The Tumor Microenvironment Innately Modulates Cancer Progression

Dominique C. Hinshaw¹ and Lalita A. Shevde¹,²

Abstract

Cancer development and progression occurs in concert with alterations in the surrounding stroma. Cancer cells can functionally sculpt their microenvironment through the secretion of various cytokines, chemokines, and other factors. This results in a reprogramming of the surrounding cells, enabling them to play a determinative role in tumor survival and progression. Immune cells are important constituents of the tumor stroma and critically take part in this process. Growing evidence suggests that the innate immune cells (macrophages, neutrophils, dendritic cells, innate lymphoid cells, myeloid-derived suppressor cells, and natural killer cells) as well as adaptive immune cells (T cells and B cells) contribute to tumor progression when present in the tumor microenvironment (TME). Cross-talk between cancer cells and the proximal immune cells ultimately results in an environment that fosters tumor growth and metastasis. Understanding the nature of this dialog will allow for improved therapeutics that simultaneously target multiple components of the TME, increasing the likelihood of favorable patient outcomes.

Introduction

The tumor microenvironment (TME) is complex and continuously evolving. In addition to stromal cells, fibroblasts, and endothelial cells, the TME comprises innate and adaptive immune cells. Previous studies have focused predominantly on adaptive immune cells in the context of cancer. T lymphocytes, in particular, have been a target of interest for their potent cytotoxic capabilities, so much so that their differentiation status became a model for other cell types and was coined the "Th1/Th2 paradigm" (1). This dichotomy posits that T cells orchestrate pathogen-dependent immune responses by differential production of cytokines: Th1 cells govern a proinflammatory phenotype and Th2 cells orchestrate an immunosuppressive phenotype. Current TME-targeted treatments have focused predominantly on T cells; prime examples include checkpoint blockade and other malignancies correlated with a negative prognosis, including breast cancer, gastric cancer, lung cancer, hepatoma, and other malignancies correlated with a negative prognosis, further establishing their role in cancer progression (8–10). Also,

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an aspect of their normal tissue remodeling abilities includes regulation of epithelial cell movement. This function of M\textsuperscript{\text{\textregistered}}s is co-opted by tumor cells within the TME; M\textsuperscript{\text{\textregistered}}s release factors (e.g., EGF) that promote the movement and invasion of cancer cells (11, 12).

While the M\textsubscript{1}/M\textsubscript{2} classification is a simplified understanding of M\textsuperscript{\textregistered} phenotype and function, in reality M\textsuperscript{\textregistered}s are plastic in nature and exist in a continuum of functional states (7). M\textsubscript{2} M\textsuperscript{\textregistered}s can further be classified into M\textsubscript{2a}, M\textsubscript{2b}, M\textsubscript{2c}, and M\textsubscript{2d} subsets (Table 1). These subsets are defined on the basis of their different inducers namely: IFN\textgamma, and LPS for M\textsubscript{1}; IL4, IL10, IL13 for M\textsubscript{2a}; TLR agonists for M\textsubscript{2b}; IL10, TNF\alpha, and glucocorticoids for M\textsubscript{2c}; and TLR and adenosine A2A receptor for M\textsubscript{2d} (13). Furthermore, these differential M\textsuperscript{\textregistered} subsets have different functional roles as outlined in Table 1. Therefore, it is unsurprising that M\textsuperscript{\textregistered}s that exhibit properties of both M\textsubscript{1} and M\textsubscript{2} exist in distinct proportions in the TME, depending on the tumor type, although the M\textsubscript{2} phenotype is typically favored. This poses a conundrum because M\textsuperscript{\textregistered}s that exhibit properties of both M\textsubscript{1} and M\textsubscript{2} exist in distinct proportions in the TME, depending on the tumor type, although the M\textsubscript{2} phenotype is typically favored. Thus, the inability of DCs to perform these functions greatly hampers the immune response to pathogens, viruses, and tumors. DCs are functionally classified into different subtypes such as classical DCs (cDC), plasmacytoid DCs (pDC), and monocyte-derived inflammatory DCs (moDC). cDCs can be further divided into cDC1 and cDC2. cDC1s develop under the control of the transcription factors IRF8, ID2, and BATF3, and cDC2s develop under the control of transcription factors IRF4, ID2, ZEB, and Notch2/KLF4 (19). These subsets are also functionally distinct: cDC1s are capable of cross-presentation and thus are able to present both

