

Tumour-associated macrophage polarisation and re-education with immunotherapy

Debra H. Josephs^{1,2}, Heather J. Bax^{1,2}, Sophia N. Karagiannis¹

¹St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences & Medicine, King's College London; NIHR Biomedical Research Centre at Guy's and St. Thomas' Hospital and King's College London, Guy's Hospital, London, UK, ²Research Oncology, Division of Cancer Studies, Faculty of Life Sciences & Medicine, King's College London, Guy's Hospital, London, UK

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1. ABSTRACT

Monocytes/macrophages constitute important contributors of cancer-associated inflammation. Through their plasticity and capacity to become polarised by tumours towards less activatory and more immunosuppressive (M2) phenotypes, tumour-associated macrophages (TAM) are thought to support tumour progression. Orchestrated by T helper 2 (Th2)-biased stimuli, macrophage recruitment, activation and polarisation in tumour microenvironments is associated with poorer clinical outcomes. Their key roles in supporting tumour progression and their capacity for plasticity have focused targeted and immunotherapeutic strategies to counteract macrophage pro-tumourigenic activities and to re-ignite their tumour-cytotoxic power. Therapeutic approaches include blockade of macrophage recruitment into tumours, suppression of TAM survival, re-polarisation towards an M1-like phenotype and antibody therapies to enhance TAM

anti-tumoural activities. Future immunotherapeutic directions may include monoclonal antibodies with enhanced effector functions. Antibodies of different classes, including those of the IgE class, shown to restrict tumour growth by harnessing monocyte/macrophage cytotoxic properties in pre-clinical cancer models, may synergise or re-educate these potent immune sentinels to destroy rather than support tumours. Opportunities for monitoring monocyte/macrophage polarisation or activatory signatures in patients may inform clinical management.

2. MACROPHAGES PARTICIPATE IN THE INFLAMMATORY INFILTRATE OF SOLID TUMOURS

In recent years, inflammation has been proposed as the seventh hallmark of cancer (following: self-sufficiency in growth signals,

insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis) (1-6). Indeed, in around 15% of cancers, tumour initiation is linked to infection (viruses, bacteria, parasites) and chronic inflammation (5, 7). A pro-tumour microenvironment where tumour initiation and progression may occur is likely to show persistent or 'smouldering' inflammation (4, 6, 8).

Innate immune cells are highly represented in the complex ecosystem of a tumour, with macrophages, first reported in human tumours by Virchow in 1863 (9, 10), being the most abundant (1, 3, 8, 11, 12). Tumour-associated macrophages (TAMs), serve as sentinels of cancer-related inflammation (4, 13, 14). It was first hypothesised that TAMs were involved in anti-tumour immunity, however clinical and experimental evidence has shown that in the majority of cases, TAMs promote tumour progression (3, 8). This is supported by a meta-analysis showing correlation between tumour macrophage density and poor patient prognosis (9). Recruitment and regulation of TAMs in tissues occurs in response to a number of factors including CCL2 (MCP-1), VEGF, PDGF, TGF- β , CSF-1, MIP-1 α , complement component C5a, RANTES, and TNF- α , all of which can be produced by cancer cells (4, 15-17).

3. THE VARIED ACTIVITIES OF TUMOUR-ASSOCIATED MACROPHAGES ARE DEPENDENT ON THEIR POLARISATION

Plasticity is a hallmark of cells of the monocytic lineage and specifically of TAMs (2, 4, 5, 13). Their polarisation is induced, not based on their location, but by the combination of diverse and distinct tissue-specific signals found in the tumour microenvironment (TME) at different stages of tumour development and progression (4, 5, 8, 11, 13, 14, 17, 18). The role of macrophages in tumour growth is complex and multifaceted (9), and tumour initiation, progression and metastasis occur depending on dynamic phenotypic changes to TAMs through their interactions with the microenvironment (4, 8, 17). Generally, TAMs have a dual influence on tumour growth and progression, acting in either a pro- or anti-tumour manner (4, 13). Once polarised and activated, TAMs produce a wide variety of cytokines/chemokines, growth factors and enzymes that have ranging roles in cancer (1).

