between ICI and TGFβ inhibition through reduced fibrosis and enhanced T-cell function[233,234,237]. High numbers of intra-tumoural T_{regs} have predicted poor response to ICI in several studies; however, anti-PD1 checkpoint blockade might also promote the survival and immunosuppressive functions of T_{regs}[238]. How inhibiting myeloid-cell-mediated suppression impacts T-cell subtypes, which T-cell subtypes are targeted by different myeloid suppressor cells and the broader changes in the TME brought about by myeloid-cell-mediated suppression has not been extensively studied.

Tumour mutational status or changes in protein expression can confer resistance to treatment. For example, loss of the tumour suppressors *STK11*[158,239-241] or *PTEN*[242,243], *CDKN2A/B* mutations or loss of chromosome 9p21 **[G]** [153,243,244], mutations or deletion of *STING*[245,246], reduction in β2 microglobulin **[G]** , or loss of heterozygosity of human leukocyte antigen **[G]** (HLA)[247-249] all associate with poor response or resistance to ICI and chemotherapy. In tumours where these features are common, myeloid cells might not be the dominant resistance mechanism suppressing T-cell activation. For example, loss of antigen presentation prevents T-cells targeting the tumour cell, so in this situation myeloid cells may no longer be a relevant resistance mechanism. Conversely in other tumours the tumour cell mutational status might define a patient segment where myeloid cells are particularly important, for example those with tumours lacking *PTEN*[242,243] or *STK11*/*LKB1* [158,239-241], where loss is associated with increased myeloid cell content.

Considering the genetic landscape of human tumours is important. Clinical trials seeking to enhance ICI or chemotherapy response often recruit unselected patients, but preclinical models are developed with specific genetic tumour drivers. For example, many preclinical studies with myeloid modulators often use pancreatic tumour models driven by *Kras* mutations and p53 loss [14,28,29,91,93] as these transgenic tumour models have a complex TME. However human pancreatic tumours commonly have the additional disruption of chromosome 9 and mutations of the tumour suppressor *CDKN2A/B*[250,251], which are associated with resistance to ICI[153,243,244]. The preclinical data do not model this additional

genetic change, and in the context of pancreatic cancer, the benefit of myeloid modulators in combination with ICI-based treatments may be greatest in the subset of patients without chromosome 9 disruption. This highlights that without careful consideration of the broader landscape the clinical trial population often does not represent the context in which the preclinical studies were performed. Understanding the resistance landscape of different tumours and building an integrated picture of resistance features that use myeloid cells as a dominant resistance mechanism will enable the development of more focused clinical trials (**Fig. 4**).

Finally, although data is often generated at a tumour cohort level, it is hard to understand the complex hierarchy of resistance features. For many tumours, it is possible that more than one feature may need to be targeted for maximal benefit, whereas an enhanced response might not be achieved for some tumours even when putative resistance drivers are targeted.

Perspective and conclusion

The evidence that TAMs, M-MDSCs, neutrophils or PMN-MDSCs are associated with poor prognosis and reduced response to therapy continues to build, but the major challenge for the field remains translating the preclinical science into clinical activity **(Box 2)**. Single-cell profiling or sequencing techniques have enabled unprecedented insight into the complexity of the myeloid cell populations in primary tumours, metastatic sites, and the peripheral blood. However, while these studies generate new biological insights, describing myeloid cell populations of ever greater complexity may not ultimately help with clinical development, although this may seem counter-intuitive. The next critical step will be to link these descriptions of complex cell populations to functionally equivalent subsets of cells and the mechanisms that can be used to target pathologically active, suppressive myeloid cells and spare classically active myeloid cells with anti-tumour functions. Having the ability to assign complex myeloid cell populations to broad functional subsets that can be targeted with specific therapeutic interventions will help in developing more rational clinical treatment strategies. Given that targeting monocytic and granulocytic suppressive myeloid cells is desirable, this would help prioritise the most important approaches to achieve comprehensive suppressive myeloid cell targeting. Considering tumour stage-specific and tissue-specific functions of different myeloid cells may be important. For example, the neutrophil or PMN-MDSC might be critical in small metastases but less important in bulky

established metastatic disease, whereas macrophages or M-MDSC may play a role in primary disease or specific metastatic settings. Alternatively neutrophils or M-MDSCs may be important in the liver TME, while the TAMs or M-MDSCs play a broader role in different primary tumour settings.

Considering the positive and negative effects of myeloid-targeted therapies and combination strategies on both short-term and long-term responses is critical. In both preclinical and clinical studies, the long-term effect of inhibitors on residual or resistant myeloid phenotypes have not been explored. It is not clear whether myeloid cells other than the inhibited populations can compensate over time, whether the doses used in clinical trials impact suppressive cells sufficiently to produce a biological effect, or whether other resistance mechanisms — such as T_{regs} — compensate for reduction in suppressive myeloid cell function.

Negative preclinical studies are rarely published but the insights they give are important as they provide insight and build confidence in those settings where positive effects are seen. To select the right patients, greater understanding of where myeloid cells are the primary drivers of resistance — versus merely being associated with poor response — will be critical for improving success in the clinic. Many preclinical studies have assessed ICI enhancement following myeloid suppression and not considered the additional combination of these therapies with chemotherapy or other tumour targeted agents. Exploring these more complete therapeutic strategies preclinically would be highly informative and help develop new combination approaches for clinical testing. Tailoring combinations to specific organs, or towards primary or metastatic disease specifically will also be important to improve success. There are a number of promising new mechanisms that can impact therapeutic outcomes if the clinical positioning, as well as the dosing and scheduling, is developed correctly **(Box 3)**.

