Tumor-Associated Macrophages

Subjects: Oncology

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Resident macrophage populations within tumors are termed tumor-associated macrophages (TAMs) and can comprise up to half of the tumor mass. In established solid malignancies, the anti-tumor functions of TAMs such as phagocytosis and cytotoxic activity are suppressed, and TAMs are subverted to facilitate tumor growth.

Keywords: tumor-associated macrophages ; resistance ; antibody-dependent cellular phagocytosis ; antibody immunotherapy ; phagocytosis checkpoints

1. Tumor-Associated Macrophages

Macrophages are highly plastic cells that respond and adapt to the TME in which they are resident [1][2]. Macrophage functions range from organogenesis, the capture and elimination of pathogens, tissue homeostasis, wound healing and tumorigenesis [3][4]. In solid malignancies, TAM populations can impact tumors through a multitude of complex and often opposing mechanisms, including those impacting; cell death, immunoregulation, and angiogenesis, with the net result being either pro- or antitumor. However, recently a consensus has emerged whereby most TAMs in large tumors are thought to contribute to tumor progression by increasing cancer cell invasiveness, angiogenesis and immunosuppression [5].

TAMs originate from both bone-marrow-derived hematopoietic and non-hematopoietic lineages [6][7]. In early tumorigenesis, tissue resident macrophages accumulate within tumors and account for the majority of TAMs [8]. In the brain, tissue resident macrophages (known as microglia) arise from the yolk sac, and are distinct from hematopoietic precursors in the yolk sac or fetal liver, and proliferate within tissues throughout adulthood [9]. Furthermore, it has been reported that in murine gliomas, typically only 25% of TAMs originate from circulating monocytes, with the majority derived from tissue resident microglia [10][11]. In the liver, macrophages called Kupffer cells arise from both the yolk sac and embryonic hematopoietic stem cells [12]. In adulthood, the tissue microenvironment determines to what extent circulating blood monocytes replace these tissue resident macrophages [8]. As tumors increase in size and intratumoral vascular networks form, monocytes become the dominant source of TAMs [3][13][14]. The recruitment of TAMs to tumor sites is mediated by previously resident TAMs, cancer cells, and fibroblasts, secreting a range of chemokines including: chemokines (C-C motif) ligand (CCL)2, CCL5, CCL7, and chemokine (C-X3-C motif) ligand 1 (CX3CL1), as well as cytokines such as macrophage colony-stimulating factor (M-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and vascular endothelial growth factor (VEGF) [15][16][17][18]. Furthermore, classical monocytes ($CD14^{hi}FcγRIIIa^{lo}$ in humans and $CD11b^{hi}Ly6C^{+}$ in mice) are recruited as a tumor progresses and differentiate into TAMs, often in a CCL2-CCR2-dependent manner. Indeed, inhibition of CCR2 signaling blocks TAM recruitment and thus reduces TAM frequency, improving the survival of tumor-burdened mice in certain murine tumor models [3].

TAMs acquire immunosuppressive or immunostimulatory gene expression patterns in response to the dynamic and varied TME in large tumors. The expression patterns of these genes can be loosely categorized as pro- or antitumor in the context of disease prognosis and their potential impact on anti-cancer therapies (**Figure 1**). TAMs typically express myeloid surface markers such as CD68, CD163 (class A macrophage scavenger receptor), CD206 (mannose receptor, C type 1), macrophage galactose-type lectin (MGL), macrophage receptor with collagenous structure (MARCO), programmed cell death ligand 1 (PD-L1) and V-domain Ig suppressor of T cell activation (VISTA) [19][20][21][22]. In particular, CD68, CD163 and CD206 are extensively used to identify and quantify TAMs, in addition to their being used as prognostic markers for several tumor types [23].