The diversity between TAM phenotypes has been extensively reviewed (4, 13, 19). In summary, 'classically activated', M1 phenotypic macrophages, known to be involved in Th1 responses to pathogens, are activated by IFN- γ , TLR engagement, LPS, TNF- α , and GM-CSF. M1 macrophages express MHC II, generate nitric oxide (NO) species, and release pro-inflammatory and immune-stimulatory mediators such as IL-12, IL-1 β , IL-6 and TNF- α (8, 11, 13, 14, 19). M1 phenotypic TAMs are primarily thought to be tumouricidal and evoke tissue destruction (4, 14), either by direct or indirect cytotoxicity (9). Interestingly, these activities may also have a role in the initiation of tumour cell formation through production of early pro-inflammatory signals and destruction of local tissue architecture (8). The 'alternatively activated', M2 phenotypic TAMs differentiate upon IL-4 and IL-13 stimulation and are involved in Th2 responses, such as parasite elimination (4, 8, 11, 13, 17, 19). These cells express low levels of MHC II, have a role in tissue repair, remodelling and tumour promotion (4) and release immunoregulatory cytokines and chemokines, such as IL-10 (14). These promote suppression of immune responses and increased regulatory T cell (Treg) recruitment (11). The diversity of surface receptor expression and molecular pathways of polarisation between these phenotypes is thoroughly reviewed by Biswas and Mantovani in their recent article (19). Overall, M1 and M2 TAM phenotypes mirror Th1 and Th2 T cells and their production of IFN- γ and IL-4, and are found in normoxic and hypoxic environments, respectively (13). However, these phenotypes represent extremes of a spectrum of activation states which are produced depending on the combination of different signals in the tumour microenvironment (TME) (4, 5, 8, 12-14, 18, 20-22).

4. TAMs ARE DIVERTED BY TUMOURS IN FAVOUR OF M2-LIKE PHENOTYPES AND MAY SUPPORT TUMOUR DEVELOPMENT, GROWTH AND METASTASIS

In the TME, macrophages have a predominantly M2-like phenotype and may be switched or redirected to a different phenotype depending on the stage of tumour development and in response to distinct tumour microenvironmental signals (4, 9, 14, 15, 20). Although, M1 phenotypic TAMs may play a role in the initiation of tumour development, changes in the TME such as hypoxia, glucose level and pH, which occur during the transition from early to advanced stage tumours, may

result in a phenotypic switch to the 'trophic' M2-like phenotype (2, 5, 8, 21). This is thought to occur through expression of CCL2, CSF-1, MSF, TNF- α , IL-10 and TGF- β in tumours (4, 19, 20). Overall, evidence demonstrates that a symbiotic relationship forms between tumour cells and macrophages, with TAMs acting as 'allies' as they orchestrate virtually all aspects of cancer (1, 4, 9, 13, 23).

Tumour cell formation may first occur due to genetic mutations caused by NO species and TNF- α from M1-like TAMs (8, 19). Secondly, M2-like TAMs are recruited to hypoxic areas and towards CCL2 released by tumour cells, to produce VEGF which stimulates angiogenesis and further tumour cell invasion (5, 8, 15, 20). Thirdly, remodelling and migration is associated with M2-like TAM production of matrix metalloproteases (MMP) (19, 20). In fact, MMP production correlates with tumour progression (1) and melanoma cell-conditioned media can favour macrophage differentiation into immune inhibitory properties which express both MMPs and CCL2 (14, 24). Additionally, MMP9 has been shown to be produced by the more metastatic rounded-amoeboid melanoma cells, supporting tumour invasion through catalytic and non-catalytic mechanisms (25). Cell-cell contact-independent migration and invasion by tumour cells may also depend on epidermal growth factor (EGF) from TAMs, which is itself released upon stimulation by CSF-1 from tumour cells (1, 19). Thus, these cells migrate together in a paracrine fashion, with TAMs acting as 'cellular chaperones' (1, 5, 8, 17, 22). In addition, tumour cell migration can also be up-regulated by TAM-modulation of the cancer cell actin cytoskeleton (1). Fourthly, M2-like TAMs are involved in the creation of a metastatic niche, as seeding and establishment of metastatic tumour cells has been shown to be macrophage and CCL2-dependent (4, 8, 13, 22). Finally, adaptive immunity is diverted, skewed, and suppressed by a number of M2-like TAM activities (4, 19). These macrophages have poor antigen-presenting ability and inhibit T cell proliferation and activity (14, 20). TAM-secreted CCL-22 is also a chemoattractant for Tregs, which themselves promote M2 polarisation by IL-10 release, creating a feedback loop for suppressed immunity and pro-tumour activities (19). Overall, expression of key factors, MCP-1 and CSF-1, in the coordinated relationship between M2-like TAMs and tumour cells directly correlates with the macrophage content in tumours and poor prognosis for patients (9, 13).

5. IN IMMUNOSUPPRESSIVE TUMOUR MICROENVIRONMENTS, MACROPHAGES MAY LACK THE REQUIRED ACTIVITY TO TARGET TUMOUR CELLS

As well as skewing the polarisation of TAMs to the immunosuppressive M2-like phenotype (2, 5, 8, 21), secretion of cytokines, such as IL-10, TGF- β , and expression of inhibitory receptors, such as galectin-9, CTLA-4 and PD-L1 by Tregs and myeloid-derived suppressor cells (MDSCs) occurs in the TME (26). Tumour cells also use mediators such as VEGF and TGF- β and ligand shedding (NKG2D, MICA and MICB) to inhibit T cell functions (26).