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Cross-talk in the tumor microenvironment. **A**. The impact of inflammatory or tumor-suppressive immune cells on tumor cells in the TME. The bold arrows show the impact that immune cells ideally have on tumor cells (TC). The interactions between NKTs, DCs, T cells, neutrophils, ILCs, M\textsuperscript{\textregistered}s, and NK cells and tumor cells are depicted. Fibroblasts are denoted with the letter “F.” **B**. The cross-talk between immune cells in the TME that have been polarized to an immune-suppressive type and the cytokines secreted by the TCs that contribute to this Th2-like polarization.

**Dendritic cells**

DCs bridge the gap between the adaptive and innate immune systems. They initiate pathogen-specific T-cell responses and are therefore important for bolstering protective immunity. It is important to note that B cells and M\textsuperscript{\textregistered}s also perform antigen presentation, albeit with lower activity than that of DCs. To effectively stimulate the adaptive immune response, DCs must recognize, capture, and present antigens, upregulate costimulatory molecules, produce inflammatory cytokines, and then travel to secondary lymphoid organs for antigen presentation to T cells. The inability of DCs to perform these functions greatly hampers the immune response to pathogens, viruses, and tumors. DCs are functionally classified into different subtypes such as classical DCs (cDC), plasmacytoid DCs (pDC), and monocyte-derived inflammatory DCs (moDC). cDCs can be further divided into cDC1 and cDC2. cDC1s develop under the control of the transcription factors IRF8, ID2, and BATF3, and cDC2s develop under the control of transcription factors IRF4, ID2, ZEB, and Notch2/KLF4 (19). These subsets are also functionally distinct: cDC1s are capable of cross-presentation and thus are able to present both
## Table 1. Innate immune cells in the tumor microenvironment

