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REVIEW

Clinical evaluation of macrophages in cancer: role in treatment, modulation and challenges

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Abstract The focus of immunotherapeutics has been placed firmly on anti-tumour T cell responses. Significant progress has been made in the treatment of both local and systemic malignancies, but low response rates and rising toxicities are limiting this approach. Advancements in the understanding of tumour immunology are opening up a new range of therapeutic targets, including immunosuppressive factors in the tumour microenvironment. Macrophages are a heterogeneous group of cells that have roles in innate and adaptive immunity and tissue repair, but become co-opted by tumours to support tumour growth, survival, metastasis and immunosuppression. Macrophages also support tumour resistance to conventional therapy. In preclinical models, interference with macrophage migration, macrophage depletion and macrophage re-education have all been shown to reduce tumour growth and support anti-tumour immune responses. Here we discuss the role of macrophages in prognosis and sensitivity to therapy, while examining the significant progress which has been made in modulating the behaviour of these cells in cancer patients.

Keywords Cancer · Immunology · Macrophage · Immunotherapy

Abbreviations

5YS	5-Year survival
AE	Adverse event
CPI	Checkpoint inhibitor
CSF1	Colony-stimulating factor 1
CSF1R	Colony stimulating factor 1 receptor
IDO	Indolamine 2,3-dioxygenase
irAE	Immune related adverse event
MDSC	Myeloid derived suppressor cell
MIP-1α	Macrophage inflammatory protein-1α
NSCLC	Non-small cell lung cancer
PAMP	Pathogen associated molecular pattern
PDAC	Pancreatic ductal adenocarcinoma
PIGF	Phosphatidylinositol-glycan biosynthesis class F protein
TAM	Tumour associated macrophage
TLR	Toll-like receptor
TME	Tumour microenvironment
uNTX	Urinary *N*-telopeptide
VCAM-1	Vascular cell adhesion protein 1

Introduction

The potential of utilizing the host immune system to eradicate cancers has been hotly debated over the course of the last century. Many doubted the ability to prime the host immune system to a tumour which has already successfully evaded detection and generated a profoundly immunosuppressive tumour microenvironment (TME). Over the last two decades a range of immunotherapies have made it to the clinic, clearly proving the point of principle, but the ability of immunotherapy to target more aggressive and less immunogenic tumours is still in doubt [1].

To comprehend the limitations of current T cell immunotherapeutics, namely T cell checkpoint inhibitors (CPIs), which skew the balance of stimulatory and inhibitory signals, it can be useful to imagine the tumours as exerting an immunosuppressive force, and the immune system as having a finite immune potential (Fig. 1). Immunosuppression will

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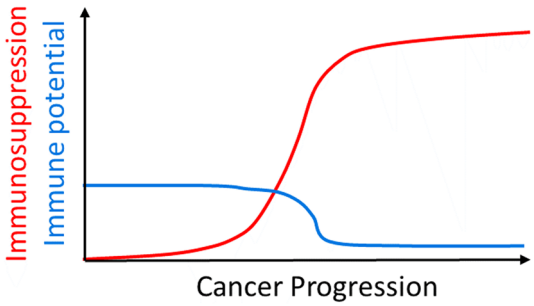

Fig. 1 Magnitude of immune potential versus tumour generated immunosuppression. Diagrammatic hypothesis representing immunosuppression over time during cancer progression graphed with immune potential. Immune potential is the ability of the immune system to mount an effective adaptive immune response

rise with cancer progression and possibly plateau, but at a level both beyond the limit of the normal immune potential and even further from the immune potential of an immunocompromised cancer patient, thus even with a plentiful supply of neoantigens the immune system is rendered ineffective. CPIs function to boost the immune potential of the host to a point at which it can feasibly compete and overcome the immunosuppression generated by the tumour. While this is desirable in an anti-cancer context, the effect of such an untethered immune response in the host can have serious deleterious effects beyond the tumour [2, 3].

The major successes in immunotherapies for cancer patients have relied upon the direct modulation of T cell activation, either by targeting T cell costimulatory proteins such as CTLA-4 and PD-1, or by adoptive T cell transfer using ex vivo T cell activation. However, the side effects associated with these drugs appear to be dose-dependant, cumulative with previous cycles of therapy and additive with other similar regimes. This apparent limit has led to a shift in research to identify suitable complimentary therapies that kill tumour cells in a way which primes the TME for T cell activation by inducing immunogenic forms of cell death [4, 5].

Developments in the field of immunology, and the elucidation of the myriad of components interacting in the TME, are leading to the development of a new range of immunotherapeutics that focus on an expanding set of targets with therapeutic and diagnostic potential.

In contrast to many approved immunotherapeutics that boost the immune potential, one interest has been in trying to actively reverse the immunosuppression generated by the tumour by disrupting immunosuppressive factors in the TME or by disrupting cells normally co-opted by tumours.

One specific vein of research has focused on a subset of the myeloid cell compartment comprising the monocyte–macrophage lineage which can be subverted and recruited to the tumour as tumour associated macrophages (TAMs). While TAMs can comprise up to 50% of the tumour mass, they have been less intensively studied than other immune subsets [6]. There is a growing body of literature showing their prognostic value, and they are emerging as promising therapeutic targets in oncology.

Synopsis of macrophage origin and classification

TAMs are predominantly derived from circulating populations of monocytes. As a simplified paradigm, macrophages have been categorized as classically activated M1 (inflammatory) which are anti-tumour, or alternatively activated M2 (wound repair) which are pro-tumour. The M1 M2 dichotomy was developed by in vitro observations but recent advances have led to a more complex spectrum of activation states. Both monocyte and macrophage populations frequently display hybrid M1/M2 phenotypes, or phenotypes that cannot be adequately defined using the M1:M2 system [7]. It has been identified that the M1/M2 system is leading to confusion and inconsistency between researchers and ultimately impeding progress [8].

Others advocate the use of in vivo function to classify M1 M2 macrophages, focusing on the iNOS (M1): arginase (M2) ratio. With cells being defined as inhibitors of cell growth and killers or as promoters of cell proliferation and wound repair (Fig. 2) [9]. Flow cytometry has, however, led to the distinction of a range of macrophage and monocyte types based on their relative expression of various cell surface markers.