Perpetuation of a pro-tumour environment is achieved by the symbiotic relationship between tumour cells and M2-like TAMs. In particular, CCL2, secreted by both cell types, increases further macrophage influx and skewing, and is thought to be self-enhancing and critical to tumour progression (4, 9, 15, 16, 23). Furthermore, tumour cells may produce IL-6 which acts through NF- κ B to regulate survival and enhance pro-tumoural macrophage polarisation (23). Although often considered an M1 phenotype-associated inflammatory cytokine participating in innate cell activation, the effects of IL-6 constitute a double edge sword in cancer inflammation. IL-6 can also be produced by tumour cells as well as by macrophages and supports tumour cell survival and promotes angiogenesis. Its release can trigger further IL-6 production by monocytes in an autocrine and paracrine manner, engendering re-education of dendritic cells and macrophages (12, 23). Following chemotherapy or radiation, release of inflammatory signals may recruit and activate tumour-promoting immune cell activities by damaged or dead tumour cells and can result in reduced tumour cell killing and M2-like TAM upregulation, which can lead to chemoresistance (3, 12).

The potent pro-tumour polarising influence of the TME has also been demonstrated in an experimental setting: macrophages were skewed to M1-like phenotypes *ex vivo* with LPS and IFN- γ stimulation, but upon reperfusion these cells showed no anti-tumour activity (9, 27). This suggests that although cells may be switched to an anti-tumoural phenotype, exposure to suppressive signals within the tumour ecosystem, results in the neutralization of tumouricidal activity. These findings may indicate that targeting the tumour microenvironment *in vivo*

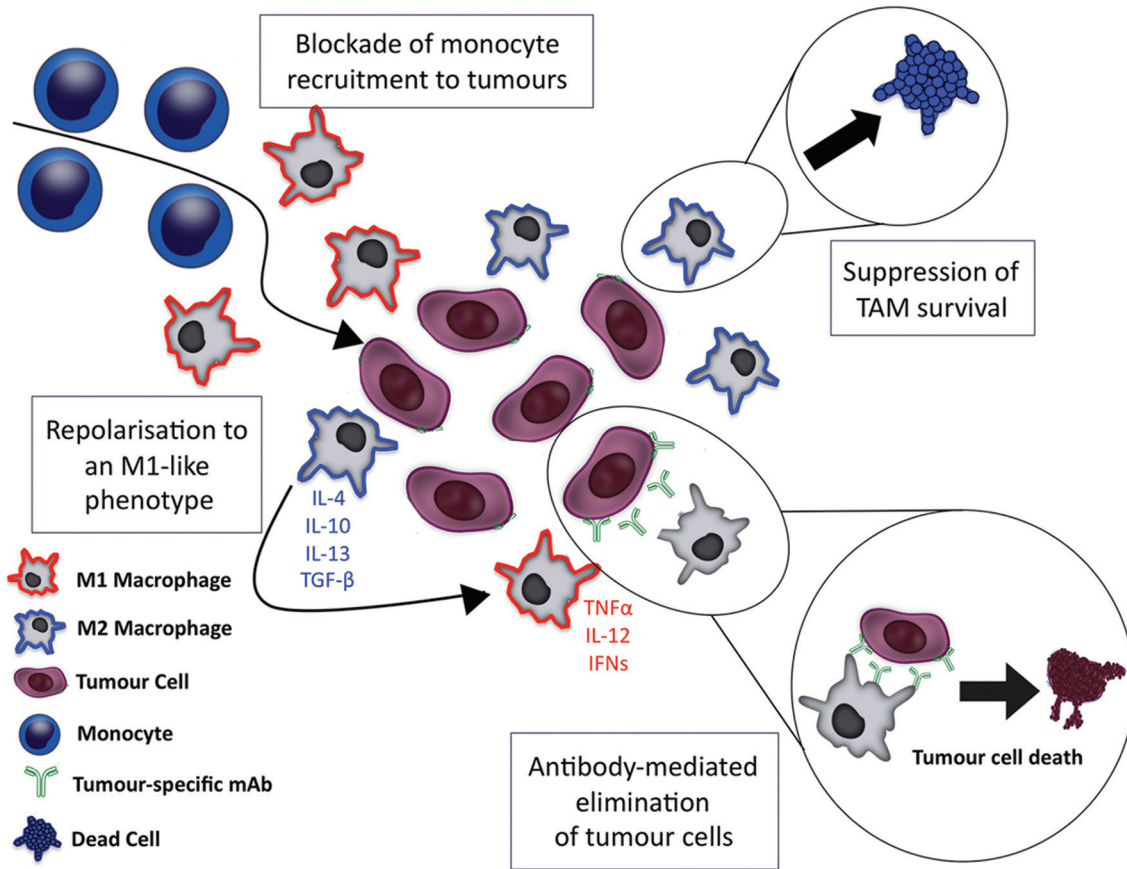


Figure 1. Tumour-associated macrophage (TAM) -targeted therapeutic strategies for cancer. The pro-tumorigenic functions of TAMs depend on their accumulation and survival within tumours and their M2-like polarisation status. Current TAM-targeted treatment strategies include: (i) blockade of monocyte/macrophage recruitment; (ii) suppression of TAM survival; (iii) repolarisation of TAMs towards an M1-like phenotype; and (iv) antibody-mediated elimination of tumour cells by monocytes/macrophages. Cytokines listed are the key cytokines required for M1- or M2- skewing of macrophages.

to divert macrophages to mediate anti-tumoural activities may prove more effective.