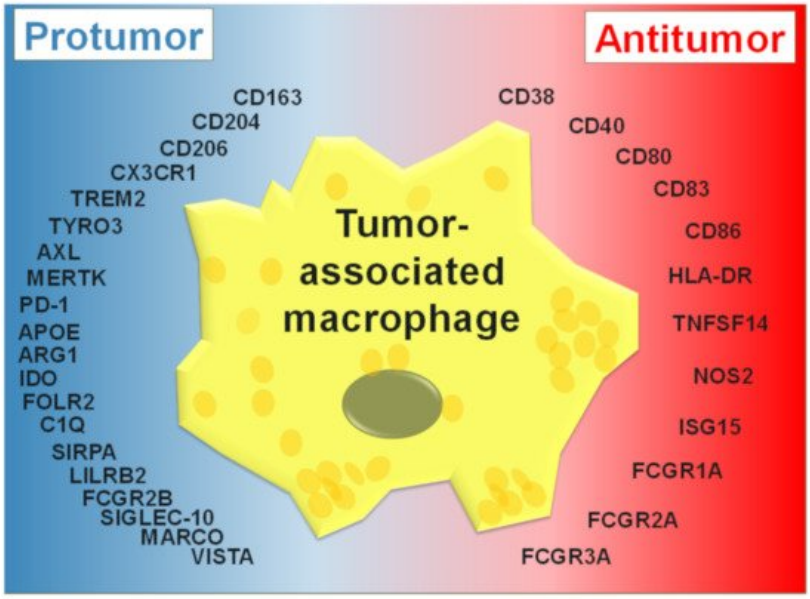

Figure 1. TAM-associated markers. Expression of genes in TAMs that phenotypically and functionally associate with protumor (blue) and antitumor (red) outcomes in the context of tumor progression and/or efficacy of direct targeting mAb therapy (adapted from [24]).

2. TAM Activation States

The intrinsic heterogeneity of macrophages was historically stratified into two broad activation states: M1 (for proinflammatory or classically activated macrophages) and M2 (for anti-inflammatory or alternatively activated macrophages) [25][26][27]. Although it is often reported that TAMs more closely resemble M2 macrophages, the M1/M2 dichotomy is now thought to be too reductionist and these states are likely to be examples within a spectrum of activation states [28][29]. M1 macrophages are generated following stimulation with the interferon-γ (IFN-γ) alone or in concert with bacterial components, e.g., lipopolysaccharide (LPS) or pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) [30]. Phenotypic regulation of M1-like macrophages is regulated via multiple transcription factors, such as IRF-1, STAT-1 and NF-κB [31][32][33]. These induce a pro-inflammatory phenotype in M1 macrophages, which is additionally regulated by the transcription factors: IRF-4, STAT6, PPAR-γ, the protein degradation adaptor protein, Tribbles homolog 1 (TRIB1), and chromatin modifiers including, histone demethylases and Jumonji domain-containing protein D3 (JMJD3) [32][34]. In contrast, M2 macrophages are polarized by several factors, and can be further subdivided into M2a, M2b, and M2c [30]. M2a macrophages are generated following exposure to IL-4 and/or IL-13. M2b macrophages are induced by immune complexes (ICs), LPS, certain Toll-like receptor (TLRs) agonists, or the IL-1 receptor antagonist (IL-1ra) [35][36][37]. M2c macrophages can be induced in response to exposure to IL-10, transforming growth factor-β (TGF-β), or glucocorticoids (GCs) [38][39]. TAMs with enhanced expression of CD163, CD204, CD206, stabilin-1, arginase-1, and matrix metallopeptidase 9 (MMP9), and elevated production of IL-10, VEGF, and prostaglandin E_2 (PGE_2), generally show M2-like characteristics [40][30][2][41]. In addition to implications for tumor neogenesis as well as “wound healing”, the status of ‘M2-like’ macrophages has ramifications for multiple treatment modalities.

3. Protumor Functions of TAMs

TAMs possess an ‘M2-like’ phenotype and function that promotes immunosuppression, metastases, and angiogenesis (**Figure 2**). Tissue resident macrophages and TAMs can phagocytose, and lyse cancer cells, activate NK cells and induce T helper 1 (Th1) immune responses [42][43][44]. However, TAMs are broadly associated with poor prognosis in several tumor types, including cholangiocarcinoma, glioma, Hodgkin lymphoma and ovarian and breast cancers [41]. Increased frequencies of $CD163^+$, $CD204^+$ and $CD206^+$ TAMs correlate with tumor progression and worse clinical prognosis [31]. Furthermore, in some malignant tumors, the density and quantity of TAM infiltration is associated with higher Ki-67 expression, indicating elevated cancer cell proliferation [45].