In summary, myeloid cells play a pivotal role in driving tumour progression and resistance to therapy. Despite a lack of robust clinical activity, it is too soon to conclude that targeting myeloid cells has no therapeutic value. Indeed, there are a number of important ongoing clinical trials using the anti-IL8-antibody BMS-986253, the CCR2/5 antagonist BMS813160, the CXCR2 inhibitor AZD5069, the PI3Kγ inhibitor eganelisib, the CD40 agonist antibody selicrelumab and the C/EBPα small activating RNA MTL-CEBPA, that will develop our

878 understanding of the potential clinical benefit and provide helpful insights. With a new
879 generation of inhibitors targeting CD47, CD11b integrin, LILRB family members and
880 prostaglandin EP2 and EP4 receptors being developed, it is critical that we refine our
881 approaches to develop more focused clinical strategies and even revisit existing molecules.
882 However, to improve success for patients it is important that we evolve preclinical modelling
883 beyond subcutaneous syngeneic tumour models and consider the preclinical-to-clinical
884 translation of concepts carefully, focusing on rational combinations of therapies that can be
885 actioned clinically.

Glossary

Tenosynovial giant cell tumours (TGCT). A rare benign tumour driven by overexpression of CSF-1, where CSF1R expressing cells such as macrophages accumulate in the tendon sheath and tissue surrounding joints.

Hepatic Kupffer cells. Specialist macrophages in the liver that break down red blood cells as one of their major functions.

Neutropenia. A reduction in neutrophils in the peripheral blood common following chemotherapy treatment. Severe reductions in peripheral neutrophils render patients susceptible to infection or febrile neutropenia and is an adverse toxicity.

Prostaglandins. Bioactive lipids produced from arachidonic acid that activate multiple G-protein coupled receptors (GCPRs) and are produced during inflammation, tissue damage and in the TME, affecting multiple cell types.

***STK11*/LKB1.** *STK11* encodes the tumour suppressor LKB1, controlling AMPK activation. Expression is lost in many tumour types, for example lung cancer, rendering tumours refractory to many current treatments associated with accumulation of myeloid cells.

Phosphatase and tensin homolog. PTEN. A lipid phosphatase and tumour suppressor that regulates PI3K-AKT pathway activation; PTEN is genetically mutated, deleted or shows reduced expression in many tumour types.

Chromosome9p21. A region of chromosome 9 that in humans encodes the genes *CDKN2A/B, MTAP* and those encoding IFNα and IFNβ. Deletions or mutations of this region occur in many diseases.

STING. A critical effector of the DNA sensing pathway that triggers inflammation-associated responses upon DNA damage.

β2 microglobulin. Part of the antigen presentation machinery.

Human leukocyte antigen. HLA. Part of the MHC antigen presentation complex in humans that is required to present antigens to T-cells.

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S. Barry and D. Gabrilovich are AstraZeneca employees and shareholders.

Box 1. Myeloid cells in cancer

The myeloid lineage consists of closely related cell populations. Myeloid cells with normal functions, such as pathogen defence or tissue remodelling and repair, co-exist with pathologically activated immunosuppressive myeloid cells that support tumour progression and metastases. They are characterized by distinct genomic, biochemical, functional, and phenotypic features[4-7] (**Supplementary Table 1**). Pathologically activated polymorphonuclear cells (PMN/neutrophils) are often referred to as polymorphonuclear myeloid derived suppressor cells (PMN-MDSCs, sometimes referred to as granulocytic MDSCs or G-MDSCs). Pathologically activated monocytes are referred to as monocytic MDSCs (M-MDSCs). For tumour associated macrophages (TAMs), the terminology M1/M2 macrophage reflect their polarization state, M1 macrophages have a normal pro-inflammatory anti-tumour phenotype, while M2 have a suppressive pro-tumour phenotype, although this is now considered an over-simplification as more detailed analysis has revealed these cells can display a spectrum of different functional phenotypes[12].

Normal and tumour-modified myeloid cells are heterogeneous and complex, and share common myeloid progenitors. Granulocytic and monocytic myeloid lineages arise from granulocyte macrophage progenitors (GMP). Dendritic cells (DC) — important in T-cell priming — arise from specialized precursors within the same differentiation program. Granulocytes include several cell types, the most prominent of which are polymorphonuclear neutrophils (PMN). Classical neutrophils can differentiate into PMN-MDSC (sometimes referred to as tumour-associated neutrophils). The monocytic lineage includes monocytes, which originate in bone marrow and differentiate to macrophages in tissues (bone marrow derived macrophages, or BMDMs). Tissue-resident macrophages (TRM) derive from non-myeloid embryonic precursors, are self-renewing, and expand within a specific tissue. Tumours contain a spectrum of tumour modified myeloid cells. In tumours, macrophages, regardless of origin, are often termed tumour-associated macrophages (TAM) [252,253]. TAMs functionally segregate into immune suppressive, tumour-promoting TAM and non-suppressive TAMs, where M-MDSC-derived TAMs are potently suppressive and classical monocyte-derived TAMs are largely less immune suppressive to T-cells, but modify other aspects of the TME. The contribution of BMDMs and TRMs to the TAM population of different tumours is poorly understood, and although monocytes can differentiate into TAMs, they can also give rise to