6. TARGETING TAMs AS AN IMMUNOTHERAPEUTIC STRATEGY FOR CANCER

6.1. Blockade of macrophage recruitment to tumours

In the large majority of tumours, TAMs are considered pro-tumourigenic, as they secrete growth and angiogenic factors as well as immunosuppressive factors (19). One approach therefore may be to deplete TAMs or to inhibit their recruitment in tumour lesions (Figure 1).

CCL2 (MCP-1) is a chemokine known to recruit macrophages to sites of inflammation.

Evidence suggests that CCL2 is progressively overexpressed by solid tumours and may play a role in their clinical progression (28, 29). Antibodies against CCL2 or its receptor CCR2 have therefore been investigated in preclinical models. There is evidence to support a role for anti-CCL2 therapy in prostate cancer (30, 31) and breast cancer (32). More recently, in mice with pancreatic cancer, CCR2 inhibition using a targeted agent (PF-04136309) as an adjunct to standard chemotherapy demonstrated blockade of monocyte recruitment to tumours resulting in enhanced anti-tumour immunity, decreased tumour growth, and reduced metastases (29) (Table 1). Based on this data, a Phase IB clinical trial using PF-04136309 combined with standard chemotherapy in pancreatic cancer patients is underway (<http://clinicaltrials.gov/show/NCT01413022>).

Activating macrophages for cancer immunotherapy

Table 1. Possible activities of cancer therapeutic and experimental interventions on the tumour cell/tumour-associated macrophage (TAM) axis

Standard therapies			
Therapy	Proposed affect on monocytes/macrophages		Refs.
Chemotherapy*	Release of signals that alter macrophage polarisation to favour M2 monocytes/macrophages, and can lead to chemoresistance.		3, 12
Radiotherapy*	Damaged or dead tumour cells can upregulate M2 macrophages that support tumour survival.		3, 12
Other therapeutic and experimental interventions			
Target/strategy	Therapy	Proposed activity	Refs.
TAM surface proteins i.e., scavenger receptor-A, CD52 and folate receptor β	Immunotoxin-conjugated mAbs, i.e., SRA-ZAP, alemtuzumab*, anti-FR β -PE38	Suppression of macrophage survival	43-45
Legumain	Legumain-based DNA vaccine	Dendritic cell activation, antigen presentation, stimulation of cytotoxic CD8+T cells and the specific abrogation of legumain-expressing TAMs	46, 47
NF- κ B pathway in TAMs	TLR agonists, i.e., PolyI: C, lipopolysaccharide (LPS), monophosphoryl A*, imiquimod* and CpG-oligodeoxynucleotide (as an adjuvant to tumour-specific antigen vaccines)	NF- κ B activation in macrophages, promoting their production of IL-12, IFN α/β and TNF α	48, 49, 50, 52-56
IL-10	Anti-IL-10R mAbs, i.e., 1B1.3a (in combination with CpG-ODN)	Repolarization of infiltrating macrophages to M1, stimulation of innate immune response and subsequent adaptive immunity	51
CD40	Anti-CD40 mAb, i.e., SGN-40	NF- κ B activation, MHC II and CD86 upregulation, increased IL-12, TNF α and IFN- γ and tumour cell destruction	57, 58, 76
Macrophage microRNAs	miR-125b overexpression	Increased IFN- γ receptor expression, and improved T-cell activation by macrophages	59
	miR-155 overexpression	Possible redirection of macrophages towards anti-tumour phenotype	60, 61
CCL2 and CCR2	anti-CCR2 agent, PF-04136309 (in combination with standard chemotherapy*)	Macrophage recruitment blockade, decreased tumour growth and reduced metastases	29, 30, 31, 32
CSF and CSF1R	Antisense oligonucleotides	Reduced macrophage recruitment and tumour growth	36-38
	CSF1/CSF1R antibodies or agents (in combination with standard chemotherapy, i.e., trabectedin*)	Blockade of macrophage recruitment, reduced metastases, angiogenesis, increased CD8+T cells, and slowed tumour growth. Downregulation of M2 polarization genes	18, 34, 35, 38
	Liposomal bisphosphonates	Cytotoxicity to macrophages, and regression of tumour growth, angiogenesis and metastasis	39-42
CD142	Anti-CD142 mAb, i.e., humanised TF8-5G9	ADCP killing of tumour cells by monocytes/macrophages	71
CD20	Anti-CD20 mAb, i.e., rituximab*	Monocyte/macrophage associated tumour cell killing by ADCC	66, 74, 75, 78
CD30	Anti-CD30 mAb, i.e., SGN-30	Monocyte/macrophage dependent tumour cell growth arrest	76
FR α	MOv18 mAb	Protection against tumour growth and improved survival mediated by monocyte effector functions and recruitment	98, 99