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Normal functions</th>
<th>Stimulatory cytokines in the TME</th>
<th>Cytokine/chemokine secretion</th>
<th>Human markers</th>
<th>Mouse markers</th>
<th>Effect</th>
<th>Source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MACROPHAGES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>Activate Th1 responses, phagocytosis, type 4 hypersensitivity</td>
<td>IFNγ, IL2, IL3, IL6, IL12, IL23, CCL10, CCL11, CCL14</td>
<td>CD64, IDO, SOCS3, CXCL10, CD80, CD86, CD68, MHC-II, ILIR, SOCS3</td>
<td>CXCL9, CXCL10, CXCL11, NOS2</td>
<td>Antitumor</td>
<td>(2, 13, 94)</td>
<td></td>
</tr>
<tr>
<td>M2a</td>
<td>Activate Th2 responses, wound healing, allergy</td>
<td>IL4, IL10, IL13, CCL2, CCL3, CCL4, CCL14</td>
<td>MRC1, TGM2, CD23, CCL22, CD163, IL-IR II</td>
<td>Mrc1, Tgm2, Fizz1, Ym1/2, Arg1, MHC-II, ILrra</td>
<td>Protumor</td>
<td>(2, 13, 94)</td>
<td></td>
</tr>
<tr>
<td>M2b</td>
<td>Th2 activation, immunomodulation, matrix remodeling</td>
<td>IL1, IL6, IL10, TNF, CCL1</td>
<td>CD86, MHC-II</td>
<td>CD86, MHC-II</td>
<td>Protumor</td>
<td>(13, 94)</td>
<td></td>
</tr>
<tr>
<td>M2c</td>
<td>Tissue repair, immunomodulation, matrix remodeling</td>
<td>IL10, TNF, CCL1</td>
<td>CD163, Mrc2</td>
<td>CD163, Mrc1</td>
<td>Protumor</td>
<td>(13, 94)</td>
<td></td>
</tr>
<tr>
<td>M2d</td>
<td>Angiogenesis, clearance of apoptotic tissues</td>
<td>TLR, adenosine A2A receptor</td>
<td>TNF, TGFβ, VEGF, A10, IL12, CCL5, CCL10, CCL14</td>
<td>VEGF</td>
<td>VEGF</td>
<td>Protumor</td>
<td>(13, 94)</td>
</tr>
<tr>
<td><strong>DCs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immature DCs</td>
<td>Recognize antigens, migrate to secondary lymphoid organs, phagocytosis, minimal</td>
<td>N/A</td>
<td>N/A</td>
<td>CD11c, HLA-DR, FLT3L, CD11c, MHCII, FLT3L, CD45</td>
<td>Depends on tumor type</td>
<td>(20, 95)</td>
<td></td>
</tr>
<tr>
<td>cDC1</td>
<td>APC to CD8 T cells, cross presentation, secretion of IL12</td>
<td>IL2, TNF, IFNγ</td>
<td>CD11c, CD141, XCR1, HLA-DR, Nec2, CLEC9A, CD80, CD86, CD40, CCR7, FLT3, TLR3, IL10, TNF</td>
<td>CD11c, CD8x (lymphoid), MHCII, Clec9a, CD103 (Non-Lymphoid), DEC205, XCR1, CD80, CD86, CD1M1, CD26, CD24</td>
<td>Depends on tumor type</td>
<td>(19, 96-98)</td>
<td></td>
</tr>
<tr>
<td>cDC2</td>
<td>APC to CD4 T cells</td>
<td>IL10, TGFβ, IL12</td>
<td>CD11c, CD11b, MHCII, CD45, Siglec H, CD80, CD86, CD172a, CD26</td>
<td>CD11c, B220, CD45, Siglec H, CD317, Gr-1, Ly6C</td>
<td>Depends on tumor type</td>
<td>(19, 96-98)</td>
<td></td>
</tr>
<tr>
<td>pDCs</td>
<td>Abundant secretory activity (IFN type 1), respond to viral infections</td>
<td>Type 1 IFN, TNF, IL12</td>
<td>CD11c, HLA-DR, CD304, CD303, CD103, FLT3, B220, PDCA1, FcRε, ILT3, ILT7, DR6, CD300A, BTLA, CD62L, CD45R4A</td>
<td>CD11c, B220, CD45, Siglec H, CD317, Gr-1, Ly6C</td>
<td>Depends on tumor type</td>
<td>(20, 96-98)</td>
<td></td>
</tr>
<tr>
<td>MoDCs</td>
<td>Produce high levels of the pro-inflammatory cytokines TNF, IL6, and IL12</td>
<td>TNF, IL1, IL2, IL23</td>
<td>CD11c, CD14, Factor XIIA, HLA-DR, CD62L, CCR5, CD208, CD86, CD164, MAR-1</td>
<td>CD11c, MHC-II, CD11b, F4/80, Ly6C, CD206, CD115, CD97b, FcRε, CD80, CD86, SLAM, PD1, PD2, DEC205, IDO</td>
<td>Antitumor</td>
<td>(19, 95, 97, 99, 100)</td>
<td></td>
</tr>
<tr>
<td>Tolerogenic DCs</td>
<td>Diminished APC, stimulate Th2 responses and Tregs to induce tolerance</td>
<td>PGE2, TGFβ, VEGF, IL10, TNF</td>
<td>TGFβ</td>
<td>CD11c, CX3AR1, CD163, CD300L, CFH, CSGALNACT1, FcyR1a, FcyR1b, P2RY14, ZBTB16</td>
<td>Protumor</td>
<td>(99-101)</td>
<td></td>
</tr>
</tbody>
</table>