From a clinical perspective, the study of macrophages faces a unique challenge, in that we find it more amenable to study discretely defined subsets of cells, but it is becoming increasingly evident that this is not possible with such a heterogeneous set of cells. While many continue to report based on two distinct subtypes, it is important to remember that the activation states of macrophages incorporate discrete populations and spectrums or continuums where cells can adopt hybrid states [10].

Contribution to tumourigenicity

Macrophages have been implicated in all aspects of tumour growth and spread, but they are also known to be critical mobilizers of the adaptive immune system (Fig. 3). As such they play an enigmatic role in tumour development and the generation of anti-tumour responses.

In line with their roles in immune stimulation and antigen presentation, there is evidence high macrophage infiltration in the early stages of tumour growth can result in tumour destruction while low levels of infiltration support tumour growth [11, 12]. Macrophages can promote anti-tumour responses but advanced tumours have been shown to polarize TAMs into an M2-like phenotype [13].

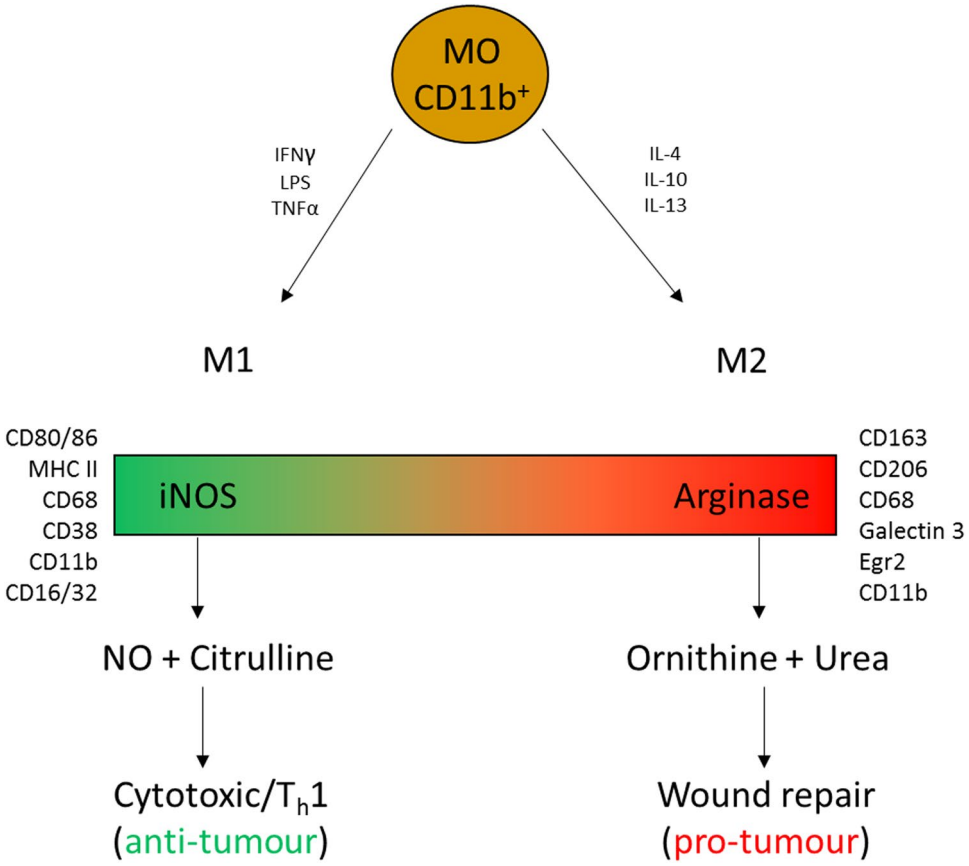

Fig. 2 Synopsis of M1:M2 macrophage dichotomy. CD11b monocytes (MO) can mature with a heterogeneity of phenotypes which together represent a spectrum with M1 and M2 macrophages representing the two extremes of that spectrum. In vitro, IFNγ, LPS and TNFα drive M1 polarization whereas IL-4, IL-10 and IL-13 drive M2 polarization. M1 macrophages express CD68, CD11b, CD38. CD16/32, MHC II and CD80/86, their primary function is dependent on the expression and function of inducible nitric oxide synthase (iNOS) which results in the extracellular accumulation of nitric oxide (NO) and citrulline which, along with other cytokines, can drive cytotoxic anti-tumour T_h1 responses. M2 macrophages express CD68, CD11b, CD163, CD206, Galectin 3 and Egr2, their primary function is dependent on the expression and function of arginase which results in the extracellular depletion of arginine and the accumulation of ornithine and urea which are key to wound repair mechanisms but can also promote immune suppression and tumour progression

Tumours can secrete a range of chemoattractants that promote recruitment of monocyte and macrophage populations [14]. TAMs become co-opted to promote tumour cell proliferation and survival, tumour vascularization and immunosuppression along with supporting extravasation and growth of tumour cells at distal sites [15].

The importance of TAMs is evident across the literature, they can affect patient prognosis and determine sensitivity to a range of therapies. Preclinical studies and early stage clinical trials have implicated them as prime therapeutic targets [16, 17].

Effect of macrophage infiltration and polarization on patient prognosis

Prognostic significance of circulating and infiltrating macrophages

A high density of macrophage infiltration into the tumour has been cited as a negative prognostic indicator in a range of solid and haematological malignancies (Tables 1, 2). Colorectal cancer displays a contrasting trend whereby high macrophage infiltration can result in increased patient survival.