*Clinically available therapeutics

A number of recent studies have demonstrated that inhibitors of the M-CSF receptor are effective in inhibiting macrophage recruitment and/or pro-tumoural differentiation (33, 34). Furthermore, Germano *et al.* recently reported that macrophage targeting *in vivo* is a key component of the anti-tumour activity of the drug trabectedin (35). This effect is attributed to the ability of trabectedin to rapidly activate caspase-8 (the key effector molecule of the extrinsic apoptotic pathway) in mononuclear phagocytes, which, unlike neutrophils and T cells, are not protected by the presence of the decoy receptor. Treatment of mice bearing trabectedin-resistant tumours resulted in slowed tumour growth, in spite of confirmed resistance of cancer cells to the drug. Additionally, reduced levels of circulating (CD14+) monocytes and tumour-associated (CD163+) macrophages were found in patients treated with trabectedin. Targeting monocytes and TAMs, with the rationale that these cells constitute a largely tumour-promoting cell population, trabectedin was thus shown to inhibit their pro-tumoural effects. Indeed, the adoptive transfer of macrophages to treated mice significantly re-instated tumour growth. Pathological examination of tumour sections revealed that in treated tumours the vessel network, VEGF and CCL2 were significantly down-modulated. Thus, in addition to direct cytotoxic activity on macrophages, trabectedin also reduced the recruitment of circulating monocytes into tumours and affected angiogenesis (35) (Table 1).

In addition, blockade of the CSF-1/receptor axis using antibodies and antisense oligonucleotides has also demonstrated efficacy in reducing macrophage recruitment and tumour growth (36-38). In a mouse model of breast cancer, chemotherapy upregulated the macrophage chemotactic factors CSF-1, CCL8/MCP-2 and IL-34, which led to an increase in CSF1R-expressing macrophages in the TME. Treatment with inhibitors of CSF1R in combination with chemotherapy, resulted in the blockade of macrophage recruitment with enhanced therapeutic activity, reduced metastases and increased T cells in tumours (38). Furthermore, administration of an anti-CSF1R antibody has also been shown to induce downregulation of a core set of M2 polarisation genes in tumour-associated macrophages (18).

6.2. Suppression of TAM survival

An alternative approach for targeting TAMs as an immunotherapeutic strategy for cancer is to deplete TAMs locally in tumours (Figure 1). Towards

this, one strategy is to directly induce macrophage apoptosis using chemical reagents, immunotoxin-conjugated mAbs or attenuated bacteria; the other is to trigger immune cells, *e.g.* T lymphocytes, to recognize and abrogate TAMs. Liposomal bisphosphonates have become prominent drugs for macrophage depletion, since they demonstrate elective cytotoxicity to macrophages resulting in regression of tumour growth, angiogenesis and metastasis (39-42). Immunotoxin-conjugated mAbs targeting the surface proteins of TAMs, such as scavenger receptor-A, CD52 and folate receptor β , have also demonstrated some clinical success (43-45).

Other than directly inducing the apoptosis of TAMs, another approach is to evoke an acquired immune response, in which cytotoxic T lymphocytes act as the scavengers of TAMs since they can naturally target macrophage membrane molecules. One such molecule is legumain, a lysosomal protease highly expressed in several tumours and also overexpressed in M2-like TAMs (46). When tumour-bearing mice were immunized with a legumain-based DNA vaccine, dendritic cells were activated, triggering antigen presentation, co-stimulation of cytotoxic CD8+ T cells and the specific abrogation of legumain-expressing TAMs (46, 47) (Table 1).

6.3. Repolarisation of TAMs towards an M1-like phenotype

A therapeutic approach that has been explored is the re-programming of M2-like TAMs to become immunosupportive M1-like macrophages instead (Figure 1). As described above, the polarisation of TAMs largely depends on the local cytokine profile. When high levels of Th1 cytokines (*e.g.* TNF α , IL-12 and IFNs) are present, M1 macrophages will predominate; whereas exposure to Th2 cytokines, (*e.g.* IL-4, IL-10, IL-13 and TGF- β), results in polarisation to M2 status (19).

The NF- κ B pathway can positively modulate the transcription of Th1-response cytokines. Attenuated NF- κ B activation in TAMs results in M2 polarisation, whereas NF- κ B reactivation can redirect TAMs to a tumouricidal M1-like phenotype (48). Several agents capable of activating NF- κ B have been reported including Toll-like receptor (TLR) agonists (*e.g.* PolyI:C, lipopolysaccharide (LPS), monophosphoryl A, imiquimod and CpG-oligodeoxynucleotide (CpG-ODN)), anti-CD40 mAbs and anti-IL-10R mAbs. CpG-ODN has been widely used as an adjuvant of tumour-specific antigen

vaccines, on the basis that the activation of TLR9 can upregulate NF- κ B activation in TAMs, promoting their production of IL-12, IFN α/β and TNF α (49, 50). The combined treatment of CpG-ODN with other agents such as the monocyte chemoattractant CCL-16 and anti-IL-10R monoclonal antibody (mAb) rapidly shifted infiltrating macrophages from M2 to M1, and triggered reduction of large tumours (51) (Table 1). Currently, CpG-ODN-based therapies are in clinical trials (52-56).

