Review

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Tumor-associated macrophages, dendritic cells, and neutrophils: biological roles, crosstalk, and therapeutic relevance

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Abstract: Tumor-associated myeloid cells constitute a series of plastic and heterogeneous cell populations within the tumor microenvironment (TME), and exhibit different phenotypes and functions in response to various microenvironmental signals. In light of promising preclinical data indicating that myeloid-based therapy can effectively suppress tumor growth, a series of novel immune-based therapies and approaches are currently undergoing clinical evaluation. A better understanding of the diversity and functional roles of different myeloid cell subtypes and of how they are associated with TME remodeling may help to improve cancer therapy. Herein, we focus on myeloid cells and discuss how tumor cells can simultaneously reprogram these cells through tumor-derived factors and metabolites. In addition, we discuss the interactions between myeloid cells and other cells in the TME that have the potential to directly or indirectly regulate tumor initiation, invasion, or angiogenesis. We further discuss the current and future potential applications of myeloid cells in the development of focused therapeutic strategies in cancer treatment.

Keywords: cell-interaction; dendritic cells; macrophages; neutrophils; therapy; tumor microenvironment.

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Introduction

Efforts to uncover the link between tumor cell genetic mutations and tumor progression have been ongoing for several decades. Recently, researchers have discovered that in addition to gene mutations, tumor progression is also regulated by tumor-associated immune and non-immune cells [1]. The tumor immune microenvironment (TIME) includes immunosuppressive cells, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T (Treg) cells, as well as anti-tumor effector cells, such as cytotoxic $CD8^+$ T cells, $CD4^+$ T helper (Th), and natural killer (NK) cell. The tumor microenvironment (TME) also contains a variety of non-immune cell populations such as endothelial cells and fibroblasts [2], with all cells in this environment exhibiting substantial diversity and plasticity.

The discovery of the cell-cell interaction in the TME has led to a revolution in cancer treatment, from the development of chemotherapy and radiation strategies that target tumor cells to the design of antibody-based immunotherapies that modulate immune responses [3]. As central mediators of adaptive immunity, T cells represent effective targets for immunotherapy. Immune checkpoint blockade (ICB) therapies targeting programmed death 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) on the surface of T cells have greatly prolonged cancer patient survival [4]. However, the number of patients who respond to these types of therapies is still limited, leading to efforts to develop new types of inhibitors. Several subtypes of myeloid cells, including macrophages, dendritic cells (DCs), and MDSCs can promote tumor development by producing tumor-promoting factors and molecules that inhibit the cytotoxic activity of T cells. Specific myeloid cell subsets also express PD-1, and some myeloid-specific immune checkpoint molecules have additionally been discovered [5]. Therein, the checkpoints on macrophage, for example, signal regulatory protein alpha (SIRPα) and colony stimulating factor 1 receptor (CSF1R), have undergone decades of research. The results show the checkpoints contribute

to the immunosuppressive function of TAMs and their therapeutic antibodies are currently being tested in clinical trials [6]. Moreover, based on the DCs antigen presentation function, DCs activating and motivating factors can be administered, leading to anti-tumor function [7]. These suggest that myeloid cells may represent potential therapeutic targets. In additional to the most studied macrophages and DCs, the importance of neutrophils in cancer has been increasing apparent recently and some neutrophils-specific therapeutic methods occur. Thus, we mainly focus on tumor-associated macrophages, DCs, and neutrophils in this review. All three cell types undergo the development from haematopoietic stem cells to tumor-infiltrating myeloid cells, which is regulated by tumors [1]. They exhibit a diverse array of phenotypes that can promote or suppress tumor growth and function, and they play important regulatory roles through their crosstalk with tumor cells, T cells, and other cell populations. A comprehensive understanding of the relationships among tumor-infiltrating myeloid cells and other populations in the TME is critical in order to provide important insights into the mechanisms underlying immune surveillance and tumor immunotherapy.

In this review, we focus on the roles of macrophages, DCs, and neutrophils, and we discuss the mechanisms whereby tumor cells regulate myeloid cells and promote their metabolic reprogramming. We then discuss the interactions between myeloid cells and other cells in the TME to highlight their crucial roles in tumor progression. Finally, we explore current therapeutic strategies targeting myeloid cells that may be beneficial in overcoming an immunosuppressive TME to promote T cell-mediated tumor clearance.

Tumor-associated macrophages

Macrophages represent a versatile subset of myeloid immune cells that execute a broad array of functions, including the regulation of tissue homeostasis, defenses against pathogens, and the promotion of wound healing [8]. Macrophages that infiltrate tumors or populate the TME of solid tumors are defined as TAMs, and can affect tumor growth, tumor angiogenesis, immune regulation, metastasis, and chemoresistance.

Phenotypic properties and functional roles of TAMs

Circulating monocytes derived from bone marrow hematopoietic stem cells (HSCs) are generally believed to be the primary source of macrophages. However, recent evidence suggests that macrophages are maintained in most healthy tissues by embryonic precursors independently of monocytes [9, 10]. The tissue-resident macrophages (TRMs) of embryonic origin and circulating monocytes are two main sources of TAMs during tumor progression. In pancreatic ductal adenocarcinoma mouse model, TAMs of different origins show various impacts on fibrosis, herein, embryonically derived TAMs have a fibrotic phenotype with higher expression of molecules involved in extracellular matrix deposition and remodeling [11]. Although the origins of macrophages have been mapped in multiple animal models, our ability to interrogate these populations in human tissues remains limited. As these technologies continue to develop, RNA velocity analyses embedded on a diffusion map can be used to infer the future fate of human cell populations. For example, in colorectal cancer (CRC) patients, a strong directional flow from $CD14^+$ monocytes towards macrophage populations has been reported based on RNA velocity analysis. Monocytes give rise to TAMs through different tissue-resident macrophages [12]. RNA velocity analyses can be expanded to other cancers, and have the potential to permit future analyses of macrophage origins [13].

Activated macrophages are often classified into the M1 (classically activated) and M2 (alternatively activated) subtypes [14]. In general, M1 macrophages promote inflammatory responses against invading pathogens and tumor cells, whereas M2 macrophages tend to exhibit an immunosuppressive phenotype, favoring tissue repair and tumor progression. The conversion between M1 and M2 macrophage subtypes is a dynamic process known as macrophage polarization that occurs in response to microenvironmental signals. Based on their functions within the tumor microenvironment, TAMs are most often characterized as M2-like macrophages, given that they express higher levels of anti-inflammatory cytokines, scavenging receptors, angiogenic factors, and proteases than do M1-type macrophages [15]. However, it is worth noting that M1 and M2 types are just relative terms and macrophages actually consist of a continuum of phenotypes. For example, although TAMs are conventionally acknowledged as M2-like macrophages, to be exact, they exhibit phenotypes anywhere in between M1 and M2.

In a recent systemic analysis of myeloid cells across 15 human cancer types, macrophages subsets in different tumor types showed heterogeneous transcriptomic patterns [16]. TAM subsets were identified with various marker genes, including *SPP1*, *C1QC*, *ISG15*, and *FN1*, and different TAM subsets presented different functional phenotypes. For example, $SPP1^+$ TAMs in CRC patients had highly correlation with angiogenesis, leading to poor prognosis and resistance

to CSF1R treatment [12]. With more and more TAMs subsets being discovered, some of them dash the stereotype as playing pro-tumor roles. The complement activation and antigen processing and presentation pathways significantly increase in *C1QC*$^+$ TAMs [12]. Meanwhile, *Cxcl9*$^+$ TAMs in CT26 colorectal carcinoma facilitate the recruitment of protective *Cxcr3*$^+$ T cells and predict the response to anti-PD-L1 treatment [17]. Since the functions of these subgroups are mainly based on bioinformatics analysis and the limitations of experimental support, we still focus on the function of TAMs to stimulate tumor progression in this review.

Tumor cell-mediated regulation of TAMs polarization and function

The regulatory roles of tumor-derived factors

Several factors produced by tumor cells can influence macrophage polarization (Figure 1A and Table 1). Tumor-derived colony stimulating factor 1 (CSF1), a primary chemoattractant and functional regulator for macrophages, functions in synergy with interleukin (IL)-4 to drive the polarization of M2 macrophages [18]. Microparticles are specialized subcellular vesicles from 100 to 1,000 nm in diameter, and macrophage uptake of tumor-derived microparticles can drive their M2 polarization and the apoptotic death of M1 macrophages, promoting tumor growth and metastasis [19]. Furthermore, hypoxia shapes and induces specific macrophage phenotypes that support tumor malignancy, given that hypoxia promotes immune evasion, angiogenesis, tumor cell survival, and metastatic dissemination. In the hypoxic TME, the cytosolic accumulation of hypoxia-inducible factors 1α (HIF-1α) induces secretion of high mobility group box-1 (HMGB1) by melanoma tumor cells and promotes M2-like macrophage accumulation [20]. Hypoxia-primed cancer cells can also attract and polarize macrophages towards a pro-angiogenic M2-polarized subtype via the release of eotaxin and oncostatin M (OSM) [21]. Hepatocellular carcinoma (HCC) progression can be accelerated via the upregulation of HIF-1α-mediated IL-10, promoting M2 macrophage polarization [22]. Hypoxia can further induce the production of key monocyte recruitment factors including C-C motif chemokine ligand (CCL) 2, CCL5, CXC-chemokine ligand 12 (CXCL12), CSF1, and vascular endothelial growth factor (VEGF) by tumor cells and the stroma [23]. Once recruited into hypoxic regions, monocytes downregulate the receptors for several of these factors, effectively trapping differentiated TAMs within hypoxic microenvironments [24].