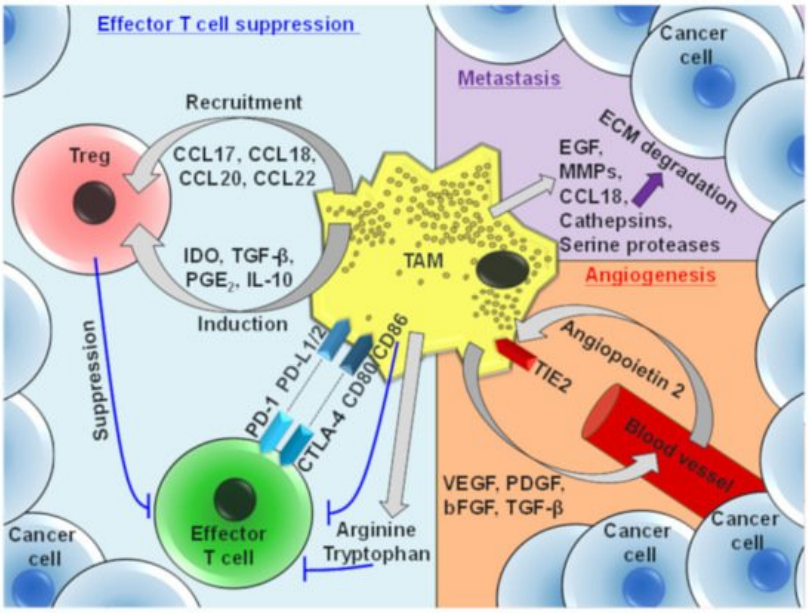

Figure 2. Major protumor functions of tumor-associated macrophages. TAMs mediate suppression of effector T cells via the secretion of soluble proteins and through the expression of inhibitory cell surface molecules. TAMs also produce several factors that promote extracellular matrix (ECM) degradation, which facilitates tumor metastasis. Furthermore, TAMs secrete cytokines that promote angiogenesis, consequently accelerating tumor growth.

TAMs also produce high levels of cytokines and chemokines, which recruit or induce immunosuppressive cell types at tumor sites. Thymically derived natural T_{reg} traffic and infiltrate to tumor sites via several chemokine receptors, in particular CCR4 [46].

In addition to inducing Treg cells and MDSCs at the tumor site, TAMs actively participate in the immunosuppression of effector T cells. TAM-derived arginine and tryptophan suppresses CD3 ζ-chain expression in T cells, resulting in the inhibition of effector T cell activation [47][48].

TAMs express several enzymes, cytokines and chemokines that promote tumor metastases, such that TAM frequencies positively correlate with cancer cell invasiveness and metastasis [49].

TAMs are also important promoters of angiogenesis in the TME. They function to degrade the tumor basement membrane, via the production of MMPs and cathepsins, and secrete proangiogenic growth factors such as VEGF, PDGF, bFGF and TGF-β that induce new vasculature in growing tumors [50][51].

In summary, TAMs promote tumor growth through multiple mechanisms that are attributed to ‘M2-like’ phenotypes induced within the TME, highlighting a need to develop strategies that either delete these cells or repolarize them to proinflammatory antitumor states. In the context of direct targeting mAb immunotherapies, TAMs can function to phagocytose mAb-opsonized cells, and novel strategies to target the so-called ‘phagocytosis checkpoints’ to enhance the phagocytic functions of these cells are also currently under investigation.