Arguably the most robust prognostic evidence is available for breast cancer and Hodgkin's Lymphoma. A distinct gene signature in breast cancer has shown high macrophage density is prognostic if combined with a high $CD4^+$ helper T cells to cytotoxic T cell ratio. The signature closely correlated to the development of secondary tumours that could

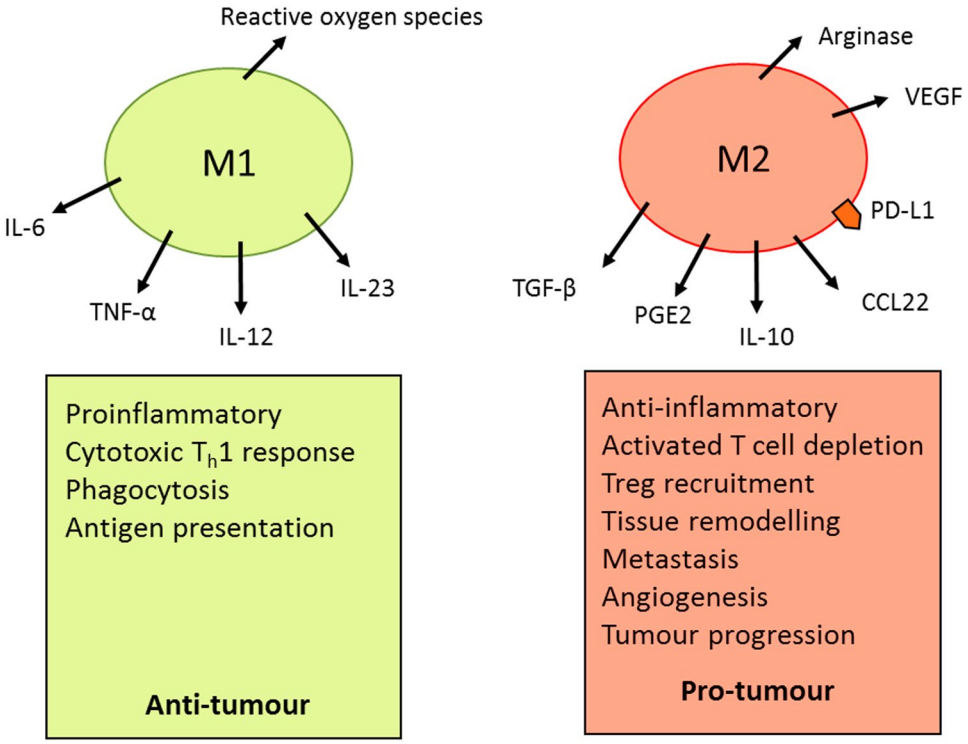

Fig. 3 Synopsis of pro- and anti- tumoural effects exerted by macrophages. Key enzymes and cytokines produced by M1 and M2 macrophages that have the effect of driving or inhibiting cancer progression. M1 cells can drive inflammation and cytotoxic T_h1 responses while M2 cells can produce factors such as vascular endothelial growth factor (VEGF) and Prostaglandin E2 (PGE2), and are involved in the depletion of activated T cells, recruitment of regulatory T cells, tissue remodelling, angiogenesis and tumour progression

accurately predict survival in women after complete resection [18]. A macrophage gene signature has been developed for Hodgkin's Lymphoma that can accurately predict survival and response to therapy, indicating that the pro-tumour effect of macrophages is not restricted to solid tumours [19].

Prognostic significance of histologic localization

Histologic examination of colorectal cancer, for which TAM infiltration is a positive prognostic indicator, revealed infiltration at the tumour front in colon cancer leads to enhanced survival and reduced liver metastasis, irrespective of CD8 T cell infiltration [20–23]. The proximity of the TME to the intestinal microbiome has been hypothesized as a potential explanation for the differential behaviour of macrophages in colorectal cancer. It is possible that the continuous supply of pathogen-associated molecular patterns (PAMPs) available to macrophages may outweigh the ability of the tumour to polarize the cells to an M2-like phenotype. This hypothesis may also explain why similar results have been seen in gastric cancer, in which tumours may have varying access to the intestinal microbiome depending on the localization of the tumour. Thus, high infiltration of macrophages in the tumour nests in gastric cancer is associated with enhanced antigen presentation and T cell activation, and a positive prognosis [24].

The histological localization of macrophages in breast cancer has shown no correlation with prognosis, while in endometrial cancer high TAM infiltration into the tumour hotspot (tumour core of necrotic cells) is associated with advanced clinical staging, myometrial invasion and histological differentiation [25–27]. Characterization in other tumour types is warranted.

Prognostic significance of polarized macrophages

It is possible the results of many studies were adversely affected by failure to distinguish pro- and anti-tumour populations. When differentiated in non-small cell lung cancer (NSCLC), it was found that high M1-like macrophage infiltration was associated with prolonged survival, while the level of M2-like infiltration had no impact on survival [28, 29]. This is in contrast to an earlier meta-study examining the prognostic relevance of overall $CD68^+$ infiltration in NSCLC that found no link with OS [30].

Similarly, in patients with hepatocellular carcinoma after curative resection, high numbers of $CD11c^+$ dendritic cells and low numbers of $CD206^+$ macrophages correlated with extended OS, whereas $CD68^+$ TAM infiltration displayed no prognostic significance [31]. In ovarian cancer there is inconsistent evidence on the prognostic effect of $CD68^+$ cell infiltration, however, differentiation of the populations revealed that a high M1-like:M2-like ratio is prognostically favourable [32–35]. Together these data indicate whole macrophage counts used to explore the prognostic effects in other cancers may not accurately reflect the true trend or

Table 1 The effect of macrophage infiltration and macrophage related biomarkers on prognosis in solid tumours

Tumour	Indicator	Prognostic Significance	Reference
Breast	High CSF1 gene expression	High grade, low estrogen receptor and progesterone receptor expression and high TP53 mutations	[155]
	High TAM infiltration	Shorter DFS	[27, 156]
	High TAM density	Late clinical staging	[157] (M)
	High $CD68^+$ TAM density	Shorter DFS	[27]
	High $CD204^+$ TAM infiltration	Shorter relapse-free survival	[158]
Myxoid Liposarcoma	High $CD68^+$ TAM infiltration	Shorter OS	[159]
Cervical	High $CD68^+$ TAM infiltration	Disease progression and high grade lesions	[160]
	High CCL2 expression and $CD68^+$ TAM infiltration	Lower relapse-free survival, lower OS, increased local and distant recurrence, vascular invasion, and larger tumour size.	[161]
Uveal Melanoma	Both High $CD68^+$ and $CD68^+CD163^+$ TAM infiltration	Shorter OS	[162]
Bladder	High $CD68^+$ TAM infiltration	Late clinical staging	[157] (M)
Oral	High $CD68^+$ TAM infiltration	Shorter OS	[157] (M)
Thyroid	High $CD68^+$ TAM infiltration	Shorter OS	[157] (M)

scale of the effect imposed by pro-tumourigenic macrophage populations. The ability to draw robust prognostic indications from TAM frequency emphasizes their central role in disease progression.