Metabolic regulation

One of the TCA cycle metabolites, α-ketoglutarate (α-KG), induces macrophage polarization towards an M2 phenotype (Figure 1A). Inhibiting glutaminase, which provides glutamate as a source for α-KG production, decreases M2 polarization while increasing M1 polarization. A recent study found that α-KG supports M2 activation by promoting the Jmjd3-dependent demethylation of histone 3 at lysine 27 (H3K27) on the promoter regions associated with M2-specific marker genes [25]. While α-KG serves as a co-stimulator for Jmjd3, succinate serves as an inhibitor thereof such that the α-KG/succinate ratio determines macrophage polarization status. While an increased α-KG/succinate ratio favors M2 polarization, a decreased α-KG/succinate ratio induces cells to undergo differentiation towards the proinflammatory M1 macrophage phenotype [25].

Adenosine is a nucleoside that can be released from tumor cells in the TME. Adenosine regulates the phagocytic activity of mononuclear cells, including macrophages, via the adenosine receptors (ADORA)1, 2A, 2B, and 3, which are G-protein-coupled transmembrane receptors. The dominant ADORA inducing M2 macrophage polarization is ADORA 2A, and based on the recent results, ADORA 2B also show this function [26]. Deletion of the ADORA 2A on macrophages favors M1 polarization and substantially reduces anti-inflammatory IL-10 production [27]. Thus, adenosine in the TME leads to immunosuppression.

The signaling functions and polarization of M2 macrophages can also be regulated by lactate in the TME. Lactate is an end-product of aerobic glycolysis, which is highly active in tumor cells. Studies demonstrate that tumor cell-derived lactate induces the expression of VEGF and the M2 polarization of TAMs via HIF1α [28]. Sensing of lactate by macrophages in the context of M2 polarization is mediated by G-protein-coupled receptor 132 (Gpr132), and a loss of Gpr132 in mice inhibits breast cancer metastasis [29]. Additionally, decreased Gpr132 expression in patients with breast cancer is correlated with improved metastasis-free survival.

TAMs promote tumor progression

As mentioned above, TAMs are influenced by tumor cells in many ways. In turn, TAMs influence a number of critical biological functions that are linked with tumor progression. Here, we primarily focus on the correlation between TAMs and tumor invasion/angiogenesis (Figure 1B). Other aspects have been reviewed in detail previously [30, 31].

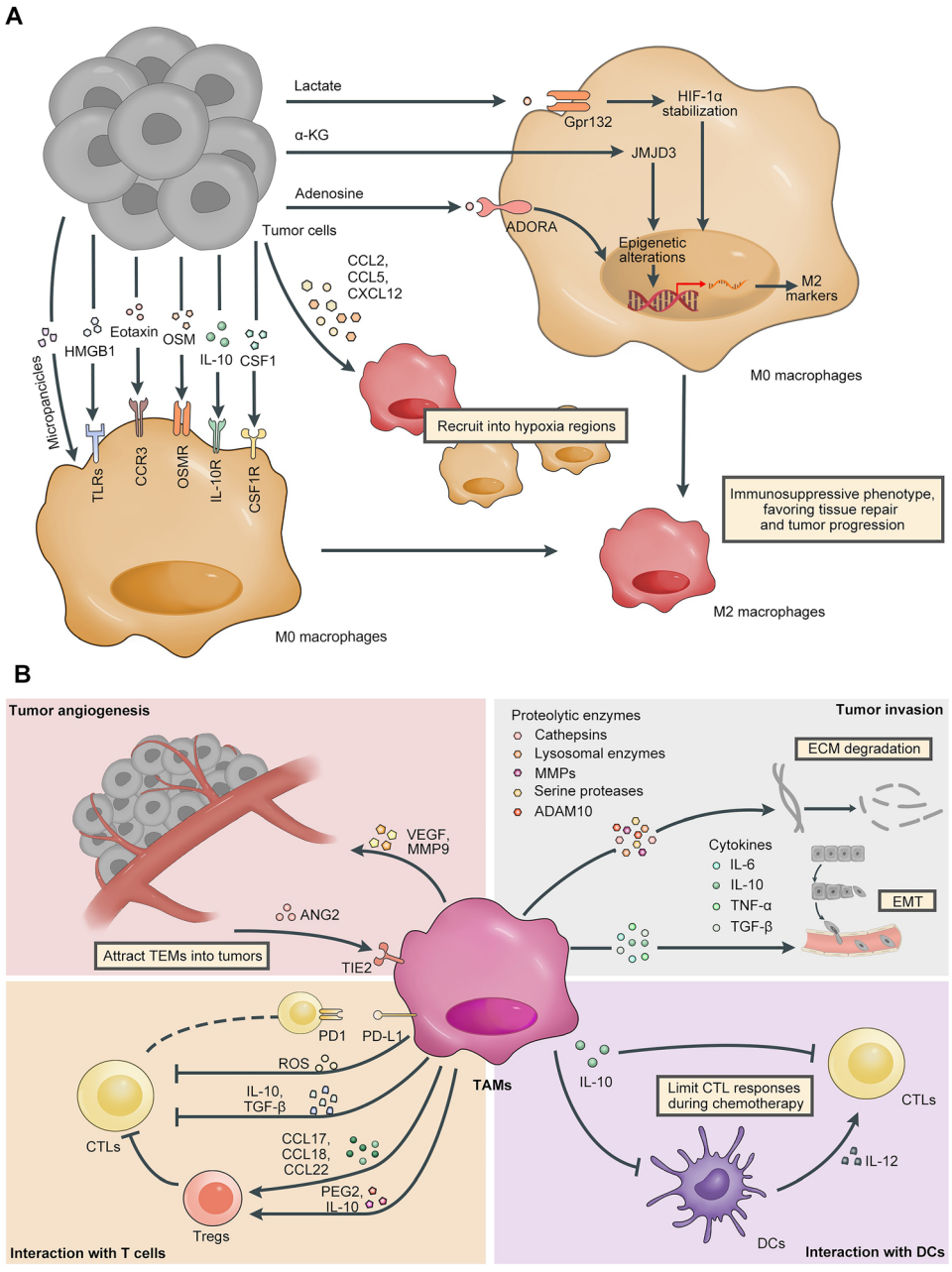

Figure 1: The roles of TAMs in tumor immunity.
(A) The polarization of M2 by tumor cells-derived factors and metabolites. To regulate TAM M2 polarization, tumor cells can secrete factors (CSF1, microparticles, HMGB1, Eotaxin, OSM and IL-10), interacting with corresponding receptors on macrophages. Tumor cells-derived chemokines (CCL2, CCL5, CXCL12) can recruit macrophages and trap them in hypoxic tumor region. Metabolites, including lactate, α-KG and adenosine, control polarization of M2 macrophages in different ways. (B) TAMs interact with other cells in TME. TAMs play particular functional roles in tumor progression, including tumor invasion (grey) and tumor angiogenesis (red). TAMs release inflammatory factors (IL-6, IL-10, TNF-α and TGF-β) to promote EMT and proteolytic enzymes (cathepsins, lysosomal enzymes, MMPs, serine proteases and ADAM10) to remodel ECM. For tumor angiogenesis, TAMs release a panel of pro-angiogenic factors (VEGF, MMP9, etc.) and endothelial cell-produced ANG2 can attract TEMs into tumors. TAMs also regulate T cells (orange) and DCs (purple) to induce immune suppression. CSF1, colony stimulating factor 1; HMGB1, high mobility group box-1; TLRs, toll-like receptors; OSM, oncostatin M; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; Gpr132, G-protein-coupled receptor 132; α-KG, α-ketoglutarate; ADORA, adenosine receptors; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; MMPs, matrix metalloproteinases; ADAM10, disintegrin and metalloproteinase domain-containing protein 10; ANG-2, angiopoietin-2; Tie2, tyrosine kinase receptor 2; VEGF, vascular endothelial growth factor; TEMs, Tie2-expressing monocytes; ROS, reactive oxygen species; PGE2, prostaglandin E2; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1.

Table 1: Tumor-derived molecules related to polarization process of macrophages, DCs and neutrophils.

Class		Molecules	Description	Ref.
Macrophages	Factors	CSF1	M2 macrophage polarization can be induced in CSF1 and/or IL-4-dependent manner	[18]
		Microparticles	Microparticles uptake leads to M2 polarization and the apoptotic death of M1 macrophages, promoting tumor growth and metastasis	[19]
		HMGB1	Hypoxia induces the secretion of HMGB1 and promotes M2 macrophages accumulation	[20]
		Eotaxin, OSM	Eotaxin and OSM are released by hyopxia cancer cells and induce M2 polarization	[21]
		IL-10	HIF-1α-mediated IL-10 can promote M2 polarization	[22]
		CCL2, CCL5, CXCL12	CCL2, CCL5 and CXCL12 recruit macrophages to hypoxic tumor regions and trap them in TME	[23, 24]
	Metabolites	α-KG	– α-KG induces M2 polarization by promoting the Jmjd3-dependent demethylation of H3K27 – α-KG/succinate ratio determines macrophage polarization status, and higher ratio presents the M2 polarization tendency	[25]
		Adenosine	Adenosine binds to ADORA, therein, ADORA 2A shows dominant M2 polarization function	[26, 27]
		Lactate	– Macrophages can sense lactate through Gpr132 and undergo M2 polarization – Downregulation of Gpr132 can improve breast cancer metastasis-free survival	[28, 29]
DCs	Factors	VEGF	– VEGF inhibits DC differentiation and maturation – VEGF recruits immature DCs to primary tumor site	[80] [81]
		IL-10	IL-10 inhibits DC maturation, activation, and the T cell stimulatory abilities of DCs	[82, 83]
		IL-6, TGF-β, CSF1	– IL-6, TGF-β and CSF1 work as negative modulators of DC maturation and activation – CSF1 induces the DCs towards suppressive TADCs via Jak2/STAT3 signaling	[78] [84]
		PSA	PSA co-cultural DCs exhibit the impaired mutation phenotypes	[85]
		MUC1	MUC1 is related with DC immaturation and anti-tumor function decrease	[86, 87]
	Metabolites	Lactic acid	Lactic acid can suppress DC activation	[89]
		IDO	IDO drives DCs toward an immunosuppressive phenotype	[90]
		Extracellular lipids	– Extracellular lipids can be imported and accumulate within DCs – High lipid content affects the DCs antigen presentation function	[91] [92]
		Wnt5	Wnt5a-induced FAO stimulates IDO, thus creating an immunosuppressive environment	[93]
		PGE2	PGE2 contributes to DC dysfunction	[96]
		COX	COX impairs the accumulation of cDC1s within tumors and suppresses their activation	[97]
Neutrophils	Factors	G-CSF	G-CSF plays an important role in inducing immunosuppressive neutrophils	[133]
		TGF-β	TGF-β leads to the presentation of a pro-tumor TAN population	[129]