4. TAM-Mediated Depletion of Cancer Cells

Tumor-targeting mAbs such as Rituximab, Herceptin and Cetuximab, recruit ADCP-mediating macrophages to directly eliminate cancer cells [52][53][54][55][56][57]. Checkpoint inhibitor mAbs such as Ipilimumab were previously thought to function solely via receptor blockade and expansion of effector T cells [58]. However, additionally, Ipilimumab has been reported to work optimally through the depletion of tumor-infiltrating immunosuppressive Treg cells, also indicating a role for ADCP-mediating myeloid cells [59][60][61]. Although several cell types are functionally capable of phagocytosing and destroying host cells, including epithelial cells, mesenchymal cells and fibroblasts, neutrophils, and monocytes [62][63][64], macrophages are ‘professional phagocytes’ and the principal effector cells in efferocytosis (clearance of apoptotic cells) and ADCP [65].

IgG antibodies can trigger ADCP indirectly via activation of the classical complement pathway, where iC3b-opsonized target cells can bind to complement receptor 3 (CR3, integrin $\alpha_M\beta_2$) to elicit engulfment by ‘sinking phagocytosis’ [66]. Importantly, the macrophage cell surface receptors required for ADCP are less varied than for efferocytosis, with ADCP in the context of mAbs like Rituximab almost entirely dependent on FcγRs that bind the Fc portion of IgG antibodies. Human

macrophages express the activating high affinity FcγRI and low affinity FcγRIIa and FcγRIIIa [67][68], as well as the inhibitory FcγRIIb. Antibody-bound target cells interact with FcγRI, FcγRIIa and FcγRIIIa for optimal ADCP (FcγRI, FcγRIII and FcγRIV in the mouse), whereas engagement with the sole inhibitory FcγR, FcγRIIb (FcγRII in mice), attenuates phagocytic function [67]. The expression levels and cellular distribution of FcγR on effector cells are of crucial importance in mAb therapy outcomes. Furthermore, human IgG1 and murine IgG2a, and IgG2c isotypes preferentially engage, activating above inhibitory FcγR, eliciting stronger ADCP (relative to human IgG2 or murine IgG1), and therefore are the preferred IgG isotypes for direct tumor-targeting mAbs [69][70][71].

After engagement, activating FcγRs cluster and phosphorylate ITAM in their cytoplasmic domains or associated gamma chains [67]. This induces the formation of the phagocytic synapse and thence, actin polymerization leads to the formation of the phagocytic cup [72]. The macrophage then extends pseudopodia around the opsonized target cell, engulfing it in a process termed zippering phagocytosis [73]. Actin filaments subsequently rearrange within the macrophage, causing its cell membrane to encompass the target cell, which leads to its inclusion into a phagosome. The phagosome fuses with endosomes and then lysosomes [74], followed by a marked reduction in pH (~4.5) and generation of ROS [75], leading to the destruction of the phagocytosed cell [76]. The inhibitory FcγRIIb possesses an ITIM in its cytoplasmic domain, and the interaction of IgG Fc regions or immune complexes results in the recruitment of src homology 2 (SH2) domain containing inositol polyphosphate 5-phosphatase (SHIP), curtailing signaling from activating FcγR and consequently ADCP [77].

A seminal study by Clynes et al. [78] observed that nude mice deficient in the common gamma chain ($FcR\gamma^{-/-}$/nu/nu mice), which consequently lack expression and signaling from the activating FcγRs, were unable to control human breast carcinoma BT474M1 growth in response to trastuzumab treatment. This implicated a role for activating-FcγR-bearing myeloid cells and NK cells in therapeutic outcomes. Importantly, mice deficient in the inhibitory FcγRIIb showed potent antibody-mediated target cell killing. The latter result not only demonstrated that FcγR-dependent mechanisms contribute substantially to the action of direct targeting mAbs, but implicated macrophages as key effectors cells in direct targeting mAb immunotherapy, given that NK cells do not express FcγRIIb in mice or humans [78].

Subsequent studies using intravital microscopy have reported that following anti-CD20 mAb therapy in murine models, Kupffer cells in the liver sinusoids, phagocytose circulating mAb-opsonized malignant B lymphoma cells [75][79][80], including in human CD20 transgenic mice [80]. Anti-CD20 mediated depletion of lymphoma cells in adoptive transfer models or the Eμ-Myc B cell lymphoma model has been shown to be dependent on activating FcγRs. Furthermore, the clodronate-mediated elimination of macrophages abrogated anti-CD20 therapy in this mouse model, further highlighting the indispensable role of macrophages in malignant B cell depletion [81].