Role of macrophages in therapeutic response

Effect of chemotherapy on macrophages

Conventional chemotherapies are considered immunosuppressive due to toxic systemic effects on rapidly proliferating

Table 1 (continued)

Tumour	Indicator	Prognostic Significance	Reference
Ovarian	High $CD68^+$ infiltration	Shorter 5YS	[20]
	High $CD68^+$ density	Early clinical staging	[157] (M)
	High $CD68^+CD163^+$ TAM infiltration	Shorter PFS and OS	[33]
	High M1:M2 ratio in TME	Increased OS	[32]
	Serum soluble CD163	Shorter DFS and OS	[163]
Gastric	High $CD68^+$ TAM infiltration	Shorter OS	[157] (M)
	High nest $CD68^+$ TAM	Higher 5YS	[24]
Prostate	High $CD68^+$ TAM density	Shorter median OS and poor clinical outcome	[164]
Glioma	High $CD163^+$ $CD204^+$ TAM infiltration	Correlates to histologic grade	[165]
Lung	M1:M2 gene signature	Increased OS	[28]
NSCLC	High $CD68^+$ $HLA\text{-}DR^+$ (M1) TAM infiltration to stroma and islets	Increased OS	[29]
	High $CD68^+$ TAM infiltration	Increased OS	[166]
Colorectal	High $CD68^+$ TAM infiltration	Increased OS	[157] (M)
	High $CD16^+$ TAM infiltration	Increased OS	[167]
	High $CD68^+$ TAM density at the tumour front	Increased OS, Reduced liver metastasis	[22, 23]

CD68 is a marker used to identify all macrophages, CD163, CCL2, CD163 is a strong M2 marker while CCL2, CD204 and CD206 are weaker markers also preferentially expressed by M2-like macrophages. Shaded in grey are indications where high M2-like macrophage numbers correspond to a positive prognosis. M denotes meta-study

Table 2 The effect of macrophage infiltration and macrophage related biomarkers on prognosis in haematological malignancies

Cancer	Indicator	Prognostic significance	Reference
Angioimmunoblastic T-cell lymphoma	High CD163: CD68 ratio in the TME	Shorter OS	[168]
Hodgkin's lymphoma	TAM gene signature, High $CD68^+$ cells in lymph nodes	Shorter PFS, increased risk of relapse after haematopoietic stem cell transplant	[169]
	High $CD68^+$ $CD163^+$ infiltration	Shorter OS and reduced event-free survival	[170]
Follicular lymphoma	High $CD68^+$ infiltration	Shorter OS	[171]

CD68 is a marker used to identify all macrophages, CD163 is used to identify M2-like macrophages

leukocytes and bone marrow progenitors resulting in leukocyte depletion. Chemotherapy has also been shown to stimulate the secretion of colony stimulating factor 1 (CSF1) by tumour cells, which is a potent chemoattractant for macrophages, and results in an accumulation of TAMs in the TME which contribute to chemoresistance [36].

Prognostic significance of macrophages in response to chemotherapy

High levels of infiltrating $CD68^+$ and $CD163^+$ cells are a negative prognostic marker for patients with esophageal cancer undergoing pre-operative neoadjuvant chemotherapy and indicates patients are less likely to respond to chemotherapy [37]. The CD8:CD68 cell ratio is a predictive biomarker for response to neoadjuvant chemotherapy in breast cancer patients [18, 38]. These effects were found to be at least in part due to the upregulation of CSF1 by tumour cells in response to CT. A high density of $CD163^+$ cells at the invasive front in oral squamous cell carcinoma was found to correlate to a poorer outcome after surgery following 5-FU based chemoradiotherapy [39].

On examination of the histologic localization of macrophages, $CD68^+$ in the parenchyma negatively correlated to lymphatic metastasis after neoadjuvant chemotherapy, in contrast to the number in the dense fibrous stroma which directly correlated to the number of positive lymph nodes, indicating the role of macrophages depends on intratumoural localization in breast cancer [38].

The role of macrophages in chemoresistance

Macrophages are central coordinators of immune responses during chemotherapy [40]. Blockade of macrophage recruitment increased the efficacy of paclitaxel in breast cancer, resulting in diminished growth of both primary and metastatic tumours [18]. Suppression of $CD8^+$ effector T cells by the production of IL-10 has been shown to reduce anticancer cytotoxicity [41]. IL-10 production by macrophages also limits the efficacy of chemotherapy in breast cancer and was subsequently shown to indirectly enhance tumour growth by down regulating IL-12 production by DCs which is required for cytotoxic $CD8^+$ T cell responses.

Macrophages are critical mediators of wound and tissue repair and it is possible that these functions can be naturally adapted by the tumour to generate chemotherapeutic resistance. M2-like macrophages derived from THP-1 cells, were shown to reduce apoptosis in addition to enhancing tissue repair and angiogenesis in response to etoposide, a topoisomerase inhibitor [42, 43].

Both macrophage depletion and re-education to an M1 state have been shown to increase the efficacy of chemotherapy [44–46]. The induction of M1 polarization using host-produced histidine-rich glycoprotein to reduce signalling by the M2 driver PIGF has been shown to restore sensitivity to chemotherapy, reduce tumour growth and reduce metastasis, indicating that M1 polarization can combat all major aspects of disease [44].

Macrophage modulating therapies have an advantage over many other immunotherapeutics because they can be used to synergistically improve outcome with chemotherapy, whereas the results of combining CPIs with chemotherapy have shown very little or no effect on OS or quality of life [18].

Effect of radiotherapy on macrophages

Conventional fractionated radiotherapy is considered immunosuppressive, as radiation primarily leads to apoptotic cell death, but it can also lead to necrotic cell death and mitotic catastrophe [47, 48].