CSF1, colony stimulating factor 1; HMGB1, high mobility group box-1; OSM, oncostatin M; CCL, C-C chemokine ligand; CXCL, CXC chemokine ligand; HIF-1α, hypoxia-inducible factors 1α; α-KG, α-ketoglutarate; ADORA, adenosine receptors; Gpr132, G-protein-coupled receptor 132; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; PSA, prostate-specific antigen; MUC1, mucin 1; IDO, indoleamine 2,3-dioxgenase-1; PGE2, prostaglandin E2; COX, cyclooxygenase; FAO, fatty acid oxidation; G-CSF, granulocyte colony-stimulating factor.

TAMs promote tumor cell invasion

Metastasis initiates when tumor cells acquire invasive activity and are able to escape from the confines of the basement membrane into the surrounding stroma. Highly invasive tumor cells always share the characteristics of a loss of intrinsic polarity and loose attachments to surrounding tissue structures [32]. Epithelial-mesenchymal transition (EMT) is a central event in this morphological transformation process, and it contributes to malignant biological properties including invasion and metastasis [33]. During the EMT process, tumor cells lose their cell-cell junctions and apical-basal polarity as a result of E-cadherin repression, leading to the acquisition of a motile mesenchymal cell phenotype [33]. Recent evidence suggests that TAMs are involved in the regulation of the EMT in various cancer types [34–36]. For example, after co-culture with TAMs, the expression of epithelial marker E-cadherin was reduced in CRC cells, while the mesenchymal marker Vimentin was upregulated, suggesting that TAMs can induce the CRC cell EMT *in vitro*. This research also revealed that TAMs-derived IL-6 induce EMT by regulating adenylate kinase 2 (AK2)/signal transducer

and activator of transcription 3 (STAT3)/miR-506-3p/Forkhead box Q1 (FoxQ1) axis [34]. Biologically, TAMs secrete a series of inflammatory factors, such as IL-6, IL-10, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β), thereby promoting EMT [37].

The extracellular matrix (ECM) provides both the biochemical and biomechanical context within which tumor cells exist. Cancer progression is dependent on the ability of tumor cells to traverse the ECM barrier, access systemic circulation, and establish distal metastases. TAMs support tumor cell migration, invasion, and metastasis via ECM remodeling, secreting a number of proteolytic enzymes including cathepsins (B, S, C, L, Z), lysosomal enzymes, matrix metalloproteinases (MMPs, including MMP1, MMP9, MMP12, and MMP14), serine proteases, and disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), which are important components mediating ECM degradation and cell-ECM interactions [38]. In another study, TAMs isolated from breast cancers were found to secrete CCL18, which signals via the breast tumor cell-specific membrane-associated phosphatidylinositol transfer protein 3 (PITPNM3) receptor. This signaling cascade activates integrin clustering on tumor cells, promoting integrin-ECM interactions and adhesion, and thereby facilitating invasiveness and metastasis [39].

In addition to TAMs, some TRMs also present their functions in cancer metastasis. For example, pulmonary alveolar macrophages prepare the appropriate microenvironment for promoting breast cancer metastasis to the lung. They accumulate in the premetastatic lungs through complement C5a receptor-mediated proliferation and alter the ratio the Th1/Th2 cells, suppressing tumoricidal Th1 and promoting Th2 generation [40]. Kupper cells are TRMs in the liver, which can rely on Dectin-2 to eliminate cancer cells then to inhibit liver metastasis, whereas bone marrow-derived macrophages cannot [41].

TAMs promote tumor cell angiogenesis

A few studies have shown that the levels of TAMs are closely associated with the vascularization of tumors. Macrophage infiltration into the nonmalignant primary tumors is followed by the formation of a vessel network and inhibition of the Response to suggestion macrophage infiltration can delay this process [42]. In addition to affecting the formation of new tumor vessels, TAMs also stimulate the remodeling of the established vasculature towards a more tortuous and leaky form that favors tumor dissemination [43]. Previous analyses have established the critical role of TAMs-derived VEGF and MMP9 in the promotion of tumor angiogenesis [44]. In addition, TAMs also release a panel of pro-angiogenic factors that include TNF-α, basic fibroblast growth factor (bFGF), thymidine phosphorylase (TP), urokinase-type plasminogen activator (uPA), adrenomedullin (ADM), and semaphorin 4D (Sema4D) [45]. There is a recently identified novel subset of TAMs expressing tyrosine-protein kinase receptor 2 (Tie-2) referred to as Tie2-expressing monocytes (TEMs) [46]. TEMs are attracted into tumors by endothelial cell (EC)-derived cytokine angiopoietin-2 (ANG-2), which interacts with its receptor Tie-2 [47]. A growing body of evidence indicates that TEMs in mice and humans significantly contribute to tumor angiogenesis [48]. Selective elimination of TEMs by TEMs specific makers on the surface may thus represent another promising approach to preventing angiogenesis and tumor progression. In addition, given the overlapping roles of TAM-derived VEGF-A and MMP9 in angiogenesis and lymphangiogenesis, this may represent a series of mechanisms whereby macrophages can stimulate lymphangiogenesis. Future studies will shed light on the role of TAMs in the complex control of tumor lymphangiogenesis via the production of cocktails of regulatory cytokines and other factors associated with this process [45].

Regulation of other cells in TME by TAMs

TAMs play a specific functional role in the context of immune suppression by interaction with T cells (Figure 1B). Cytotoxic T lymphocyte (CTL) are the major killers of tumor cells [49], while TAMs directly inhibit CTLs responses through the expression of programmed death-protein ligands 1 (PD-L1) on their surfaces, by producing inhibitory cytokines (such as IL-10 and TGF-β), and by depleting L-arginine via expression of inducible nitric oxide synthase (iNOS) or arginase I, which results in reactive oxygen species (ROS) production [23]. Tregs are specialized in suppressing anti-tumor immune responses and their infiltration into tumor tissues is often associated with poor prognosis [50]. TAM-derived prostaglandin E2 (PGE2) and IL-10 can promote the induction of Tregs, meanwhile, TAM-derived CCL17/18/22 can recruit additional Tregs, further resulting in CTL suppression [31]. In addition, macrophage production of IL-10 suppresses IL-12 expression by DCs in breast cancer, limiting cytotoxic $CD8^+$ T cell responses during chemotherapy (Figure 1B) [51]. In pancreatic ductal adenocarcinoma, IL-33-induced TAMs-CXCL3 production targets CXC-chemokine receptor 2 (CXCR2) on fibrotic cells to cause a fibroblast-to-myofibroblast transition which associates with an increased risk of cancer metastasis [52].

Therapeutic targeting of TAMs

Targeting TAMs recruitment and activation

As discussed above, a majority of TAMs originate from bone marrow monocyte procurers. Recruitment of TAMs to the tumor sites is a consequence of the continuous presence of tumor-derived chemoattractants. As such, cutting off those signals may represent a promising approach to modulating TAMs responses to suppress tumor growth (Table 2). For example, CSF1 and its receptor, CSF1R, regulate the migration, differentiation, and survival of macrophages and their precursors [53]. This CSF1/CSF1R axis has been heavily investigated in preclinical models and clinical trials. In a phase 1 study, anti-CSF1R therapy exhibited an ability to efficiently deplete TAMs in cancer patients and to promote a switch from infiltration primarily by $CD4^+$ T cells towards infiltration primarily by $CD8^+$ T cells [54]. Additionally, CSF1/CSF1R blockade improves the efficacy of a diverse range of immunotherapeutic treatment regimens, including CD40 agonists, PD-1 or CTLA-4 antagonists, and adoptive T cell therapy. These findings have spurred the development of several clinical trials combining CSF1 and/or CSF1R inhibitors with immune checkpoint blockade agents. In one promising study of patients with pancreatic cancer, who did not traditionally respond to immunotherapy, researchers found that some patients responded to a combination of CSF1R and PD-1 antagonists [55], and these studies are now moving forward towards a multi-arm phase II clinical trial. However, the efficacy of CSF1R-based therapies remains somewhat controversial. According to a single-cell analysis, such CSF1R blockade exhibits a cell cycle preference and is thus insufficient to deplete all macrophage populations [12].

Table 2: Therapeutic strategies targeting tumor-associated macrophages, DCs, and neutrophils.