(Continued on the following page)
ILC1 Non-NK: Macrophage activation, chronic inflammation, CD8 T-cell activation

N/A: IFNγ, TNFα

NKG2A, CD56, CD15, CD27, ICOS, IL17, IL23, IL33, IL12, NKG2D, CD11c, CD11b, CD1a, CD14

Antitumor

Antitumor

Antitumor

ILC1 Non-NK: Macrophage activation, chronic inflammation

N/A: IFNγ, TNFα

ICOS, IL10, IL12, IL23, IL27, IL33, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22, IL23, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22

Antitumor

Antitumor

CD56⁺ CD16⁺ NKs: Promote antibody-dependent cellular cytotoxicity, high perforin production, enhanced killing

N/A

CD56⁺, CD16⁺, NKG2A, CD56, CD11b, CD1c, CD14, CD15, CD27, ICOS, ICOSL, IL12, IL23, IL33, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22, IL23, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22

Depends on tumor type

ILC2: Stimulate T-cell responses through Th2-related cytokines, promotes skin inflammation

IL5, IL13

ICOS, IL10, IL12, IL23, IL33, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22, IL23, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22

Protumor and antitumor

ILC3: Chronic inflammation, intestinal homeostasis, lymphoid development, bacterial immunity

IL22, IL23, IL17

CD56, CD127, CD117, CD161, NKG2D, NKG2A, CD56, CD11b, CD1c, CD14, CD15, CD27, ICOS, ICOSL, IL12, IL23, IL33, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22, IL23, IL36, IL4, TNF, TGFβ, IL17, IL6, IL22

Protumor

Protumor

Abbreviation: N/A, not applicable.

Table 1. Innate immune cells in the tumor microenvironment (Cont’d)

NEUTROPHILS

N1: Phagocytosis, release of NETs, inflammatory cytokines, tumor and ROS; respiratory burst, promotion of tumor cell apoptosis

N/A

TNFα, IL1, IL6, NKP30, PGE2, DEFs, RNases, Along with toxic substances and reactive oxygen species, NETs

TNFα, NKP30, PGE2, DEFs, RNases

Antitumor

(23, 30, 33)

N2: Support angiogenesis, cancer cell migration and invasion, immune surveillance, and metastasis as well as secrete chemokines, cytokines and ROS/RNS

TGFβ, Angiotensin II

Oncostatin-M, MMP-9, CXCL1, CXCL8, CXCL10, TGFβ, IL33

Angiogenase, CCL2, CCL5

Protumor

(23, 30, 33)

MDSCs

M-MDSCs: Suppress innate and adaptive immune responses

CSF-1, CCL2, CCL7, HiF1α, CXCL1

NO, NOS, CCL5, Arg1, PGE2, IL4

CD11b⁺, CD14⁺, CD127⁺, CD161⁺

CD11b⁺, Ly6C⁺, Ly6G⁺, CD103⁺

Protumor

(41-45)

PMN-MDSCs: Suppress innate and adaptive immune responses

ROS, Arg1, PGE2, IL4

CD11b⁺, CD14⁺, CD127⁺, CD161⁺

CD11b⁺, Gr-1⁺, Ly6G⁺, Ly6C⁺

Protumor

(41-45)

eMDSCs: Suppress innate and adaptive immune responses

N/A

N/A

N/A

Not well characterized

Protumor

(42)

NK CELLS

CD56⁺ CD16⁺ NKS: Produce inflammatory cytokines

TGFβ, PGE2, IDO, IL10

IFNγ, TNFα

CD16⁺, CD56⁺, NKG2A⁺, CD56⁺, NKG2A⁺

CD16⁺, CD56⁺, NKG2A⁺, CD56⁺, NKG2A⁺

Depends on tumor type

Depends on tumor type

(48, 51)