inflammatory DCs, while monocytic precursors can give rise to PMN-MDSC[254]. Over time there has been an evolution in terminology from less-well-defined populations, for example tumour associated macrophages or neutrophils, to the more segmented populations outlined above [2,3]. In recent years, transcriptional and phenotypic profiling have described multiple populations of macrophages, monocytes, and neutrophils differentially associated with tumour progression and response to treatment [5,11,21,22,26,30,56,87,88,222-225,229,231]; however, these detailed analyses often lack a clear definition of functional specialization for these cells. This is a source of confusion in the field, and does not help define therapeutic targeting strategies.

[Box 1 figure]

HPC, Haematopoietic progenitor cell; CMP, common myeloid precursor; GMP, granulocytic myeloid precursor; MDP, monocyte dendritic cell Precursor; PreDC, pre-dendritic cell; DC, dendritic cell; PMN, polymorphonuclear cell (neutrophil); PMN-MDSC, polymorphonuclear-myeloid derived suppressor cell; Mon, monocyte; M-MDSC, monocyte-myeloid derived suppressor cell; Classical MPhage, classically activated macrophage; TRM, tissue-resident macrophage; TAM, tumour-associated macrophage

Box 2. Considerations to improve the success of clinical studies of myeloid therapies

Combination strategies for optimal response

- Selectively target pathologically activated or tumour-modified myeloid cells, or comprehensive inhibition of monocytic and granulocytic suppressive myeloid cells.
- Build preclinical concepts to support combinations of chemotherapy, tumour targeted therapy or checkpoint inhibitors in models that recapitulate segments of human disease base on genetics and tissue of origin chosen to test specific hypotheses.
- Assess duration of response in diverse preclinical models representative of human tumours.
- Use combination approaches to target the tumour cell, immune system and the myeloid cell mediated resistance to maximize therapeutic response.

Test specific clinical combinations in biomarker-defined disease segments

- Identify biomarkers to enable the selection of patients with tumours predominantly dependent on one myeloid subtype; for example, patients displaying high neutrophil-to-lymphocyte ratios or high levels of PMN-MDSCs that might be more dependent on neutrophil-mediated resistance mechanisms.
- Consider tumour genetics and other features in the TME. For immunotherapy combinations, segment tumours based on genetic features such as mutations associated with intrinsic resistance to immune-oncology.

Optimize dose and schedule for combination therapies

- Explore intermittent dosing of macrophage and neutrophil modulators or different timings of treatments relative to combination partners and assess the impact of these approaches on suppressive and immune-promoting subtypes in the TME.
- Manage toxicity (for example, chronic CSF1R-inhibition-induced liver toxicity and periorbital oedema or CXCR2-inhibitor-driven neutropenia) through optimal dosing of combinations.

Box 3. Alternative treatment strategies that could be considered to optimize use of myeloid-targeting agents. ICIs are most effective when used with chemotherapy. A more effective therapeutic strategy could involve “priming” the immune system or TME by depleting or

inhibiting suppressive cells prior to treatment with chemotherapy and ICI. Intermittent dosing could allow “normalised” myeloid cells to repopulate the tumour and enable a sustained tumour response.

[Box 3 figure]

1007 **Table 1. Selected clinical trials with myeloid cell modulators targeting CSF1R, CXCR2 and PI3Kγ**