Antibodies against membrane receptors up-stream of the NF- κ B pathway have also been used for TAM repolarisation. One such membrane receptor is CD40. Buhtoiarov *et al.* demonstrated that ligation of CD40 with an agonist anti-CD40 mAb restored the activity of NF- κ B and induced tumour cell destruction (57). In another study, treatment with an anti-CD40 mAb resulted in up-regulation of MHC II and co-stimulatory molecule CD86 in macrophages, and elevated serum levels of IL-12, TNF α and IFN- γ , positively correlating with the regression of pancreatic carcinoma in humans and mice (58).

Aside from CD40 and the NF- κ B pathway, signalling through the CSF1R was recently shown to be critical for defining the phenotype of TAMs as either pro- or anti-tumour. In a mouse model of glioma, CSF1R inhibition was found to induce a shift in the genetic signature and phenotype of TAMs from pro- to anti-tumour, prolonging the survival of tumour-bearing mice (34). Blocking the CSF1R has also been shown to promote immune surveillance by CD8+ T cells when combined with chemotherapy in a mouse model of breast carcinoma (38), suggesting that reprogramming macrophages *in vivo* may support the development of a productive anti-tumour T cell-mediated immune response.

With an improved understanding of the genetic signatures that define pro- vs. anti-tumour macrophages, novel strategies to reprogram macrophages with properties supportive of anti-tumour T-cell immunity are now being explored. One such approach involves the use of microRNAs, short non-coding RNAs that can inhibit the expression of a panel of target genes. The microRNA miR-125b is enriched in macrophages and its overexpression was found to increase the expression of the IFN γ receptor, allowing macrophages to respond to co-stimulatory molecules and hence acquiring an improved ability to induce T-cell activation (59). Another microRNA, miR-155, is upregulated in macrophages responding

to inflammatory stimuli (60). In a mouse model of breast cancer, the knockdown of miR-155 accelerated tumour growth, a process that was associated with the skewing of tumour-infiltrating macrophages toward a pro-tumour phenotype (61). Taken together, these findings suggest that augmenting the expression of distinct microRNAs to re-direct the biology of TAMs toward an anti-tumour phenotype may support the development of anti-tumour T-cell immunity (Table 1).

6.4. The Role of Macrophages in Mediating the Elimination of Tumour Cells by Monoclonal Antibodies

A key strategy for harnessing the anti-tumour potential of macrophages is through treatment with a tumour antigen-specific antibody (Figure 1). Therapeutic mAbs, tightly retained on Fc receptor (FcR)-expressing macrophages, might be sufficient to overcome immune suppression within the TME, and to stimulate macrophage-mediated tumour cell killing.

Therapeutic mAb use has dramatically increased in the last decade and is now a mainstream approach for the treatment of cancer. However, the mechanisms by which mAbs mediate tumour cell death are vastly diverse and not completely understood. Direct mechanisms include the induction of apoptosis, inhibition of proliferation, or sensitization of tumour cells to chemotherapy (62, 63). In addition, most of the currently approved mAbs are of the IgG1 subclass, and therefore activate the complement cascade via their Fc regions, leading to complement-dependent cytotoxicity (CDC). The Fc regions of IgG antibodies also interact with IgG Fc receptors (Fc γ receptors) expressed on immune effector cells leading to Fc γ receptor-mediated mechanisms of tumour cell-death including antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) as well as complement activation (64). These mechanisms of antibody-mediated tumour cell death have recently been demonstrated to be unaffected by concomitant chemotherapy (65). Several Fc γ receptor-expressing immune cells have been proposed to execute the elimination of tumour cells during mAb therapy. In general, natural killer (NK) cells are considered the main effector cells, acting via ADCC (66). However, macrophages have also been shown to have cytotoxic capacity, which can involve diverse mechanisms, including ADCC, release of reactive oxygen species and reactive nitrogen species (ROS and RNS), and ADCP (67, 68).

The extent by which tumour cell killing by IgG1 is mediated by ADCC, ADCP or CDC depends on which immune effector cell type is activated, since different effector cells express specific Fc γ receptors through which the different types of tumour cell killing are mediated (69). A number of parameters may be responsible for antibody effector functions, including the antibody class/subclass, antibody Fc region engineering and Fc receptor binding characteristics; these characteristics are reflected in differential Fc-mediated mechanisms of clinically available therapeutic antibodies in oncology (62).