CD56⁺ CD16⁺ NKs: Promote antibody-dependent cellular cytotoxicity, high perforin production, enhanced killing

TGFβ, PGE2, IDO, IL10

IL22, IL10

CD16⁺, perforin⁺

Not well characterized

Not well characterized

(48, 51)

ILCS

ILC1NK Cells: Cytotoxicity, macrophage activation, chronic inflammation, CD8 T-cell activation

N/A

IFNγ, TNFα

CD56, NKP46, NKP44, IL12RB2, DNAM-1

CD56, NKP46, NKP44, IL12RB2, DNAM-1

Antitumor

(57, 62)

ILC1 Non-NK: Macrophage activation, chronic inflammation

N/A

IFNγ, TNFα

ICOS, IL10, IL12RB2, CCR6

ICOS, IL10, IL12RB2, CCR6

Antitumor

(57, 62)

ILC2: Stimulate T-cell responses through Th2-related cytokines, promotes skin inflammation

IL33, IL25

IL5, IL13

CD17, CD27, ICOS, CD294, IL10, IL33, IL17β, CD161, NKP30, PDI, CRTH2

CD17, CD27, ICOS, CD294, IL10, IL33, IL17β, CD161, NKP30, PDI, CRTH2

Protumor

(57, 62)

ILC3: Chronic inflammation, intestinal homeostasis, lymphoid development, bacterial immunity

IL22, IL17, GM-CSF

CD27, CD17, CD25, IL10, IL13, IL23R, MHCII, CCR6, NKP44, NKP30, CD46, NKP46

CD27, CD17, CD25, IL10, IL13, IL23R, MHCII, CCR6, NKP44, NKP30, CD46, NKP46

Protumor

(57, 62)

Endogenous and exogenous antigens, whereas cDC2s only present exogenous antigens and do not typically perform cross-presentation. cDCs and pDCs are present and active during steady-state conditions, while moDCs tend to only arise during inflammation. DCs specialize in different functions dependent on their stage of maturation and differentiation (Table 1). DCs can localize and acclimate to different tissues such as skin, lung, intestine, and liver and efficiently respond to environmental stimuli (20).

Analogous to Møs, DCs are plastic in nature and can be stratified into specific subtypes. In the context of cancer, DCs are broadly referred to as tumor-infiltrating dendritic cells (TIDCs), which will be the predominant focus of this section. TIDCs can be immunogenic or tolerogenic dependent upon environmental signals. Examples of DCs that contribute to immune suppression include CD56⁺ cDC2s that stimulate Th2, Th17, and T regulatory responses (19). It is important to note that each of the subtypes
Tumors classically reprogram their microenvironment to support their survival. In the context of DCs, they do so by secreting cytokines to upregulate transcriptional and metabolic pathways that promote a tolerogenic phenotype, such as those that involve IDO, Arg1, INOS, and STAT3 (21). These pathways trigger alterations in DC metabolism, metabolite production, energetic shifts, and/or alterations of chromatin accessibility (22). These modifications impact every aspect of DC functionality, including their abilities to secrete inflammatory cytokines and to prime effector T cells. Generally, DCs patrolling the TME encounter immune-suppressive factors such as VEGF, IL10, TGFβ, prostaglandin E2 (PGE2), and other cytokines (seen in Fig. 1B) that inhibit DC maturation into immunogenic cells and promote their development into a tolerogenic phenotype, not only stunting their Th1-priming capacities, but also affording them the ability to promote Th2 and T regulatory responses (20). Once removed from the TME, these DCs regain their ability to effectively process antigen and prime T cells (23), demonstrating that stimulating DC inflammatory functions in the TME may be an effective therapeutic strategy.

Further complexity regarding DC plasticity arises when considering different tumor types. DCs have been reported to be tumor-promoting in some TMEs, and tumor-suppressive in others. For example, TIDCs correlate with a positive prognosis and prime T cells (23), demonstrating that stimulating DC inflammatory functions in the TME may be an effective therapeutic strategy.