Effects	Class of agents	Agents	Combination	Cancer type	Phase	Trial ID
Targeting TAM recruitment and activation	CSF1R inhibitors	Pexidartinib	Durvalumab (PD-L1 antagonists)	Metastatic/advanced pancreatic or colorectal cancers	Phase 1	NCT02777710
		LY3022855	Durvalumab or tremelimumab	Advanced solid tumors	Phase 1	NCT02718911
		IMC-CS4	Monotherapy	Advanced solid tumors	Phase 1	NCT01346358
		Cabiralizumab	Nivolumab (PD-1 antagonists)	Selected advanced cancers	Phase 1	NCT02526017
		Cabiralizumab	Monotherapy	Diffuse type tenosynovial giant cell tumor	Phase 1/2	NCT02471716
		Pexidartinib	Monotherapy	Giant cell tumor of the tendon sheath	Phase 3	NCT02371369
		ARRY-382	Pembrolizumab (PD-1 antagonists)	Advanced solid tumors	Phase 2	NCT02880371
		ARRY-382	Monotherapy	Selected advanced or metastatic cancers	Phase 1	NCT01316822
	CCR2 antagonists	MLN1202	Monotherapy	Bone metastases	Phase 2	NCT01015560
Targeting TAM reprogramming and DC activation	CD40 agonists	APX005M	Monotherapy	Solid tumors	Phase 1	NCT02482168
		APX005M	Nivolumab (PD-1 antagonists)	Non-small cell lung cancer or metastatic melanoma	Phase 1/2	NCT03123783
		CP-870,893	Tremelimumab (CTLA4 antagonists)	Metastatic melanoma	Phase 1	NCT01103635
		ChiLob 7/4	Monotherapy	Advanced malignancies refractory	Phase 1	NCT01561911
		Selicrelumab	Atezolizumab (PD-L1 antagonists)	Locally advanced and/or metastatic solid tumors	Phase 1	NCT02304393
		HCD122	Monotherapy	Multiple myeloma	Phase 1	NCT00231166
		Selicrelumab	Vanucizumab or bevacizumab	Metastatic solid tumors	Phase 1	NCT02665416
Targeting neutrophil infiltration	CXCR2 antagonists	Reparixin	Paclitaxel	Metastatic triple-negative breast cancer	Phase 2	NCT02370238
		Reparixin	Paclitaxel	HER2 negative metastatic breast cancer	Phase 1	NCT02001974

C-C chemokine receptor 2 (CCR2) inhibition leads to monocyte retention within the bone marrow, resulting in a depleted pool of circulating cells and reduced numbers of TAMs in primary and metastatic sites [56]. In preclinical models, CCL2 or CCR2 blockade can improve the efficacy of chemotherapy, radiotherapy, and immunotherapy. Several CCR2 blockade combination clinical trials are therefore ongoing. The CCR2 inhibitors PF-04136309 and CCX782 both show safety and efficacy in patients with pancreatic cancer. When combined with FOLFIRINOX, a greater than 40% increase in responsiveness to chemotherapy has been observed together with the prolongation of patient overall survival [57, 58]. Biomarker analyses have also suggested that combination therapy was associated with increased $CD4^+$ and $CD8^+$ T cell infiltration together with reductions in levels of Tregs [57]. Similar observations have also been made in some preclinical models, facilitating further combinations with checkpoint immunotherapy. Clinical testing of this triple combination of CCR2 inhibition, chemotherapy, and checkpoint blockade is now ongoing. Although CCR2 inhibitors play a promising role in therapy, they remain subject to significant shortcomings. Indeed, the cessation of CCL2 and/or CCR2 blockade can lead to a release of the monocytes previously trapped within the bone marrow, and this has been shown to exacerbate metastasis in a murine model of breast cancer [59].

Targeting TAM reprogramming

As discussed above, one of the key features of macrophages is their plasticity, which enables them to change their phenotype in the tumor microenvironment. Reprogramming TAMs to an anti-tumor phenotype thus represents an attractive therapeutic strategy. One of the most effective approaches to such reprogramming identified to date has been the use of a CD40 agonist antibody. CD40-activated macrophages are indicative of M1 phenotype correlating with enhanced pro-inflammatory cytokine production [60]. In mouse model, CD40 pathway can be harnessed to restore tumor immune surveillance by targeting TAMs [61]. As CD40 is mainly expressed by classical dendritic cells (cDCs), the relative contribution of TAMs and cDC activation is unclear. However, enhanced responses to PD-1 and CTLA-4 antagonists have been observed following such treatment [62]. β-glucan, a yeast-derived polysaccharide, has also been shown to promote TAM differentiation into an M1 phenotype and is a potent immunomodulator with anti-cancer properties [63].

Potential therapies targeting TAMs

Tumor-expressed CD24 could interact with inhibitory receptor sialic-acid-binding Ig-like lectin 10 (Siglec-10) on TAMs to promote immune evasion were validated in ovarian cancer and breast cancer [64]. Research showed that blockade or knocking out Siglec-10 augmented the phagocytic ability of macrophages, which demonstrated the potential of Siglec-10 as immune checkpoint in immunotherapy [64]. In addition, there is increasing attention to the unique subset of macrophages expressing the cell surface receptor TREM2, which are present in different types of tumors [65, 66]. Lack of TREM2 influences the macrophage subsets proportion, diminishing the immunosuppressive clusters while the clusters which express immunostimulant gene increase [67]. Meanwhile, anti-TREM2 treatment curb tumor growth in mice and enhance the anti-PD-1 efficiency, showing its potential in combination therapy [67]. Emerging technological advances, such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics technology that preserves spatial information, will further accelerate the search for novel potential targets on TAMs.

Tumor cells can regulate TAMs polarization, both through tumor-derived factors and metabolites. In turn, TAMs can promote tumor progression. This crosstalk between TAMs and tumor cells makes TAMs as one of the most essential pro-tumor cells and treatment strategies targeting TAMs are anticipated to be feasible.

Dendritic cells

DCs are central mediators of the initiation and regulation of anti-tumor immunity. DCs migrate to the tumor sites, internalize portions of moribund tumor cells, and respond to stimuli that can drive DC maturation, emigration from the tumor site, and homing to regional lymph nodes where they can present tumor-derived antigens to antigen-specific T cells. Activated T cells upregulate the expression of chemokine receptors, enabling circulating CTLs to infiltrate the tumor and to destroy malignant cells [60]. Here, we review the DCs phenotypes driven by interactions with the TME and with T cells. Therapies targeting these interactions are also discussed.

DC subset functions

DCs are professional antigen-presenting cells that prime effector $CD4^+$ or $CD8^+$ T cell responses [68]. Previous work on the development of DCs from bone marrow progenitors

identified a population named as the common dendritic cell precursors (CDPs), which give rise to plasmacytoid DCs progenitors (pre-pDCs) and classical DCs progenitors (pre-cDCs) [69]. pDCs and cDCs are the most common and best-studied DC subsets (Figure 2A) [70], with cDCs being further separated into $CD103^+$ cDC1 and $CD11b^+$ cDC2 subtypes based on their phenotypic and functional characteristics. cDC1s support stronger $CD8^+$ T cell immunity and induce the Th1 cells polarization of $CD4^+$ T cells through the secretion of IL-12 [71, 72]. Th1 cells are responsible for cell-mediated immunity and phagocyte-dependent protective responses [73]. While, cDC2s appear to be essential for the priming of anti-tumor $CD4^+$ T cell responses [74]. In addition, inflammatory conditions can lead to the formation of monocyte-derived DCs (moDCs), which play an essential role in defenses against pathogens by participating in the induction of both adaptive and innate immune responses [75]. Advancements in high-throughput single-cell analysis technologies have enabled the identification of novel subsets of dendritic cells in human cancers. For example, lysosomal associated membrane protein 3 (*LAMP3*)$^+$ cDCs have been identified via scRNA-seq data of most cancer types, wherein they exhibit an enhanced migratory capacity and the potential to develop from both cDC1s and cDC2s [16]. Owing to the heterogeneity of cDC2 cell populations, T-bet and RAR-related orphan receptor gamma (RORyt) expression can be assessed to define distinct cDC2 subsets, including anti-inflammatory T-bet^+ cDC2A and pro-inflammatory T-bet^- cDC2B cells [76]. Single-cell analyses have extended current knowledge of DC heterogeneity, but further such studies are needed to dissect their functional roles in tumors and related therapeutic contexts.

Tumor microenvironmental remodeling of DC characteristics

Tumors can evolve multiple mechanisms that enable them to thrive under adverse conditions while actively suppressing the protective function of immune cells. Previous studies have reported that DC tolerization is involved in tumor-mediated immune evasion [77]. Herein, we discuss the extrinsic mediators and metabolic program changes in TME that can influence DC functionality.

Extrinsic mediators

Several mediators present in the tumor site can directly alter the activaity of infiltrating DCs to promote malignant progression, including secreted proteins (growth factors, cytokines, chemokines), tumor antigens, and other factors (Figure 2A and Table 1) [78].

VEGF is a secreted heparin-binding protein produced by a majority of tumors that is responsible for angiogenic activity [79]. VEGF inhibits the differentiation and maturation of DCs via binding and activating the tyrosine kinase receptors, VEGFR-1 and VEGFR-2 [80]. VEGF is also known to regulate DC migration and homing by recruiting immature myeloid cells from the bone marrow to the primary tumor site, thereby generating a population of immature DCs [81]. IL-10 is an anti-inflammatory cytokine produced by tumor cells, macrophages, regulatory T cells, and other stromal components. Tumor-derived IL-10 has been shown to inhibit DC maturation, activation, and the T cell stimulatory abilities of DCs [82, 83]. In addition, IL-6, TGF-β, and CSF1 also play suppressive roles as modulators of DC maturation and activation [78]. Studies show that tumor-derived factors in the TME, especially CSF1, induce the development of immature, tolerogenic tumor-associated DCs (TADCs) that contribute to tumor progression via Janus kinase 2 (Jak2)/STAT3 signaling [84].