Drug	Dosing Strategy	Phase	Combination Partner	Disease	Trial Identifier	Comments
CSF1R antagonists						
Turalio /pexidartinib/PLX3397 (Daiichi Sankyo/Plexxicon) (inhibits CSF1R, Kit, Flt3) (SM)	Continuous	PhI	Radiation and Temozolamide	Recurrent GBM	NCT01790503	Terminated. Safety, PK and efficacy data reported*
	Continuous	PhI	Durvalumab	CRC, Pancreatic, Metastatic Cancer Advanced cancer	NCT02777710	No results reported
	Continuous	PhI	Pembrolizumab	Melanoma and other solid tumours	NCT02452424	Terminated no efficacy*
	Continuous	PhIb /II	Ebirubulin	Metastatic Breast Cancer	NCT01596751	Safety and efficacy data reported*
ARRY-382 (Array/Pfizer) (selective CSF1R) (SM)	21-day treatment cycles continuous ARRY-382	PhIb /II	Pembrolizumab	PD1/PDL1 resistant patients, Platinum resistant ovarian, pancreatic cancer	NCT02880371	Combination tolerated but limited efficacy signal [75]
	Continuous	PhI	Monotherapy	Solid tumours	NCT01316822	Dose finding no results reported
LY3022855 (Lilly) (Ab)	IV every 4 weeks	PhI	Durvalumab (anti-PDL1) or Tremelimumab (anti-CTLA4)	Advanced Solid Tumors	NCT02718911	No efficacy [77]
	IV every 4 weeks	PhI	Monotherapy	Breast and Prostate cancer	NCT02265536	Immune PD reported, no efficacy [66]
	IV every 4 weeks	PhI	Cyclophosphamide GVAX Pembrolizumab	Pancreatic cancer	NCT03153410	No results reported
	IV every 4 weeks	PhI/II	Cobemetinib, vemurafanib	Melanoma	NCT03101254	No results reported
Cabiralizumab, FPA-008, BMS936558 (Five Prime/BMS) (Ab)	IV every 2 weeks	PhII	Nivolumab	HCC	NCT04050462	No results reported
	IV every 2 weeks	PhI/II	Nivolumab	Solid tumours	NCT03335540	No results reported
	IV every 2 weeks	PhII	SOC chemotherapy	Pancreatic cancer	NCT03336216	No results reported
	IV every 2 weeks	PhII	Nivolumab + gemcitabine	Pancreatic cancer	NCT03697564	No results reported
	IV every 2 weeks	PhII	Nivolumab	Biliary Tract Cancer	NCT03768531	Withdrawn
	IV every 2 weeks	PhII	Nivolumab	Relapsed Refractory T cell lymphoma	NCT03927105	Safety data reported*
	IV every 2 weeks	PhI	Nivolumab + radiation	Advanced metastatic cancers	NCT03431948	No results reported
CSF1 antagonist						
Lacnotuzumab, MCS-110 (Novartis) (Ab)	IV every 3 weeks	PhI/II	PDR001 (anti-PD-1)	Solid tumours	NCT02807844	Safety reported*
	IV every 3 weeks	PhII	Carboplatin, gemcitabine	TNBC	NCT02435680	Safety reported, no efficacy*
	IV every 3 weeks	PhI/II	Spartalizumab + LAG525	TNBC	NCT03742349	No results reported
CCR2/MCP-1						
PF-04136309 (Pfizer) (SM)	Continuous	Discont PhIb/II	FOLFIRINOX	PDAC	NCT01413022	Encouraging efficacy signal [83]
	Continuous	Discont PhIb/II	Nab-paclitaxel	PDAC	NCT02732938	Safety concerns no efficacy [255]
BMS813160 (BMS) (CCR2/5 inhibitor) (SM)	Continuous neoadjuvant pre-surgery	PhII	Nivolumab	HCC/NSCLC	NCT04123379	No results reported, compares CCR2/5i and IL8 blockade
	Continuous	PhI/II	GVAX, radiation, Nivolumab	PDAC	NCT03767582	No results reported
	Continuous	PhI/II	Gemcitabine, Paclitaxel, Nivolumab	PDAC	NCT03496662	No results reported
CXCR2/IL8						
AZD5069 (AstraZeneca) (SM)	Continuous + PDL1	PhI/II	Durvalumab (anti-PDL1 mAb)	HNSCC	NCT02499328	Safety data reported, no efficacy*
	Continuous	PhI/II	Durvalumab	Pancreatic cancer	NCT02583477	No results reported
	Continuous	PhI/II	Enzalutamide	mCRPC	NCT03177187	No results reported
	IV every 2 weeks	PhI/II	Nivolumab+Degarelix	Hormone-Sensitive Prostate Cancer	NCT03689699	No results reported
	IV every 2 weeks	PhI/II	Nivolumab	HCC	NCT04050462	No results reported

HuMax-IL8/BMS-986253 (BMS) (Ab)	Once IV neoadjuvant pre-surgery	PhI	Nivolumab	HCC, NSCLC	NCT04123379	No results reported compares CCR2/5i and IL8 blockade
	IV every 2 weeks	PhI/II	Nivolumab or Nivolumab + Ipilimumab	Metastatic or unresectable solid tumors	NCT03400332	No results reported
	IV every 2 weeks	PhI	SBRT (radiaotherapy) + Nivolumab	Metastatic solid tumors	NCT04572451	No results reported
SX-682 (Syntrix Pharmaceuticals) (SM)	SX-682 monotherapy for 21 days, 90days pembro	PhI	Pembrolizumab	Metastatic Melanoma	NCT03161431	No results reported
PI3Kγ						
Eganelisib (IPI-549) (infinity Pharmaceuticals) (SM)	Continuous	PhI	Nivolumab	Advanced solid tumors	NCT02637531	Early data reported encouraging efficacy [256]
	Continuous	PhII	Nivolumab	Advanced Urothelial Carcinoma	NCT03980041	No results reported
	Continuous 3 weeks	PhII	Tecentriq and Abraxane (TNBC)/ bevacizumab (RCC)	TNBC and RCC	NCT03961698	No results reported
	Continuous	PhII	Monotherapy prior to surgery	Head and neck cancer (HPV+ and HPV-)	NCT03795610	No results reported
	Continuous	PhI	Etrumadenant + Pegylated liposomal doxorubicin (PLD) or nanoparticle albumin-bound paclitaxel (NP)	TNBC and ovarian cancer	NCT03719326	No results reported

*Data published on ClinicalTrials.gov, SM – small molecule, Ab – antibody

Figure 1. Points of potential therapeutic intervention to modify tumour myeloid cells.