Indeed, Gul *et al.* recently demonstrated in a murine tumour model that macrophages play a prominent role in mAb-mediated eradication of tumour cells by ADCP (70). Phagocytosis by macrophages was dependent on both the high-affinity (Fc γ RI) and the low-affinity (Fc γ RIV) IgG Fc receptors and antibody therapeutic efficacy was lost after macrophage depletion (70). Furthermore, in a mouse breast carcinoma model, TAMs isolated from tumours were shown to express Fc γ receptors and were capable of killing tumour cells by ADCP in the presence of a tumour-targeting anti-CD142 mAb (71). In this model depletion of macrophages reduced the therapeutic efficacy of the anti-CD142 mAb, suggesting that macrophages may play an important role in the therapeutic efficacy of mAb therapy in breast cancer (71). In the human setting, IgG antibodies are recognized by the Fc gamma receptor family that includes the activatory Fc γ RI (CD64), Fc γ RIIA (CD32A), Fc γ RIIIA (CD16a) and Fc γ RIIIB (CD16b), and the inhibitory Fc γ RIIB (CD32B). Although the inhibitory receptor Fc γ RIIB may be upregulated in some tumours (72), and may be expressed by TAMs, tumour antigen-specific antibodies can function through triggering effector functions such as ADCP of tumour cells by both M1 and M2 monocytes/macrophages. This supports the potential of antibodies to activate patient effector cells including tumour-polarized macrophages (65, 71, 73).

Macrophages may also play a significant role in the success of mAb therapy in patients with haematological malignancies. In a murine lymphoma model, macrophage depletion abrogated the therapeutic efficacy of an anti-CD20 mAb (74, 75), and severe combined immunodeficiency (SCID) mice engrafted with a Hodgkin-derived cell line and treated with either anti-CD30 or anti-CD40 mAbs, demonstrated poorer survival following macrophage depletion (76, 77). Furthermore, clinical responses

to rituximab therapy have been correlated with polymorphisms in human Fc γ RIIIa (78). Since macrophages, but not NK cells, express Fc γ RII, a role for macrophages as effector cells in B cell lymphoma depletion following anti-CD20 mAb treatment is strongly supported.

6.5. Monoclonal antibodies of the IgE class can harness the anti-tumour potential of macrophages *in vitro* and *in vivo*

Since mAb interaction with immune cells is mediated by its Fc domain, the class of antibody critically determines its effector functions, and may influence efficacy. Therefore, one strategy to optimise antibody-immune system interactions is the use of alternative immunoglobulin classes, such as IgE, IgA or IgM (79-82). Human IgA antibodies have demonstrated superior efficacy compared to IgG antibodies in recruiting neutrophils, and may also have the additional advantages of forming natural dimers with improved signaling capacity on tumour cells, and being actively transported into mucosal secretions with the potential for improved targeting of certain luminal surface carcinomas (81). IgM antibodies, in addition to their role in the innate immune response, also participate in the recognition and removal of transformed cells as an important defense against cancer (82). Monoclonal antibodies of the IgM class have been isolated from the tumours of cancer patients, and have demonstrated the ability to specifically kill malignant cells by inducing apoptotic pathways (83, 84), highlighting the potential use of monoclonal IgM antibodies in the development of new anti-cancer treatments.

Key immune-activating properties of IgE contributing to protection against parasitic infections and to the allergic response may render IgE a novel anti-cancer modality (85, 86). Epidemiological evidence also suggests cross-talk between allergy and some malignancies (reviewed in Josephs *et al.*, 2013 (87)). These have prompted research groups to develop approaches aimed at triggering IgE functions to target tumour cells (80, 88-97).

Several studies to date have been conducted with the aim of investigating the effector mechanisms of therapeutic IgE antibodies targeting tumour cells. In a nude mouse xenograft model of a human ovarian carcinoma, in which a mouse/human chimeric monoclonal IgE antibody (MOv18 IgE) recognising the tumour-associated antigen folate receptor- α (FR α) was co-administered with human peripheral blood mononuclear cells (PBMcs),

MOv18 IgE provided greater protection against tumour growth compared to its IgG1 counterpart. In that system, human monocytes were found to be the key immune effector cells infiltrating the tumours. Immunohistochemical analysis of tumour xenografts demonstrated that human monocytes infiltrated tumour lesions in MOv18 IgE-treated mice only. This indicated that these IgE receptor-expressing effector cells may play an important role in the anti-tumour effect of this antibody (98). The role of monocytes in this model was confirmed by using monocyte-depleted PBMC, which resulted in a loss of the survival advantage conferred by MOv18 IgE (99).

Using *in vitro* flow cytometric assays and corresponding imaging experiments, tumour cell killing by human monocytes was demonstrated by ADCC (via the IgE high affinity receptor Fc ϵ RI constitutively expressed by a proportion of monocytes/macrophages) and ADCP (via CD23, the low affinity IgE receptor, expressed on the surface of IL-4-activated monocytes/macrophages). ADCC and ADCP are both known IgE mechanisms of action in the defence against parasitic infections (98-102). These findings highlight the potential importance of monocytes/macrophages, key IgE receptor-expressing cell populations, in mediating the anti-tumour efficacy of therapeutic antibodies of the IgE class.