The accumulation of macrophages in the TME after radiotherapy is due to the ability of MOs to survive clinically relevant doses of radiotherapy coupled with an influx of monocytes after radiotherapy [49, 50]. While this may seem attractive in the generation of an abscopal effect, there is much research showing that the influx of monocytes and macrophages is responsible for therapy failure due to their role in vasculogenesis and angiogenesis [51, 52].

Role of macrophages in radioresistance

Murine models of oral and brain cancer have shown macrophages infiltrating the tumour after radiotherapy were primarily M2-like and supported vasculogenesis and tumour growth [52–55].

Curiously, ionizing radiation skews macrophages from an M2-like to an M1-like phenotype, suggesting an enigmatic role of macrophages in radiotherapy [56]. Characterization of the TME post irradiation reveals decreased levels of the anti-inflammatory markers CD163, IL-10, VCAM-1, and MRC1 while significantly increasing the inflammatory markers iNOS, CD80, CD86 and HLA-DR [57]. However, irradiated macrophages were still able to enhance tumour cell invasion and supported the angiogenic process of tumour cells indicating the retention of M2-like traits. Blocking macrophage influx into the TME after RT has been shown to enhance response in murine models [49].

Prognostic significance of macrophages in response to radiotherapy

Prognostically, there is limited evidence on the effect of macrophages in patients undergoing radiotherapy. Macrophages have been shown to predict response to short course pre-operative radiotherapy for colon cancer, with data suggesting a high infiltration of M1-like macrophages is likely to result in a reduced response, no effect was seen by M2-like macrophages [58].

Role of macrophages in response to checkpoint inhibitors

CPIs have been the most notable achievement in the development of immunotherapy for cancer patients, but there has been limited interest in the role of myeloid cells in their clinical application to date.

Macrophages are key coordinators of adaptive immune responses, and express a range of T cell costimulatory and co-inhibitory molecules, known as the B7 family [59]. Crosstalk between tumour cells and macrophages can regulate the expression of B7 family molecules on both tumour cells and macrophages [60]. The TME is abundant in IL-10 and TNF-α, which can both upregulate PD-L1 expression on macrophages, via STAT3 signalling, which is responsible for the inactivation and depletion of activated T cells [61–63]. PD-L1 has been implicated as a major signalling molecule associated with immune escape by tumours [64].

In addition to their role in facilitating T cell responses, macrophages are critical mediators of many therapeutics that employ antibodies with fully humanized Fc domains. While the primary function of antibodies is the activation or neutralization of their targets, the choice of antibody Fc domains are known to influence their efficacy. CD16, the receptor for IgG1 is expressed primarily by macrophages and NK cells and is responsible for the neutralization of antibody targets via antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis [65, 66]. The capacity to generate ADCC responses is dependent on two variables. Firstly, the ability of the Ab used to bind FC receptors, and secondly on the activation state of the FcR expressing cell.

Anti-CTLA4

Ipilimumab is a fully human IgG1 mAb that interacts with FcγRIIIA (CD16) expressing cells. Ipilimumab efficacy relies on two mechanisms. Firstly, interference with CTLA4 binding on effector T cells, and secondly, FcγR mediated depletion of Tregs by ADCC [67, 68].

In a small study of 29 patients receiving Ipilimumab for the treatment of melanoma, responders had a higher number of $CD68^{+}CD163^{+}$ macrophages in the TME before treatment and decreased Treg infiltration after therapy. Responders had the highest level of circulating non-classical $CD16^{+}CD14^{low}$ macrophages at baseline [69]. In a study of 209 melanoma patients receiving Ipilimumab, low absolute monocyte counts and low circulating $Lin^{-}CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs were significantly associated with improved survival [70]. These studies indicate macrophages play an active role in response.

Anti-PD-1/PD-L1

Both PD-1 and PD-L1 are expressed by macrophages, and as such the effect of these neutralizing antibodies may have a depletory effect on macrophage numbers. PD-1 is expressed on infiltrating macrophages and lymphocytes of melanoma patients responding to anti-PD-1 therapy [71]. Response was primarily correlated to the proliferation of intratumoural $CD8^{+}$ T cells and the role of PD-1^{+} macrophages was not examined. A reduction of the proinflammatory cytokine CCL3 is associated with prolonged survival in metastatic renal cell carcinoma patients receiving Atezolizumab [71, 72].

Evaluation of immunologic correlates during CPI administration is required to improve our understanding of the biology of response and development of resistance. Due to the very limited number of patients receiving CPIs, our understanding of the global effect of CPIs on non-T cell immune subsets is still in its infancy.

Macrophage modulation in cancer

A wide range of efforts have been made to enhance anti-tumour responses by modulating the behaviour of macrophages. These can be distinguished into three groups:

(1) Skewing of monocyte/macrophage polarization.
(2) Inhibition of macrophage migration to the TME.
(3) Depletion of monocyte/macrophage populations.

Interest has been shown in a wide range of modulatory mechanisms with varying degrees of success. The most promising include granulocyte–macrophage colony-stimulating factor (GM-CSF), the CCL2/CCR2 axis and the CSF1/CSF1 receptor (CSF1R) axis.

Treatment with GM-CSF

GM-CSF promotes the expansion of granulocytes and monocytes, polarizes macrophages to an M1-like anti-tumour phenotype and can skew cells towards a type one phenotype capable of driving anti-tumour Th1 responses [73–77]. GM-CSF has been approved for the second line treatment of paediatric high-risk neuroblastoma in combination with IL-2 and 13-cis-retinoic acid, and has been recommended for the amelioration of febrile neutropenia in solid and haematological malignancies by The American Society of Clinical Oncology [78].

There is currently a phase 2/3 trial in the recruitment phase examining the administration of recombinant GM-CSF, BCG and 4 lethally irradiated melanoma cell lines for the treatment of pre-malignant melanoma (NCT01729663).