5. Antibody-Mediated Modulation of TAM Recruitment, Survival, and Effector Functions

Strategies to diminish the protumor functions of TAMs include the suppression of TAM generation, monocyte recruitment, and the repolarization of TAMs to proinflammatory phenotypes. Additionally, a compelling TAM targeting strategy has emerged that aims to target ‘phagocytosis checkpoints’ to enhance ‘eat me’ and block ‘don’t eat me’ signaling in tumors (**Figure 3**). **Table 1** summarizes TAM-targeting mAbs in early phase trials that have been developed to reduce protumor TAM frequencies or augment antitumor immune responses in cancer patients.

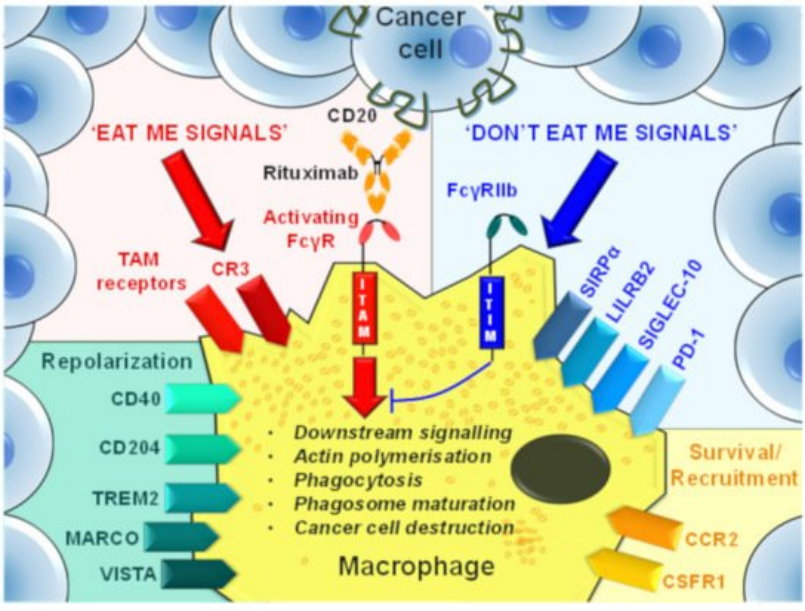

Figure 3. TAM cell surface molecule candidates for mAb targeting. TAM cell surface molecules that can potentially be targeted by mAbs to modify TAM frequencies or repolarization to a proinflammatory phenotype in the TME. These mAb targets are grouped according to the predominant effect resulting from stimulation of their natural ligand. However, mAb-mediated targeting of these molecules may exert further functional changes in TAMs and healthy mononuclear phagocytes.

Table 1. TAM-targeting mAbs in completed or active trials. These mAbs have been investigated in or are in active clinical trials, either as single agents, or in combination with chemotherapeutic agents, checkpoint inhibitors, Fc fusion proteins or TLR agonists.