There are different ways the tumour myeloid cell recruitment and differentiation process or immunosuppressive myeloid function can be targeted by therapeutics. Myeloid cells recruited to the tumour either differentiate in the bone marrow and are released into the peripheral blood or are co-opted from tissue-resident macrophages. Adoption of a suppressive phenotype can occur at multiple points from the bone marrow to the tumour TME. Blocking the release of myeloid cells from the bone marrow or their recruitment to the tumour prevents accumulation of suppressive cells in the TME (blue arrows). Inhibiting mechanisms involved in the differentiation of myeloid cells to suppressive phenotypes, or stimulating pathways that drive classical myeloid cell activation will change the balance of suppressive to pro-inflammatory cells in the TME (gold arrows). Neutralizing suppressive factors generated by suppressive myeloid cells will prevent T-cell suppression (green arrows).

Figure 2. Influence of selected myeloid cell targeted therapies on myeloid cells and the TME.

Cells of the myeloid lineage have been targeted in multiple clinical trials with therapeutics inhibiting CXCR2, CCR2, CSF1R, PI3Kγ and STAT3 signalling. These agents all inhibit both immunosuppressive and inflammatory myeloid cells in a context-dependent manner. The net benefit with these therapies is determined by the balance of the impacts they have on the pro-tumour and anti-tumour effects of different myeloid cells. The anti-tumour and pro-tumour function of the cells controlled by each mechanism are illustrated, as well as the breadth of myeloid cells and functions that are impacted by treatment.

Figure 3. Selected therapeutic strategies to reprogramme or stimulate anti-tumour macrophage function.

Anti-tumour macrophage activity can be stimulated by a number of approaches. Antibodies targeting cell surface proteins CD40 activate tumour cell killing and antigen presentation. Blocking macrophage receptor with collagenous structure (MARCO) or the mannose receptor CD206 reprograms macrophages to a classical macrophage phenotype, ultimately resulting in T-cell activation through loss of suppressive activity. Reprogramming or activation can also be achieved by stimulating the STING cytosolic DNA sensing pathway or Toll-like receptors (TLRs), inhibiting PI3Kγ, or modifying the epigenome using histone deacetylase (HDAC) inhibitors. Disrupting the interaction between CD47 and SIRPα potentially promotes the phagocytosis of

tumour cells. Suppressive macrophage or M-MDSC activity can be inhibited by targeting immune-suppressive metabolites released by the tumour that drive immune-suppressive TAM or M-MDSC macrophage phenotypes and T-cell suppression. Metabolites used or generated by TAMs, M-MDSC or PMN-MDSC also contribute to the immunosuppression within the TME.

Figure 4. Myeloid cell-mediated therapy resistance in the context of the broader tumour-immune landscape.

(A) Driving more effective anti-tumour immune responses requires development of an integrated view of how different factors influence the response to ICI and other therapies in the broader context to find the optimal therapeutic strategy. Tumour cell properties, anti-tumour immune cell content and the suppressive TME work together to influence tumour progression and therapeutic response. Immunologically, tumours can been classified into four main phenotypes based on T-cell distribution: cold (no T-cells), suppressed (sparse T-cells), excluded (T-cells trapped in the stroma), and hot (heavily infiltrated with T-cells) (figure shows bladder cancer tissue sections stained for CD8 T-cells). Both tumour and stromal features influence the anti-tumour immune response. Tumour cell mutation burden (TMB), microsatellite instability (MSI), mutation of specific genes (*PTEN*, *LKB/STK11*, *CDKN2A/B*), loss of antigen presentation, and loss of STING or IFN expression are all features associated with positive or negative outcome to ICI or chemotherapy. In the suppressive TME, different neutrophil and macrophage-like myeloid cells, dense stroma, T_{reg} cells, suppressive immune metabolites and cytokines suppress T-cell activation and reduce drug response. All of these factors influence the overall outcome to therapy, and it is possible that targeting multiple mechanisms or selecting patients carefully using different biomarkers will be required to broaden efficacy in larger patient cohorts. **(B)** Improved tumour immune responses could be achieved with comprehensive therapeutic strategies targeting the tumour cell (kill tumour cells), tumour micro-environment (remove suppression) and stimulating T-cells with ICI (PD1/PD-L1 or CTLA4).