Sipuleucel-T is a therapeutic vaccine approved for castration-resistant prostate cancer, composed of autologous PBMCs cultured ex vivo with PAP-GM-CSF. Despite gaining approval, it only modestly enhanced OS (25.8 vs. 21.7 months) with no improvement in time to progression [79]. GVAX is a vaccine comprised of a patient's own cancer cells stimulated to secrete GM-CSF and then irradiated to prevent further proliferation. GVAX has recently been given breakthrough designation for pancreatic cancer in combination with CRS-207, a listeria-based therapeutic vaccine, after positive phase 2 results. Interestingly, GVAX has been shown to induce PD-L1 positive 'post-immunotherapy lymphoid aggregates' in murine models of pancreatic adenocarcinoma that may prime the tumour into an immunogenic state [80, 81]. Building on that work the authors performed an early stage clinical trial with GVAX and Ipilimumab which showed clinical benefit [82]. These studies were performed before the approval of anti-PD-1 antibodies, and it is likely this combination will offer enhanced outcomes. A clinical trial is now recruiting (NCT02648282).

There have been fears surrounding the administration of GM-CSF due to observations of constitutive GM-CSF expression by advanced cancers [83]. GM-CSF can induce pleiotropic effects depending on its concentration and receptor, including differing effects on survival and proliferation. Tumour cells can utilize GM-CSF in an autocrine or paracrine mechanism to stimulate growth and proliferation [84, 85].

Rationale for the modulation of the CCL2/CCR2 axis

CCR2 is a chemokine receptor present on inflammatory monocytes that it is required for mobilization from the bone marrow and recruitment to the TME. Tumours can upregulate CCL2 expression, its cognate ligand, from both tumour cells and stromal cells resulting in an upregulation of $CCR2^{+}$ inflammatory monocytes and matrix metallopeptidase 9^{+} (MMP-9) neutrophil infiltration [86–93].

CCL2 has been shown to increase the survival of PBMCs and clearance of apoptotic cells which may be beneficial in an anti-tumour context,; however, CCL2 also drives M2 polarization suggesting it is more likely to play a negative role in cancer patients [94, 95]. Inhibition of the CCL2/CCR2 pathway has been shown to potently inhibit the development of metastasis in murine models of hepatocellular carcinoma, breast and prostate cancer [96–99]. Murine models of pancreatic ductal adenocarcinoma (PDAC) have shown that CCR2 inhibitors can induce a 3-fold reduction in tumour burden [100].

Both chemotherapies and radiotherapy have been shown to upregulate CCL2 production by tumour cells and stromal cells [101, 102]. Addition of anti-CCL2 antibodies is additive with chemotherapy in models of ovarian and prostate cancer, and with radiotherapy in models of PDAC [98, 103–105].

Prognostic significance of CCL2 and CCR2

CCL2 expression has been linked to cancer progression in hepatocellular carcinoma, prostate cancer, colorectal cancer, breast cancer and gastric cancer and has been shown to promote the induction of tumour growth, tumour cell migration, neovascularization and metastasis [88, 92, 97, 106–117]. Prognostically, high CCL2 in combination with VEGF in tumour conditioned media has been shown to increase the chance of early relapse in breast cancer [118]. High intratumoural CCL2 expression is related to a lower 5-year survival (5YS) in gastric cancer [119]. Intratumoural expression of both CCL2 and CCR2 are associated with a lower OS and increased risk of recurrence in non-metastatic clear-cell renal cell carcinoma [120].

Clinical modulation of the CCL2/CCR2 axis

Clinical inhibition of CCL2 initially failed to generate significant effects. Carlumab—a mAb against CCL2—was found to be safe and tolerable in patients but reduction in free CCL2 was short lived and failed to achieve an objective response in solid tumours (NCT01204996) [121, 122]. MLN1202, a similar antibody, was trialled in patients with bone metastasis from solid tumours, and resulted in reduced urinary *N*-telopeptide (uNTX) levels but with minimal therapeutic success [123]. Further to the poor therapeutic responses; in murine models a bounce back effect in CCL2 levels was observed in which levels quickly returned to baseline or higher than pre-treatment levels resulting in accelerated death [124].

An orally active CCR2 antagonist PF-04136309, has been shown to reduce growth of PDAC and enhance survival. Phase 1b trials with FOLFIRINOX have shown that it is safe, tolerable, and enhances survival [125]. Levels of peripheral circulating monocytes are inversely related to survival in pancreatic cancer [100]. Systemic CCR2 inhibition inhibits the mobilization of inflammatory monocytes from the bone marrow, consequently lowering monocyte infiltration to the TME. Preclinical models suggest the results in PDAC may translate into other tumour types, however, the unique TME of PDAC, with high innate immune cell infiltration and T cell immune privilege, must be considered unique so recapitulation of the results in other tumour types is uncertain [126, 127].

$CCR2^+$ macrophages suppress the infiltration of $MMP\text{-}9^+$ neutrophils to the TME. In murine models of cervical cancer, when macrophages are depleted in the TME, protumourigenic neutrophils are recruited. Consequently, no major difference in tumour incidence or tumour burden is seen between CCR2 null and wild type mice, with only a small delay from dysplasia to carcinoma being noted [128]. It is possible that this compensatory influx of neutrophils may be inhibited by the dense desmoplastic in pancreatic cancer, indicating the therapeutic benefit of PF-04136309 may be restricted to pancreatic cancer.

Rationale for modulation of the CSF1/CSF1R axis

CSF1 is a secreted cytokine that binds CSF1R on cells and which can control the production, migration, function and differentiation of macrophages. CSF1R is predominantly expressed on myeloid cells of the monocyte–macrophage lineage and its inhibition has been used in various preclinical models for local macrophage/monocyte depletion. CSF1R mediated depletion has been shown to increase the efficacy of chemotherapy, radiotherapy, angiogenic inhibitors, and CPIs [36, 55, 129–131]. In addition to enhancing monocyte migration, CSF1 binding has been shown to promote the development of M2-like macrophages [132, 133].

Targeting CSF1R has the added advantage of being highly expressed on potently immunosuppressive MDSCs and can inhibit the migration of both macrophages and monocytic MDSCs to the TME [119, 120]. Along with M2-like macrophages, MDSCs secrete high levels of indolamine 2,3-dioxygenase (IDO) and have been implicated in resistance to CPIs and rapid outgrowth of B16 cell line tumours [119].