In addition to mediating monocytes/macrophage tumour cell killing by ADCC and ADCP, it is possible that a tumour-specific monoclonal IgE antibody may have the ability to repolarise TAMs, towards a different M phenotype. Since it is known that Fc ϵ RI receptor occupancy by IgE increases Fc ϵ RI receptor levels at the surface of mast cells and basophils (103), it could be hypothesized that administration of a therapeutic monoclonal IgE antibody may act to enhance IgE receptor expression on monocytes/macrophages, thereby amplifying their anti-tumour response.

7. CONCLUDING THOUGHTS

Originally localising in tumours in response to signals of stress or inflammation, monocytes subsequently differentiate into alternative macrophage phenotypes featuring immunosuppressive properties and impaired cytotoxic capacity, due to exposure to Th2-biased inflammatory signals produced in tumour microenvironments. Monocytes/macrophages can also promote tumour growth through tissue destruction and remodelling, as

well as through contributing to changes in the pre-metastatic tumour niche that favour metastasis and by supporting metastatic tumour cell survival at distant sites by protecting circulating tumour cells during intravascular transit (104). Constantly evolving tumours can dynamically recruit monocytes/macrophages and re-educate these cells *in situ* to support tumour growth at all stages of cancer development. On the other hand, monocytes/macrophages are characterised by their plasticity, providing both a challenge, as tumour cells can continuously influence and control TAM functions, as well as an opportunity in the form of targeted and immunotherapeutic interventions to reverse tumour-induced immunosuppression through re-educating macrophages. The principles of targeting TAMs either by destroying specific immunomodulatory subsets or by activating them to kill, rather than promote cancer cells has been demonstrated by numerous interventions.

Monoclonal antibodies constitute an increasingly appreciated therapeutic direction with capacity to activate monocytes/macrophages. The functions of many antibodies may rely on engagement of Fc receptor-bearing macrophages in tumours; TAMs constitute locally accessible and potentially cytotoxic effector cells which require key activatory signals to destroy cancer cells. Indeed, existing clinically-available therapeutic antibodies are now known to function by mediating monocyte/macrophage-mediated effector functions, ADCC and ADCP, as part of their therapeutic profile. Specific antibody Fc engineering to selectively bind activatory Fc receptors on the surface of effector cells including monocytes/macrophages and also NK cells is expected to yield promising outcomes. New exciting avenues may arise through engineering antibodies of different classes like IgA and IgE, which may be better suited to function in Th2-biased environments. These engineering approaches may harness the anti-tumoural properties of local immune sentinels like TAMs and re-educate these cells to target cancer.

New opportunities may also lie with a future utility for monocytes/macrophages as biomarkers within the patient circulation and the tumour microenvironment. Specific TAM and circulating monocyte signatures identified through emerging imaging and bioinformatics technologies could signify re-education of these key immune sentinels and reflect responses to therapies. Monitoring monocyte/macrophage profiles could facilitate

patient stratification and yield clinical algorithms for personalised medicine.

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- MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; CSF, colony stimulating factor; FcR, Fc receptor; ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; FR α , folate receptor α ; CCL2, chemokine (C-C motif) ligand 2; MCP-1, monocyte chemoattractant protein-1; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β ; MIP-1 α , macrophage inflammatory protein-1 α ; RANTES, regulated on activation normal T cell expressed and secreted; TNF- α , tumour necrosis factor- α ; IFN- γ , interferon- γ ; GM-CSF, granulocyte-macrophage colony stimulating factor; MHCII, major histocompatibility complex II; IL, interleukin; MSF, macrophage slowing factor; MMP, matrix metalloproteases; EGF, epidermal growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-L1, programmed death ligand 1; NKG2D, natural killer group 2, member D; MICA/B, MHC-class-I-polypeptide-related sequence A/B; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; CCR2, chemokine (C-C motif) receptor 2; M-CSF, macrophage colony stimulating factor; CpG-ODN, CpG-oligodeoxynucleotide; SCID, severe combined immunodeficiency; PBMC, peripheral blood mononuclear cells

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Send correspondence to: Sophia N. Karagiannis, NIHR Biomedical Research Centre at Guy's and St. Thomas's Hospitals and King's College London, St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Faculty of Life Sciences and Medicine, Kings' College London, Guy's Hospital, Tower Wing 9th Floor, London, SE1 9RT, UK, Tel: 440207188 6355, Fax: 440207188 8050, E-mail: sophia.karagiannis@kcl.ac.uk

Abbreviations: TAMs, tumour-associated macrophages; mAb, monoclonal antibody; TME, tumour microenvironment; Th, T helper; TLR, Toll-like receptor; LPS, lipopolysaccharide;