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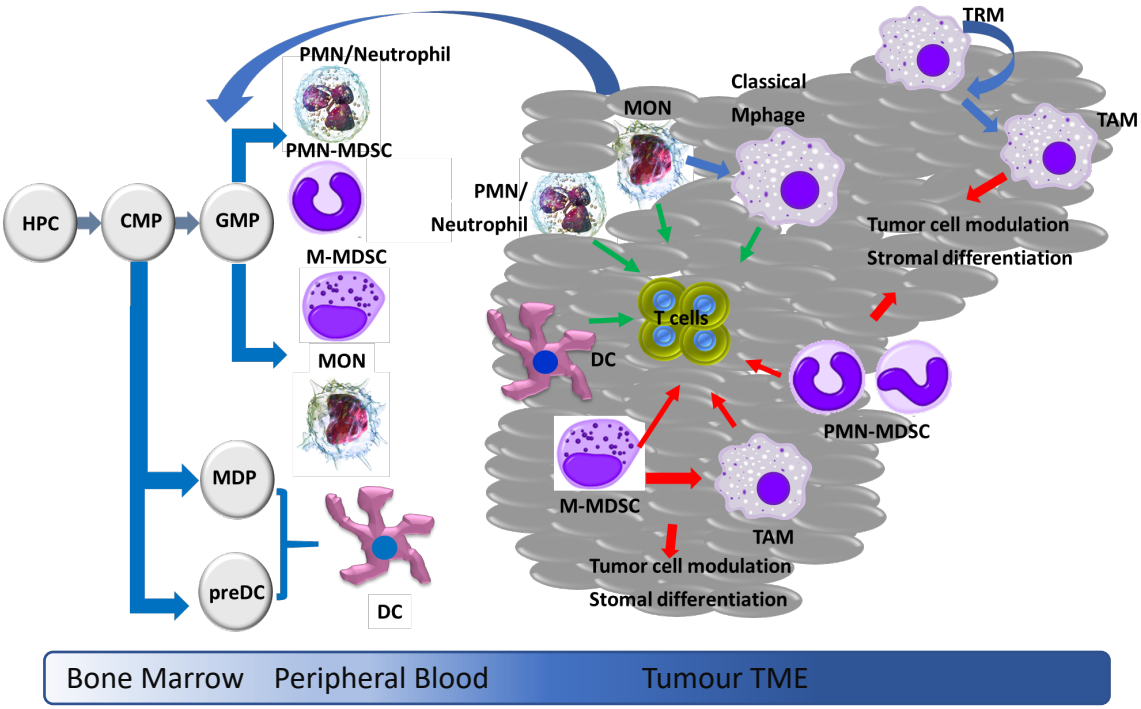
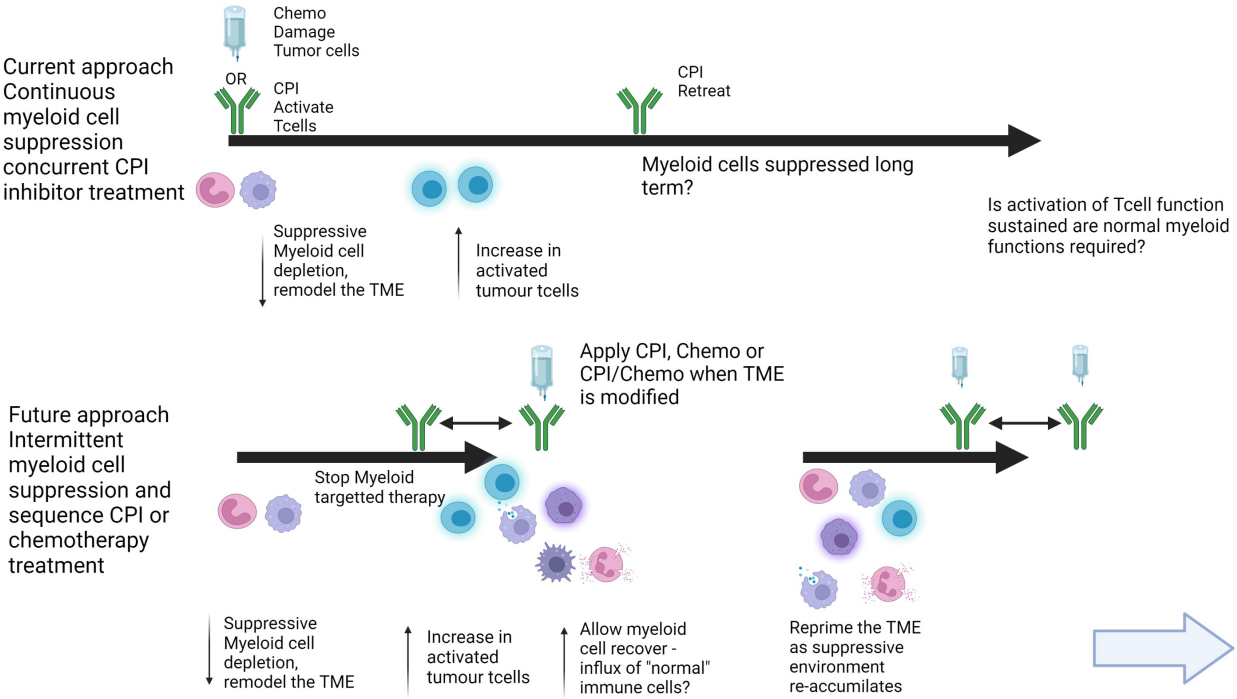
Box1 Illustration. Development of myeloid cells in cancer.



Box 2 Illustration. Considerations to improve success with myeloid therapies.



Box 3. Myeloid cell mediated ICI resistance in the context of the broader immune resistance landscape.