Unlike GM-CSF which results in upregulation of PD-L1 expression on immune infiltrates, inhibition of CSF1 signalling appears to upregulate CTLA-4 on tumour infiltrating $CD8^+$ CTLs in addition to enhancing PD-L1 expression on macrophages and tumour cells, but with a concomitant decrease in PD-1 expression by monocytes and macrophages [131]. Inhibition of signalling by CSF1R on macrophages has been shown to enhance antigen presentation and T cell effector functions. Combination with CPIs was shown to induce tumour regression in murine models of PDAC [131].

While CCL2:CCR2 inhibitors can inhibit the mobilization of monocytes from the bone marrow and may result in a build-up of potentially pro-tumour cells elsewhere, anti-CSF1R antibodies deplete macrophages. There has been evidence that CSF1/CSF1R inhibition can increase metastasis in breast cancer via a compensatory increase in expression of G-CSF, however, this has not been seen in other tumour models [134].

Prognostic significance of CSF1 and CSF1R

CSF1R overexpression is associated with a negative prognosis in breast cancer patients [135]. In murine models, CSF1R overexpression is associated with reduced survival in endometrial, hepatocellular and colorectal cancer and targeting of both CSF1 and CSF1R have been shown to increase survival [136].

Clinical modulation of the CSF1/CSF1R axis

There are a range of anti-CSF1R antibodies currently in clinical trials designed to generate ADCC of tumour cells over expressing CSF1R and TAM depletion (Table 3).

CSF1R is a member of the KIT family of tyrosine kinases. Imatinib Mesylate can act as a tyrosine kinase inhibitor to these kinases. A trial using Imatinib in KIT^+ patients showed clear clinical efficacy with 20/27 achieving stable disease, 1 complete response and 4 partial responses. Because of the promiscuity of Imatinib, toxicities due to off target effects were significant with 1 in 4 discontinuing treatment due to intolerable AEs [137–140].

There have been efforts to design tyrosine kinase inhibitors that target CSF1R, but they have lacked specificity

Table 3 Clinical trials involving CSF1R inhibitors (as of September 2017)

Name	Type	Cancer	Combination	Result	References
IMC-CS4 (Eli Lilly)	Fully human IgG1 mAb CSF1R	Breast, prostate	Monotherapy	Phase 1 ongoing	NCT02265536
		Advanced solid tumours	Monotherapy	Phase 1 ongoing	NCT01346358
			Anti-PD-L1 or Anti-CTLA-4 Ab	Phase 1 ongoing	NCT02718911
AMG 820 (Amgen)	Fully human IgG1 mAb CSF1R	Advanced solid tumours	Monotherapy	Phase 1 completed (tolerable, 38% stable disease	NCT01444404
		Pancreatic, colorectal, NSCLC	Anti-PD-1 Ab	Phase 1b/2 recruiting	NCT02713529
RG7155 (Roche)	Humanized IgG1 mAb CSF1R	Advanced solid tumours	Monotherapy	Phase 1 ongoing	NCT01494688
			Anti-CD40 Ab	Phase 1 ongoing	NCT02760797
			Anti-PD-L1 Ab	Phase 1 ongoing	NCT02323191
		Diffuse-type giant cell	Monotherapy	Phase 1 complete (74% objective tumour response)	[172]
PLX3397 (Plexxikon)	Orally active small molecule inhibitor of CSF1R and other KIT kinases	Glioblastoma	Monotherapy	No efficacy	[173]
		Breast	Monotherapy	No efficacy	I-SPY-2 trial NCT01042379
		Advanced solid tumours	Monotherapy	Phase 1/2 ongoing	NCT02584647 NCT02071940 NCT02975700 NCT01499043 NCT01004861
		Advanced haematological malignancies	Monotherapy	Phase 1/2 ongoing	NCT01349049 NCT02390752
		Tenosynovial giant cell tumours	Monotherapy	Phase 3 ongoing	NCT02371369
		Hodgkin Lymphoma	Monotherapy	Tolerable, limited efficacy	[174]
		Advanced solid tumours	Anti-PD-1 Ab	Phase1/2a ongoing	NCT02452424
		Pancreatic or colorectal cancers	Anti-PD-L1 Ab	Phase 1 ongoing	NCT02777710
		GIST	c-Kit inhibitor	Phase 1b ongoing	NCT02401815
		Malignant peripheral nerve sheath tumours	mTOR inhibitor	Phase 1 ongoing	NCT02584647
		V600E-mutated melanoma	BRAF inhibitor	Phase 1b ongoing	NCT01826448
		Glioblastoma and prostate cancer	Radiotherapy	Phase 1b/2 ongoing	NCT01790503 NCT02472275
		Breast cancer and advanced solid tumours	Chemotherapy	Phase 1b ongoing	NCT01596751 NCT01525602
PLX7486 (Plexxikon)	Tyrosine kinase inhibitor of CSF1R and TrkA, TrkB, and TrkC,	Advanced solid tumours	Monotherapy	Phase 1 ongoing	NCT01804530
FPA008 (FivePrime)	Humanized mAb CSF1R	Tenosynovial giant cell tumours	Monotherapy	Phase ½ ongoing	NCT02471716
		Selected advanced solid tumours	Nivolumab	Phase 1a/b	NCT02526017

to CSF1R and induced intolerable side effects unrelated to macrophage behavior. A novel compound, DCC-3014, displays remarkable specificity and was due to be used in a First-In-Human trial by the end of 2016 but is yet to commence [141].

While the efficacy of CSF1R inhibitors has not yet led to their clinical approval, effective depletion of TAM numbers has been a positive development which may effectively compliment other therapies.

Combination of macrophage modulation and T cell checkpoint inhibitors

Progress has not been aided by a relative under characterization of macrophage behaviour during the administration of current immunotherapeutics and analysis of how they may impact response. This is more striking when considering the central role monocytes and macrophages play in shaping the immune response. There has been limited publication of the relationship between response to CPIs and myeloid cells, but the level of immunological interrogation of patients focusing on myeloid subsets is not clear.