Tumor antigens can also potentially function as DC-suppressive factors, For example, prostate-specific antigen (PSA), which is a serine protease overexpressed in most prostate cancers, was the first tumor-associated antigen shown to inhibit DC maturation, longevity, and function [85]. DCs cultured in the presence of active PSA exhibited significantly reduced CD83, CD80, CD86, and human leukocyte antigen (HLA)-DR expression consistent with impaired maturation. Mucins such as MUC1, a glycoprotein overexpressed in many tumor cells, are responsible for impaired DC maturation and function [86]. When cultured in the presence of MUC1, immature DCs exhibited CD83, CD80, CD86, and CD40 upregulation together with the production of higher levels of IL-6, TNF-α, and IL-10, but they fail to produce IL-12, and thus do not induce Th1 cells responses which are crucial to anti-tumor immunity [87].

Metabolic reprogramming

The unique features of the TME including hypoxia, scarce nutrient availability, and competition for amino acids can induce metabolic perturbations within TADCs [88], thereby interfering with the development of robust anti-tumor immune responses. For example, tumor-derived lactic acid can suppress DC activation *in vitro*, and blocking of lactic acid production reverts the TADCs phenotype to normal [89]. The metabolism of tryptophan by indoleamine 2,3-dioxygenase

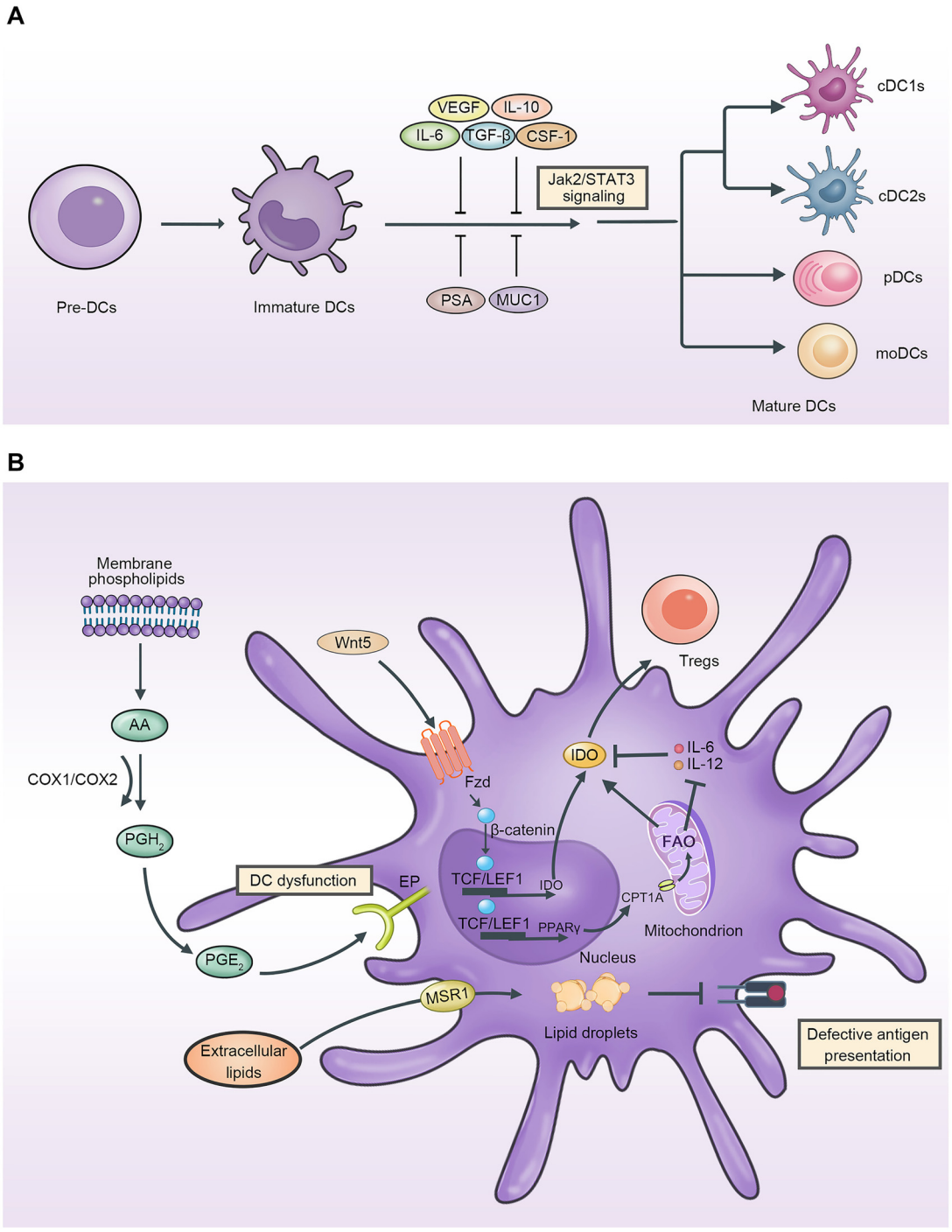

Figure 2: DCs origin and reprogramming in TME.
(A) Tumor cells influence on DCs development. cDC1s, cDC2s, pDCs and moDCs are predominant DCs subsets. They present various phenotypes and functions. Tumor-derived factors (VEGF, IL-10, IL-6, TGF-β, and CSF-1) and tumor antigens (PSA, MUC1) interfere the DCs maturation. (B) Lipid-mediated DCs metabolic reprogramming. DCs lipid metabolism and bioactive lipid sensing can drive tolerogenic TADC polarization. High lipid content defect DCs antigen presentation function. Tumor-derived Wnt5a triggers DC FAO via a β-catenin-PPAR-γ pathway, thereby supporting IDO enzymatic activity and subsequent Tregs differentiation. PEG2 leads to DC dysfunction, suppressing the differentiation of Th1-inducing DCs. pDCs, plasmacytoid DCs; cDCs, classical DCs; moDCs, monocyte-derived DCs; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-β; CSF-1, colony stimulating factor 1; PSA, prostate-specific antigen; MUC1, mucin 1; AA, arachidonic acid; COX, cyclooxygenase; PGH2, prostaglandin H2; PGE2, prostaglandin E2; EP, E-prostanoid receptors; FZD, frizzled; FAO, fatty acid oxidation; IDO, indoleamine 2,3-dioxgenase-1; MSR1, macrophage scavenger receptor 1.

(IDO) in the TME can drive the generation of Tregs, and thereby inhibiting T cell-mediated anti-tumor immunity [90].

Lipid metabolism plays a particularly important regulatory role in this context (Figure 2B). Owing to tumor-related metabolic disturbances, lipids in lipid droplets (LDs) can accumulate within cancer cells, allowing survival in a microenvironment with high energy demands [91]. Extracellular lipids can be imported and accumulate within the intracellular space by increased uptake of extracellular lipids due to the upregulation of the macrophage scavenger receptor 1 (MSR1), and DCs with high lipid content have defects in processing of tumor-associated proteins [92]. A recent study indicated that tumor-derived Wnt5a can induce β-catenin-peroxisome proliferator-activated receptor-γ (PPARγ) signaling in TADCs, thus shifting DC metabolic activity away from glycolysis and towards fatty acid oxidation (FAO). This metabolic program effectively inhibits the activation of effector T cells while driving the differentiation of Tregs [93]. Furthermore, Wnt5a-induced FAO plays a critical role in regulating DC metabolism, as it suppresses IL-6 and IL-12 expression, in addition to stimulating IDO enzymatic activity, creating an environment conducive to the generation of Treg cells [93]. Blocking FAO in combination with anti-PD-1 treatment may represent an effective therapeutic strategy. Notably, bioactive lipids, such as PGE2, have been shown to function as key signaling molecules that modulate TADC function. Membrane lipids can be metabolized to generate PGE2 via the cyclooxygenase-2 (COX)/PGE2 pathway [94, 95]. Notably, tumor-derived PGE2 can disrupt the early stages of DC differentiation, contributing to DC dysfunction in cancer [96]. Tumor-derived COX also impairs the accumulation of cDC1s within tumors and suppresses their activation, including IL-12 production. The implantation of COX-deficient transplantable tumors in basic leucine zipper transcription factor ATF-like 3 (Batf3)-knockout mice lacking cDC1s does not lead to impaired tumor growth [97]. Overall, current evidence indicated that intracellular lipid metabolism and bioactive lipid sensing in the TME can effectively drive tolerogenic TADC polarization.

Signals involved in the interplay between DCs and T cell

In the TME and tumor-draining lymph nodes (TDLNs), DCs present tumor-associated antigens to T cells, thereby promoting antigen-specific T cell activation and proliferation. However, such antigen presentation alone is insufficient to prime effective anti-tumor immunity. Many other signals also influence these interactions between DCs and T cells, including costimulatory molecules, cytokines, and chemokines (Figure 3).