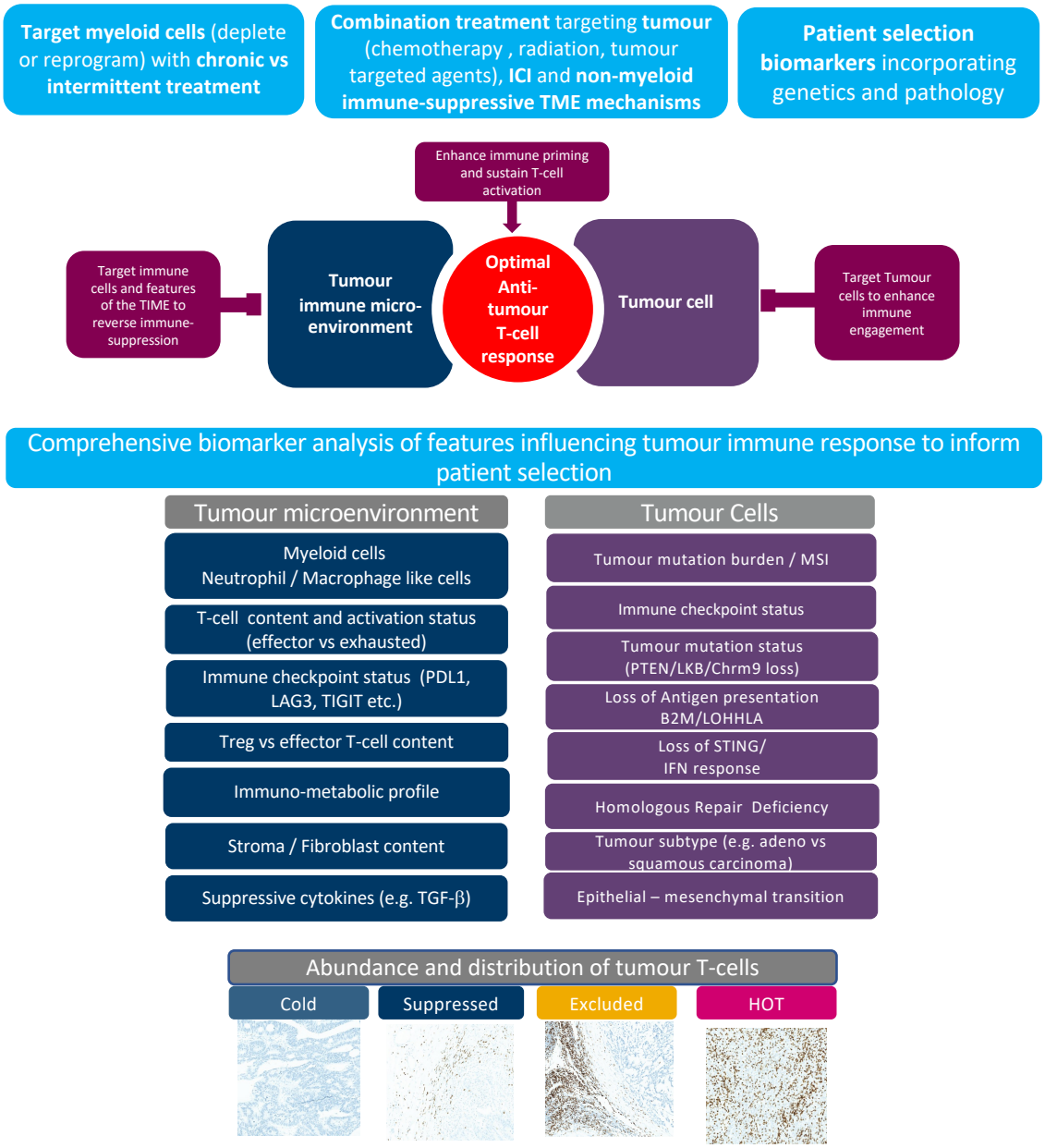


Figure 1. Points of potential therapeutic intervention to modify tumour myeloid cells.