Target	Compound	Sponsor	Phase	Indication	Status	ClinicalTrials.gov identifier
CCR2	Plozalizumab	Southwest Oncology Group	II	Metastatic cancer, unspecified adult solid tumor	Completed	NCT01015560
CCL2	Carlumab	Centocor Research & Development, Inc.	II	Prostate cancer	Completed	NCT00992186
CSF-1R	AMG820	Amgen	I	Solid tumors	Completed	NCT0144404
	Emactuzumab (RG7155)	Roche	I	Solid tumors	Completed	NCT01494688
	IMC-SC4	Eli Lilly	I	Breast and prostate cancer	Active	NCT02265536
CD40	SEA-CD40	Seagen Inc.	I	Non-small-cell lung carcinoma, squamous solid tumors	Active	NCT02376699
	LVGN7409	Lyvgen Biopharma Holdings Limited	I	Solid tumors	Active	NCT04635995
	CDX-1140	Celldex Therapeutics	I/II	Melanoma	Active	NCT04364230
	APX005M	Apexigen, Inc.	II	Soft tissue sarcoma	Active	NCT03719430
	ADC-1013	Janssen Research & Development, LLC	I	Advanced solid neoplasms	Active	NCT02829099
	ChiLob 7/4	Cancer Research UK	I	B-cell lymphoma	Completed	NCT01561911
	Selicrelumab	Hoffmann-La Roche	I/II	Pancreatic adenocarcinoma	Active	NCT03193190
FcγRIIb	BI-1206	BioInvent International AB	I/II	Indolent B-cell non-Hodgkin lymphoma	Active	NCT03571568
SIRPα	BI 765063	OSE Immunotherapeutics	I	Solid tumor	Active	NCT03990233
	CC-95251	Celgene	I	Neoplasms	Active	NCT03783403
	GS-0189	Gilead Sciences	I	Non-Hodgkin lymphoma	Active	NCT04502706
VISTA	CI-8993	Curis, Inc.	I	Solid tumor	Active	NCT04475523

6. TAM Recruitment and Survival

6.1. CSF-1R

CSF-1R is a tyrosine kinase receptor expressed on all myeloid cells. Its ligands are M-CSF (CSF-1), GM-CSF (CSF-2) and IL-34, and their binding to CSF-1R induces differentiation, recruitment to tumor sites, and the survival of monocytes and macrophages [82]. The ‘M2-like’ TAM phenotype has been reported to be mediated by the growth factor M-CSF in addition to the Th2 cytokines: IL-4/IL-13, and Treg-cell-derived IL-10, in the TME [30]. Since the presence of CSF-1R$^+$ TAMs correlates with poor survival in several tumor types [83], targeting CSF-1R represents an attractive strategy to eliminate or potentially repolarize these cells. Mononuclear phagocytes are almost completely absent in *CSFR1*$^{-/-}$ mice [84]. Accordingly, mAbs targeting either CSF-1R or its ligand M-CSF have been developed. The antitumor and

antimetastatic activities of anti-CSF-1R mAb have been demonstrated in subcutaneous EL4 lymphoma and MMTV-PyMT breast tumor models [85].

6.2. CCR2/CCL2

CCL2 is a key chemokine which mediates macrophage recruitment to tumor sites. The anti-CCR2 mAb MLN1202 has been successfully used in patients at risk for atherosclerotic cardiovascular disease to reduce markers of inflammation [86]. Targeting CCR2 or its ligand, CCL2, with mAbs to block TAM recruitment has also been investigated in mice with orthotopic MDA-MB-231 human breast cancer tumors. Here, treatment with anti-CCL2 mAb reduced TAM accumulation, consequently reducing angiogenesis and tumor growth [87]. Furthermore, Carlumab, a human IgG1 anti-CCL2 mAb, has been investigated in clinical trials for patients with various solid tumors. However, this strategy was not sufficiently efficacious, even when combined with chemotherapy [88]. Likely explanations include the broad redundancy in the chemokine system, which contains dozens of different ligands and receptors. Indeed, tissue resident macrophages in particular, which differentiate into the most protumor fraction of the myeloid compartment, may be independent of regulation by any single chemokine receptor or ligand [89].

7. Conclusions

Although TAMs are indispensable effector cells in direct targeting mAb immunotherapy, the immunosuppressive TME markedly reduces their ability to elicit ADCP and deplete mAb-opsonized targets. Currently, several early phase clinical trials are investigating different TAM-targeting mAbs, and in particular, anti-CD40 agonistic mAbs hold great potential to repolarize TAM activation states in the TME. Furthermore, mAb-mediated FcγRIIb blockade is also a promising candidate for the enhancement of ADCP in the context of anti-CD20 mAb therapies. Recently, high-dimensional single-cell RNA sequencing has shed new light on the variety of myeloid cells in the TME. Furthermore, this has also revealed novel TAM-associated cell surface markers and signaling pathways, with the potential for targeted intervention to reshape the tumor myeloid cell landscape to in turn enhance clinical outcomes.

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