The expression of CD80 and CD86 on DCs can control the activation or suppression of T cells through interactions with CD28 and CTLA-4, respectively [98]. TADCs express a high level of PD-L1 and the expression is upregulated during antigen-presentation to protect DCs from cytotoxicity of activated T cells. However, DC-derived PD-L1 suppress T cell activation and cytokine production, thus dampening the anti-tumor immune responses [99]. Besides, there are many DCs costimulatory molecules working on anti-tumor effect. CD40 on DCs has been shown to interact with CD40L on T cells, enhancing T cell stimulatory capacity and favoring Th1 cells responses [100]. Th1-driving effector DC (DC1) is a special DCs subtype which expresses elevated levels of intercellular adhesion molecule 1 (ICAM-1) but produces only low levels of IL-12, excluding the influence of IL-12 on Th1 polarization [101]. ICAM-1-expressing DC1 drive Th1 polarization, and blocking ICAM-1/LFA-1 interactions in co-cultures of DCs and naïve T cells can attenuate Th1 polarization [101]. Similarly, DCs that express OX40L give rise to a primary Th1 cells response and vaccination of such $OX40L^+$ DCs results in significant enhancement of therapeutic anti-tumor effect [102]. Moreover, the expression of both CD70 (CD27L) and glucocorticoid-induced TNFR-related protein (GITRL) GITRL on DCs can support $CD8^+$ T cell priming, thereby inducing anti-tumor immunity [103].

DC-derived cytokines can promote the development of either a pro-inflammatory or anti-inflammatory microenvironment. The effector activity of T cells is dependent upon DC-derived cytokines, including IL-12 and type I interferons (IFN-α/β), which are pro-inflammatory factors that shape Th1 cells development [104, 105]. IL-12 production by DCs requires both CD40L and IFN-γ signals [106]. Type I interferons can inhibit IFN-γ signaling, thereby restricting IL-12 expression by DCs, suggesting that these two signaling pathways can antagonize one another [107]. It has also been shown that DC-derived IL-10 and TGF-β1 are associated with the initiation and progression of cancer [108]. They can support Treg development and serve as anti-inflammatory mediators [109, 110].

DCs can also produce chemokines in the TME that attract T cells. CXCL9 and CXCL10, which bind to CXCR3, are key chemokines necessary for the intratumoral trafficking of effector T cells [111]. It is important to note that cDC1s serve as the primary source of CXCL9 and CXCL10, and BATF3 deletion or a lack of cDC1s can result in impaired effector T cell trafficking and defective anti-tumor immunity [111]. Moreover, CXCR3 can also be expressed by Treg cells. As such, cDC1s may recruit other immunosuppressive cells into the TME,

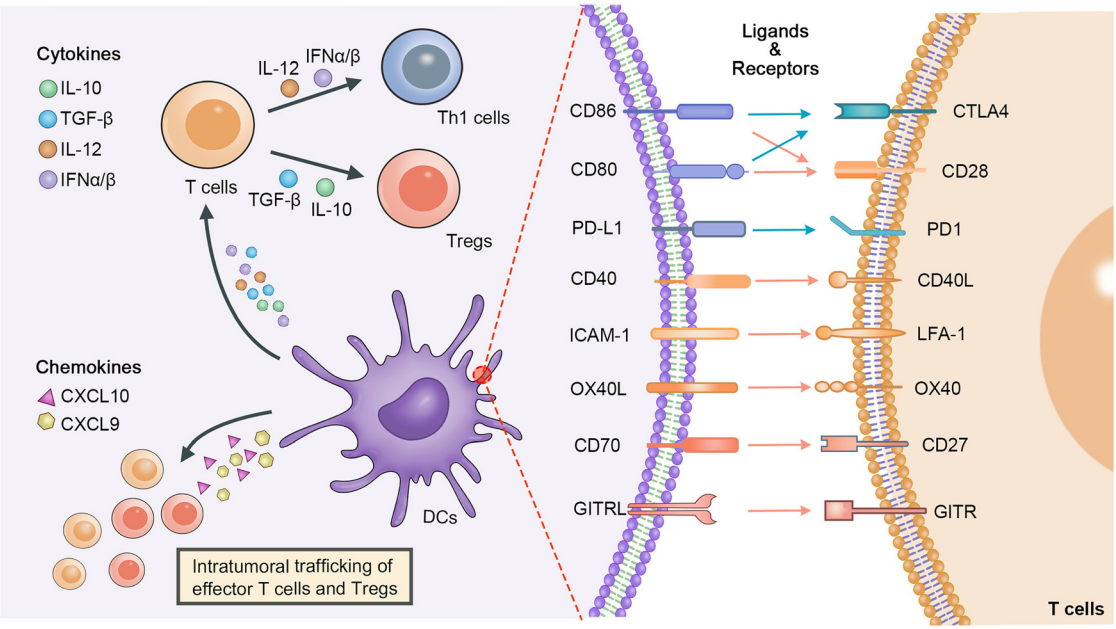

Figure 3: Signals between DCs and T cells.
Costimulatory molecules play contrary roles. Some of them suppress T cell activation (blue arrows and receptors), while others favor Th1, CTL cell responses (orange arrows and receptors). DCs secrete IL-12 and IFNα/β to shape Th1 cells development, meanwhile, TGF-β and IL-10 can support Treg development. CXCL9/10 are produced by TADCs, binding to CXCR3 on effector T cells and Treg cells and then recruiting them into TME. CTLA-4, cytotoxic T lymphocyte antigen 4; PD-1, programmed cell death protein 1; PD-L1, programmed cell death 1 ligand 1; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; GITR, glucocorticoid-induced TNFR-related protein; GITRL, GITR ligand; TGF-β, transforming growth factor-β; CXCL, CXC chemokine ligand.

suggesting that the interactions between DCs and T cells may be even more complex than previously recognized. In the context of cancer, TDLN DCs may initially prime naïve, tumor antigen-specific T cells, while DCs in the TME may further license the migration of antigen-primed T cells [112].

DCs-based immunotherapy in cancer treatment

DC-based immunotherapies are being actively developed, and can be broadly classified into *ex vivo* and *in vivo* approaches. *Ex vivo* approaches include traditional DCs vaccines, which rely on *in vitro* DC antigen loading, activation, and cytokine treatment prior to injection back into patients [113]. *In vivo* approaches, in contrast, target DCs within patients in an effort to enhance their anti-tumor activity. Approaches to enhancing DC activation and mobilization have been discussed in detail in prior reviews [7, 114]. Herein, we will focus on discussing approaches to restoring DC immune functionality through targeting *in vivo* DCs ligands, blocking inhibitory signals, and interfering with metabolic pathway activation (Table 2).

Ligation-based *in vivo* DC targeting

Ligation-based approaches to DC targeting *in vivo* include the ligation of CD40, and C-type lectin receptors (CLRs). CD40, a member of the tumor necrosis factor receptor (TNF-R) family, is a surface receptor best known for its ability to initiate multifaceted activation signals in normal B cells and DCs [115]. CD40 agonist antibodies have been used to provide activation signals necessary for DC-derived pro-inflammatory cytokine production and the enhancement of T cell activation. Moreover, agonistic CD40 antibodies can be combined with chemotherapy, checkpoint inhibitory antibodies, and other immune modulators to alter therapeutic outcomes [116]. A phase I study in combination with gemcitabine in patients with advanced pancreatic cancer found these antibodies to be well-tolerated and to exhibit anti-tumor activity [117]. In a mouse model, CD40 agonistic antibody treatment can overcome resistance to checkpoint blockade treatment and improve survival [62]. Recent single-cell analyses have highlighted changes in immune cell number and function upon anti-CD40 treatment. In MC38 tumor models, the cDC1s population is specifically amplified by such treatment. In addition, anti-CD40 treatment can impact the expansion, migration, and transition of tumor-infiltrating $CD8^+$ effector

memory T (T_{em}) and tissue-resident memory T (T_{rm}) cells, enhancing the crosstalk of basic helix-loop-helix family member E40 (*BHLHE40*)$^+$ Th1-like cells and cDC1s [12].

CLRs are also attractive therapeutic targets, as DC subsets are known to express different CLRs that are involved in the recognition and capture of many glycosylated antigens [118]. The CLR protein family consists of DEC205, Mincle, C-type lectin domain family 9 (CLEC9A), and DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). Different from directly activating DCs like CD40 antibody, CLRs work as targets receptors for antigen delivering. In previous study, researchers have generated human chimeric antibodies specific for CLEC9A and DEC205, with antigens on C-terminus. Those antibodies can specifically deliver antigens to DCs for processing and presentation to $CD4^+$ T and $CD8^+$ T cells, underscoring the potential clinical utility of this strategy [119]. Human studies using antigens targeted to DEC205 have been shown to specifically induce tumor-specific T cell responses in subsets of patients [120].

Blocking inhibitory signals

Overcoming the immunosuppressive activities of TADCs represents another approach to enhancing DC function. One advantage of this approach is that it allows for the systemic administration of inhibitors, as opposed to the local administration approach required for many immune agonists. One of the first examples of this approach was the targeting of VEGF, given that its suppressive effects on DCs have been discussed previously. VEGF inhibitors are already in clinical use for the inhibition of angiogenesis, and anti-VEGF antibodies have been shown to enhance the numbers and functionality of DCs in tumor-bearing mouse model systems [121, 122]. VEGF inhibition has also been shown to enhance DC maturation in human patients, suggesting that this may contribute to the efficacy of VEGF inhibitors in clinical settings [123]. Another potent immunosuppressive signal in TME is STAT3, and STAT3 inhibitors, which can promote DC maturation and activation, are currently being evaluated in clinical trials [124, 125].