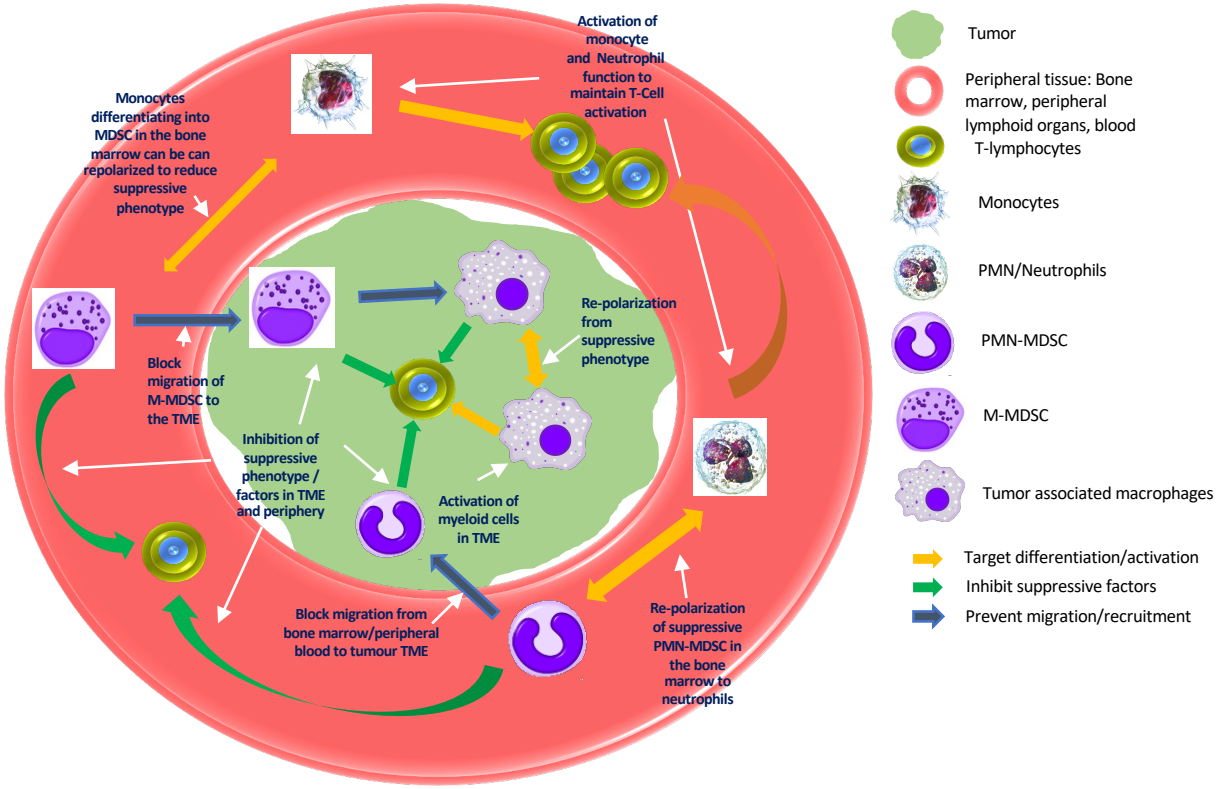


Figure 2. Influence of selected myeloid cell targeted therapies on myeloid cells and the TME.

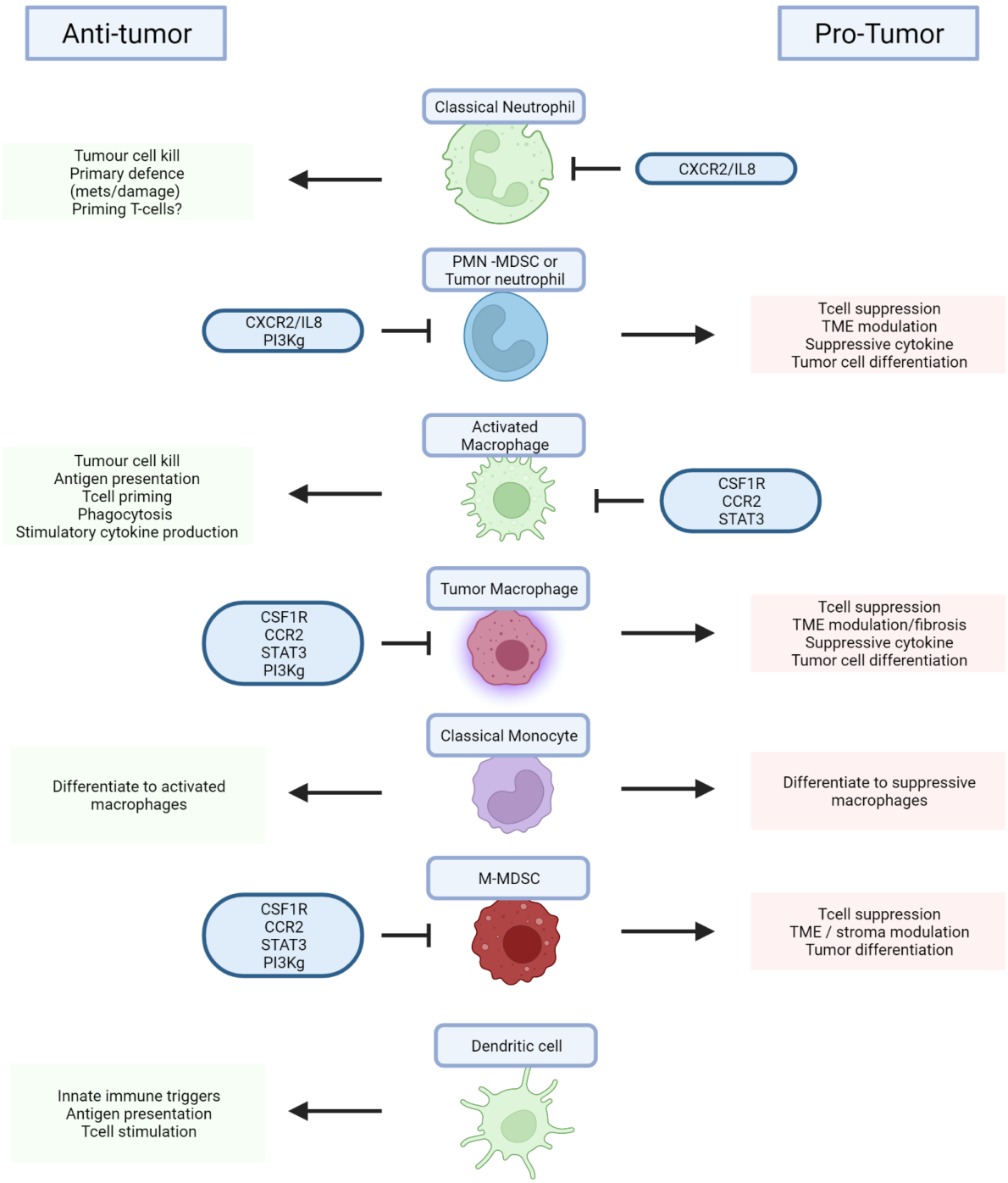


Figure 3. Selected therapeutic strategies to reprogram or stimulate anti-tumour macrophage function.