Ipilimumab (10 mg/kg) has been successfully trialled with subcutaneous recombinant GM-SCF in metastatic melanoma with an enhanced OS of 17.5 vs 12.7 months, and was better tolerated than Ipilimumab alone [142]. The mechanism resulting in reduced toxicities is not known; however, there was no difference in the objective response rate and no significant change in PFS. There is currently a phase 2/3 clinical trial examining the combination of Nivolumab and Ipilimumab with or without GM-CSF in unresectable melanoma (NCT02339571).

Positive results of clinical trials examining macrophage modulation will intuitively result in future trials combining them with CPIs. Some of these combinational approaches are entering early stage clinical trials, but there have also been a number of trials which have indirectly combined CPIs with macrophage modulation and seen positive results.

Trabectedin is a drug approved for soft tissue sarcoma that binds the minor groove on DNA resulting in a poorly characterized DNA damage in all cells, but critical to its anti-tumour efficacy is its ability to selectively induce apoptosis in monocytes and macrophages, reduce recruitment of $CD68^+$ monocytes to the TME and reduce CCL2 and CXCL8 levels [143–145]. Trabectedin has been shown to be synergistic with anti-PD-1 antibodies in murine models of ovarian cancer with the generation of systemic anti-tumour immunity [146]. It has been approved for the treatment of soft tissue sarcoma under the trade name Yondelis, and is currently in clinical trials for use in breast, prostate and paediatric sarcomas. The prolonged period of treatment required to see an effect on macrophage populations makes it unlikely to exert an observable effect in fast growing or late stage tumours.

MGN1703, a DNA-based TLR agonist is being trialled in advanced solid malignancies with Ipilimumab (NCT02668770). Similarly, IMO-2125, a synthetic TLR-9 agonist which is expressed by plasmacytoid dendritic cells but also to a lesser extent by monocytes and macrophages, is being trialled in combination with ipilimumab in patients with metastatic melanoma (NCT02644967). If successful data emerges from these trials it will increasingly turn focus towards the role of innate immune cells in response to CPIs [147].

Data emerging from the phase 3 clinical trial KEYNOTE-252/ECHO-301 suggests that Epacadostat—an IDO inhibitor—in combination with Pembrolizumab can improve outcome for stage III/IV unresectable or metastatic melanoma patients. IDO is primarily secreted by M2 macrophages but can also be produced directly by tumour cells in cancer patients. A phase 3 trial is currently recruiting 600 patients to further test this combination (NCT02752074).

Discussion

Side effects associated with CPIs are dose dependant (Table 4), it appears they are also cumulative to the cycles received and additive with other CPIs [148]. The most recent evidence to emerge from CheckMate 067 examining Ipilimumab and Nivolumab in advanced melanoma, has suggested the side effects are not cumulative but remain high with 58% of patients experiencing grade 3 or 4 adverse events (AEs). Intuitively this has led to a shift in therapeutic design, which has been predominantly focused on engineering or stimulating T cells ex vivo. However, it is uncertain if these cells will be able to overcome the immunosuppressive environment that acts to 'turn off' these cells after readministration.

The most notable and promising examples of successful macrophage modulation have been found in murine models on PDAC and these are now beginning to show efficacy in the clinical setting, but the unique composition of the pancreatic cancer TME may not accurately reflect the potential of macrophage modulation in other tumour types. It is hypothesized that the success seen may be due to the restricted flow of cells into and out of the microenvironment resulting in a reduced ability to compensate for a loss of macrophage function and consequent tumour inhibition. It however appears likely that macrophage modulating therapies will compliment CPIs, and it will be of keen interest to see if the reduced AEs seen with GM-CSF and Ipilimumab will be seen with other therapies designed to reduce immunosuppressive factors in the TME.

Table 4 Incidence of immune related AEs (irAE) seen in patients receiving ipilimumab

	0.3 mg/kg (%)	3 mg/kg (%)	10 mg/kg (%)
Incidence irAEs	26	56	70
Incidence grade ¾ irAEs	0	7	25
Incidence of drug discontinuation due AEs	13	10	27

Figures taken from [148]

While some have been quick to suggest that the ability to understand and direct MO behaviour represents an immunotherapy breakthrough it is clear from recent clinical evaluation that manipulation of macrophages as a stand-alone therapy in its current state is insufficient for therapeutic success [149]. However, it appears macrophage depletion may be a more effective strategy than macrophage re-education due to the profound immunosuppressive force exerted by advanced tumours [150].

In addition to the combination of macrophage modulation and immunotherapies, there is significant scope and promise for their combination with other therapies. For example, the anti-tumour effect of BRAF inhibitors was noted to be reliant on host tumour-directed immune responses [151]. 50% of advanced melanomas are BRAF positive and initially respond to therapy, but tumours develop mechanisms of acquired resistance and become refractory [152]. In preliminary studies, inhibiting monocyte and MDSC influx to the TME synergistically enhanced the effect of BRAF inhibition [153, 154]. There is mounting preclinical evidence to justify the use of macrophage modulating therapies with BRAF inhibitors in advanced melanoma.

Preclinical data in murine models has shown that the effect of immunotherapy in mouse models is more effective in the early stages of disease progression, which is generally defined by a low concentration of immunosuppressive elements in the TME. While the reversal of this immunosuppression may restore sensitivity, delineation of the primary immunosuppressive factors responsible for the reduction in efficacy is difficult due to the plethora of interacting factors and systems in the TME. Significant literature is available on many factors, but their relative importance in determining sensitivity to therapy has not been fully elucidated. The clinical prognostic evidence on immunosuppressive factors in patients undergoing treatment is limited, but do suggest that they are the key to the development of systemic and durable anti-cancer responses.

Targeting of macrophages has been shown to profoundly shape the immune response and we now have a range of sophisticated therapeutics that are beginning to make impacts in the clinic. Rational design of immunotherapeutics that will increase their efficacy, response rates and generate systemic and durable response rates will require a holistic mind-set towards understanding the immune system. Given the central role that macrophages play in shaping the immune response they will play an integral role in immunotherapeutic design.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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