Metabolic pathway targeting

The regulation of immunometabolism as a means of enhancing anti-tumor immunity is a growing area of active research in the field of cancer therapy. Interfering with lipid metabolism represents a particularly attractive approach to enhancing TADC-mediated anti-cancer immunity. PGE2 acts as an anti-inflammatory mediator, inhibiting inflammatory chemokine release from activated DCs [126]. Blocking COX enzymes to limit PGE2 production represents a promising immunotherapeutic approach that may enhance antitumor therapy by interfering with lipid metabolic pathways. For example, aspirin, a nonsteroidal anti-inflammatory drug (NSAID) that can block the COX-1/2 pathway, as well as celecoxib, a COX-2 inhibitor, have been found to improve anticancer treatment outcomes when combined with anti-PD-1 therapy in a murine model of melanoma [97]. Moreover, IDO inhibition is actively being studied in mice and in clinical trials. The potential combination of IDO inhibitors with DC-based cancer vaccines is also a topic of ongoing study [127]. Inhibiting fatty acid catabolism may also offer therapeutic potential as a means of restoring TADC functionality. Inhibiting FAO using etomoxir has been shown to improve the ability of DCs to induce T cells activation and suppress Treg cell differentiation *ex vivo* [93]. Furthermore, a combination of FAO inhibition and anti-PD-1 blockade treatment was shown to exhibit significant improvements in host survival driven by enhanced anti-tumor immunity [93].

Generally, DCs are antigen-presenting cells supporting stronger T cell anti-tumor immunity. However, the complexity of TME interferes DC maturation and antigen presentation, and even remodels DCs into pro-Tregs differentiation phenotype. Immunotherapy can exclude the immunosuppressive signals or directly activate DCs through receptors on their surface, enhancing DC anti-tumor ability.

Tumor-associated neutrophils

Neutrophils, which originate from myeloid precursors, compose the significant cellular parts of the leukocyte compartment and are the primary responsive cell type associated with innate immune responses [128]. The TME can control neutrophil recruitment, and tumor-associated neutrophils (TANs) can regulate tumor progression and growth.

Neutrophil classification

The role of neutrophils in cancer has long been a matter of controversy. In 2009, in an effort to mirror the M1/M2 TAM classification model, neutrophils were classified into N1 (anti-tumor neutrophil) and N2 (pro-tumor neutrophil) subsets [129]. This classification model was based on the discovery that differences in neutrophil polarization were evident following TGF-β treatment in mouse model systems. However, the utility of such N1/N2 polarization is limited given that their surface markers, cytokine expression patterns, transcription

factor regulators, and other hallmarks of activation are largely unknown. Moreover, neutrophils can be divided into high-density neutrophils (HDNs) and low-density neutrophils (LDNs). LDNs can be further separated into immature MDSCs and mature cells that are derived from HDNs in a TGF-β-dependent manner (Figure 4). LDNs exhibited impaired levels of activity as compared to HDNs with respect to several basic neutrophil functions, and LDNs are tumor permissive, whereas HDNs play an anti-tumor role [130]. Although the neutrophil with high RNAase content that makes it with lower RNA content under the 10X Genomics platform and requires setting low filtering thresholds to allow their detection [131]. Multiple scRNA-seq data on the development of neutrophils and neutrophils from cancer tissues are emerging. For example, six neutrophil subpopulations were found in mouse lung tumors and five subpopulations were found in human lung tumors, and three subpopulations show conserved gene expression within mouse and human neutrophils [132]. In order to further detect the low gene transcripts of neutrophils, scRNA-seq needs to be performed by other platforms (such as BD Rhapsody) to restore a higher proportion of neutrophil gene transcripts, but still lack related scRNA-seq in tumor tissues to distinct neutrophil subpopulations.

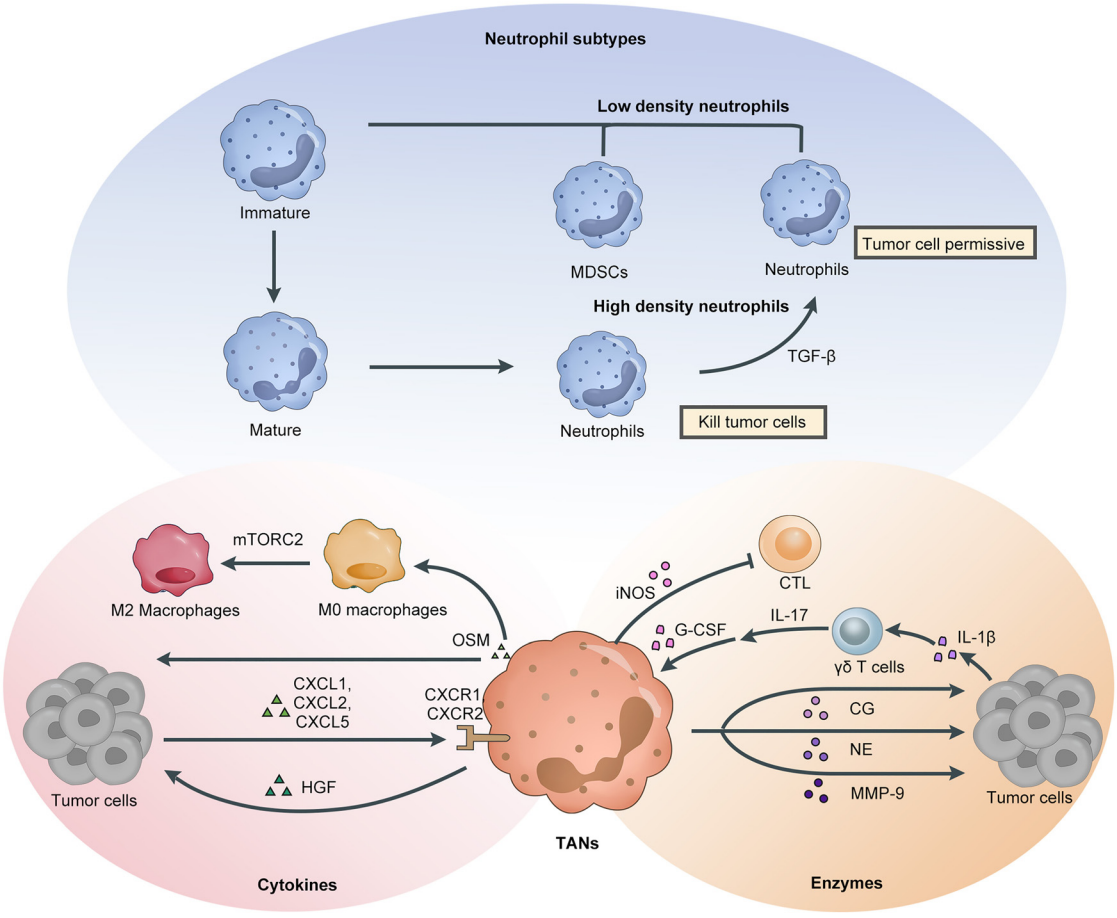

Figure 4: TANs effects on promoting tumor progression.
Neutrophils can be roughly divided into low-density neutrophils (LDNs) and high-density neutrophils (HDNs). Therein, LDNs consist of both immature MDSCs and mature cells that are derived from HDNs in a TGF-β-dependent mechanism. TANs can both release cytokines (red) and enzymes (orange) to reprogram tumor cells. High level of OSM and HGF increases the invasiveness of various cancer cell types. Moreover, OSM plays a part in M2 macrophage polarization. TANs can be recruited into tumors upon the binding of specific chemokines to CXCR1 and CXCR2. The majority of neutrophil-released proteases play a pro-tumor role, including CG, NE and MMP-9. The factors produced by γδ T cells alter neutrophil phenotypes, producing iNOS that suppresses the activity of anti-tumor CTLs. MDSCs, myeloid-derived suppressor cells; HGF, hepatocyte growth factor; OSM, oncostatin M; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; mTORC2, mechanistic target of rapamycin kinase signaling complex 2; CG, cathepsin G; NE, neutrophil elastase; MMP-9, matrix metallopeptidase 9; G-CSF, granulocyte colony-stimulating factor; iNOS, inducible nitric oxide synthase.

Tumor-derived factors regulating TAN functions

The phenotypic classification of TANs is partly based on specific tumor-derived factors. Granulocyte-colony stimulating factor (G-CSF) and TGF-β are the best-studied molecules in this context (Table 1). G-CSF is the only hematopoietic growth factor that exhibits increased serum expression during early tumor development. Prolonged G-CSF stimulation is both necessary and sufficient to promote the development of tumor-induced immunosuppressive neutrophils [133]. TGF-β production within the TME induces a population of TANs with a pro-tumor phenotype. TGF-β blockade can increase the production of neutrophil-attracting chemokines, resulting in an influx of TANs that are hypersegmented, more cytotoxic to tumor cells, and express higher levels of proinflammatory cytokines, thereby inducing a more robust anti-tumor phenotype [129].

Neutrophil effects on tumor and immune cells

TANs can play dichotomous roles in the context of tumor progression, alternatively functioning in an anti-tumor and pro-tumor manner under the influence of complex TME conditions [134]. Below, we primarily discuss the ability of TANs to promote tumor progression (Figure 4).

Reactive oxygen species

Several lines of evidence suggest that neutrophils may be linked to carcinogenesis through ROS-dependent and -independent mechanisms. Owing to their robust phagocytic activity, neutrophils can produce high phagolysosomal levels of ROS that can aid in pathogen killing [135]. However, the release of these ROS by neutrophils can result in DNA damage and mutations that are linked to cancer initiation [136]. ROS can also result in epithelial damage and pro-oncogenic inflammation [128].

Cytokines and chemokines

Neutrophils release various cytokines and chemokines into the TME. Neutrophil-derived secreted factors primarily act in a pro-oncogenic manner. OSM is a pleiotropic cytokine in the IL-6 family that reportedly promotes tumor progression by enhancing angiogenesis and metastasis. When cocultured with breast cancer cells, neutrophils express and release high levels of OSM, which increases cancer cell detachment and invasive capacity [137]. Similarly, neutrophils cultivated in tumor-conditioned medium derived from HuCC-T1 or HepG2 cells secrete high levels of hepatocyte growth factor (HGF), which can increase the invasiveness of various cancer cell types, thus suggesting a possible role for TANs in tumor invasion [138]. Neutrophil recruitment into tumors appears to be dependent upon the binding of specific chemokines to CXCR1 and CXCR2 expressed on the surface of neutrophils. Inhibiting CXCR2 signaling can significantly reduce the numbers of tumor-infiltrating neutrophils both *in vitro* and *in vivo* in tumor model systems, and is associated with slower tumor growth [139]. In papillomas mouse models, CXCR2 deficiency can reduce tumor microvessel density by approximately 50% while reducing the size of tumors formed. Moreover, compared with adjacent normal tissues, papillomas upregulate several CXCR2 ligands, including CXCL1, CXCL2, and CXCL5 [140].

Neutrophil-derived secreted proteins can also disturb the functions of other immune cells. For example, OSM can promote M2 macrophage polarization in the TME in a manner dependent upon the mechanistic target of rapamycin kinase signaling complex 2 (mTORC2) [141].

Enzymes

Neutrophils contain four types of granules: primary (azurophil), secondary, and tertiary granules, as well as secretory vesicles. These granules contain a range of different proteases, with the best-studied of these being cathepsin G (CG), neutrophil elastase (NE), and MMP-9 [142]. These factors have been found to play a pro-metastatic role through mechanisms associated with the EMT and ECM remodeling [143]. CG is a serine protease that is presynthesized in promyelocytes in the bone marrow and then stored in neutrophil primary granules in an active protease form. In breast cancer MCF-7 cells, CG can bind to the tumor cell surface in a manner independent of its catalytic site, thereby inducing cell aggregation in a manner dependent upon its enzymatic activity [144]. The formation of these tumor cell aggregates can permit tumor cell dissemination through the circulatory system to distant sites and where new metastases can be established. The inhibition of CG was found to result in reduced osteolysis in breast cancer, highlighting CG as a potential therapeutic target in this oncogenic context [145]. NE is also a serine protease that is released upon neutrophil degranulation. NE can directly induce tumor cell proliferation in the context of both human and murine lung adenocarcinoma by gaining access to

the endosomal compartment within tumor cells, wherein it degrades insulin receptor substrate-1 (IRS-1). IRS-1 degradation results in increased interactions between phosphatidylinositol 3-kinase (PI3K) and the potent mitogen platelet-derived growth factor receptor (PDGFR), thereby skewing the PI3K axis to favor tumor cell proliferation [146]. This pro-tumor function has also been observed in other tumor types including esophageal cancer, gastric cancer, and breast cancer [147–149]. In these cancer types, NE was found to mediate the release of transforming growth factor-alpha (TGF-α) from the cell surface. Higher levels of NE in breast cancer patients are correlated with lower survival rates, suggesting that NE is an independent prognostic biomarker in at least certain cancers [150]. NE and CG, as serine proteases, can also promote lung metastasis by degrading the anti-cancer protein thrombospondin 1 (Tsp1) *in vitro* and *in vivo* [151]. In addition to NE and CG, neutrophil-derived MMPs including MMP-8 and MMP-9 are associated with cancer progression [152]. Although MMP-9 is expressed in many cell types, studies suggest TANs are major contributors of highly angiogenic MMP-9 [153], and MMP-9 is stored in secondary granules in neutrophils wherein it is not associated with tissue inhibitor matrix metalloproteinase 1 (TIMP-1), rendering it better able to promote angiogenesis [147].

Enzymes secreted by neutrophils can influence the function of other immune cells. For example, tumor-derived IL-1β activates γδ T cells to produce IL-17. IL-17-producing γδ T cells can promote the upregulation of G-CSF in mammary tumors, leading to subsequent alterations in the neutrophil phenotypes discussed above. These phenotypically altered neutrophils can produce iNOS that suppresses the activity of anti-tumor $CD8^+$ T cells, thereby promoting breast cancer metastasis [154].

Therapeutic targets in cancer

Although there is substantial evidence that neutrophils play a deleterious role in tumor progression, therapeutically targeting this cell type in cancer remains very challenging. Neutrophils are critical mediators of host defenses against infection, and the depletion of these cells can thus result in dangerous levels of immunosuppression [152]. TANs themselves or specific neutrophil-promoting factors may represent potential immunotherapeutic targets (Table 2). One such promising therapeutic approach is the inhibition of CXCR2, which is the positive regulator of neutrophil mobilization. Very few tumor cells express CXCR2, suggesting that the effects of agents targeting this chemokine receptor are specifically attributable to immune cells. The combined inhibition of CXCR2 and PD-1 efficiently improves survival and suppresses metastasis, confirming the ability of CXCR2 to confer sensitivity to anti-PD-1 therapy [155]. In clinical trials, CXCR2 antagonists have mainly been used in patients with respiratory diseases such as chronic obstructive pulmonary disease and asthma [156, 157]. Their therapeutic utility in cancer patients remains an area of active study.

Rather than targeting neutrophils directly, inhibiting specific neutrophil-derived enzymes known to promote tumor growth and invasiveness may also represent a viable anti-tumor treatment strategy. The function of NE in the context of tumor progression has been discussed in detail. However, recent NE-related clinical trials have focused on patients with bronchiectasis [158]. The application of these NE inhibitors to treat cancer patients largely sought to alleviate the side effects of other cancer treatments. For example, after receiving a NE inhibitor, patients with thoracic esophageal carcinoma showed an improved systemic inflammatory response [159]. Other therapies, such as inhibiting TGF-β or targeting tumor necrosis factor-related apoptosis-inducing ligand receptor (TRAIL-R) or SIRPα may also represent effective treatment strategies [160]. However, these signals can be detected by many other immune cells, and are not specific to TANs [161, 162]. As such, we have elected not to discuss these pathways in detail in the present review. Overall, further research into the therapeutic utility of TANs is needed, as they are not as well studied as TAMs or DCs. Most importantly, additional preclinical and clinical studies are needed to better understand the therapeutic effects of targeting neutrophils in cancer patients.

In this review, we specially focus on their detrimental roles to the host by secreting cytokines and enzymes. Nevertheless, TAN anti-tumor function shouldn't be ignored. Since the incomplete research on exact roles, recruitment pathways, subpopulations and mechanisms of action of TANs, corresponding specific therapies remain to be developed.

Concluding remarks

With the recent and remarkable success in the development of novel cancer immunotherapies and the targeting of traditional T cells, there is an urgent need to elucidate the relationship between different immune components of the TME in order to improve the understanding of ICB and to establish reliable tumor biomarkers and effective combination treatment strategies. In this review, we have summarized key factors secreted by multiple myeloid cell types that can regulate or be affected by cell heterogeneity,

cancer type, and individual differences. We further dissected the cross-talk between myeloid cells and other cell populations within the TME and provided clinical insights regarding the application of myeloid cells in the field of patient treatment.

Additional work is still necessary to identify specific cytokines with potential therapeutic utility. With respect to cellular interplay, a majority of studies to date have focused on the ability of tumor cells to reprogram other cell types, with the interactions between different immune cells and between immune and non-immune cells remaining less well studied. Moreover, most *in vitro* experiments consist of two kinds of cells exposed to specific culture conditions, thus failing to recapitulate the true complexity of the TME. Recent developments in the generation of three-dimensional (3D) tumor models offer a better opportunity to mimic the TME, and thus warrant broad application [163]. There is now substantial evidence to support the utility of myeloid-based targeted therapies that can impact solid tumor progression and provide clinical survival benefits. New checkpoints associated with different types of cells in the immune system have the potential to further advance the field of immunotherapy. Precisely eliminating the pro-tumor myeloid cells within the TME may represent another viable therapeutic strategy. Although techniques such as the CIBERSORT and XCell algorithms can estimate the abundance of tumor-infiltrating myeloid cells by using gene expression data from bulk tissues and flow cytometry in order to highlight the complexity of major myeloid lineages, these approaches are insufficiently detailed or scalable to permit effective phenotypic differentiation [164, 165]. With recent advances in the development of single-cell RNA sequencing technologies, more interesting myeloid cell subtypes have been identified, that may explain the variable efficacy of certain myeloid-targeted antibodies [16]. Considering the challenges associated with capturing and identifying immune checkpoints and immune cell populations in transcriptomic datasets, further technologies and approaches will be necessary to validate these observations.

Future research efforts should seek to clarify which myeloid cell subtypes are crucial to human disease, to elucidate their functional roles, and to establish whether they can be manipulated in precision medicine applications. Toxicity is an additional important consideration in the context of myeloid-based targeted therapy, and it remains very challenging to patient samples with immune-related adverse events (irAEs) in clinical trials. As little is known regarding the mechanistic basis for such irAEs, it may be difficult to link these myeloid-based targeted therapies with irAE incidence. Further studies also need assess the safety of myeloid-based targeted therapy. In addition, cancer is a systemic disease that affects the entire immune system. In addition to focusing on local immune responses within the TME, we must assess the systemic immune landscape. As such, it is necessary to further study the mechanisms underlying myeloid-based targeted therapies in order to evaluate the safety of these drugs, and to then conduct additional research and clinical trials.

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Competing interests: Authors state no conflict of interest.

Ethical approval: The local Institutional Review Board deemed the study exempt from review.

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