Neutrophils

Neutrophils account for up to 70% of circulating leukocytes and are the first line of defense against pathogens (28). These cells are typically short-lived, persisting up to five days in circulation (29). Upon tissue damage or infection, epithelial cells secrete neutrophil homing chemokines, compelling them to extravasate from circulation and enter the damaged tissue where they secrete inflammatory cytokines, release neutrophil extracellular traps (NET), and phagocytose invading microorganisms (30). NETs are composed of a chromatin backbone as a vehicle for antimicrobial peptides and toxins and are released as a further method of attack, although to the detriment of the neutrophil (31, 32). In the context of cancer, tumor-associated neutrophils (TAN) also follow the Th1/Th2 paradigm and exhibit an N1 (tumor-suppressive) or N2 (tumor-promoting) phenotype (Table 1). The phenotype of neutrophils in the TME depends on the tumor type and the stage of disease progression. Neutrophils are inflammatory during early tumor stages, but as the tumor progresses, they adopt an immunosuppressive phenotype (33). Neutrophils mediate inflammation via production of reactive intermediates (ROS/RNS). They also reconfigure the extracellular matrix through secretion of neutrophil elastase (NE) and matrix metalloproteinases (MMP8/9) in the TME and promote angiogenesis (Oncoaptin-M), tumor progression (PGE2), and invasion (through the release of ROS/RNS, NE, MMP-9). NETs are comprised of MMPs, cathepsin G, and NE (34, 35). These proteases degrade proinflammatory cytokines and reposition the TME to enhance tumor progression and aid in metastasis (36).

The plasticity of circulating neutrophils is an important feature in patients with cancer. These neutrophils, called high-density neutrophils (HDN) or low-density neutrophils (LDN), correspond to N1 and N2 phenotypes, respectively. In many cancer types, LDNs, which exhibit a more immature phenotype, predominate in the circulation and may contribute to cancer progression and metastasis (29). A detailed understanding of neutrophils and signals that pivot neutrophils to become immune suppressive holds much promise toward reprogramming the TME. This is important given that they are present in the tumor in large numbers. The unique mechanism of NET-osis (NET formation) may prove to be a promising therapeutic target. While preclinical models demonstrate effectiveness of NET targeting, evidence on the clinical front is awaited.

Myeloid-derived suppressor cells

Another cell type that can be found in the TME includes myeloid-derived suppressor cells (MDSC). Some argue that MDSCs are a subtype of neutrophils (33), as there are several overlapping markers between MDSCs and TANs that make distinguishing between these cell types challenging. It is still debated whether MDSCs represent a separate lineage of cells or are polarized immature neutrophils (37). Despite this quandary, MDSCs are defined as, “a heterogeneous population of cells of myeloid origin that comprise myeloid progenitor cells and immature macrophages, immature granulocytes, and immature dendritic cells” (38). Accordingly, MDSCs and TANs clearly differentiate into distinct cell types even though they both stem from myeloid progenitor cells. Other than being hypodense, MDSCs are divergent from neutrophils in several ways, including reduced expression of CD16 and CD62L, and increased expression of Arg1, CD66b, and CD11b (39, 40). MDSCs can be further categorized into subsets: monocytic MDSCs (M-MDSC), which are distinguished by a CD11bhi, Ly6Chi, and Ly6Glo phenotype, and early-stage MDSCs (eMDSC) that are CD13+ and CD14+, and CD33+ in humans (41, 42). It is noteworthy that both M-MDSCs and PMN-MDSCs present within the TME have an enhanced suppressive phenotype when compared with MDSCs present within peripheral lymphoid organs, due to increased expression of suppressive molecules by MDSCs in the TME (43).

MDSCs present in the TME contribute to immunosuppression, including T-cell suppression and innate immune regulation, through various mechanisms (Table 1; ref. 43). Furthermore, MDSCs sculpt the primary TME and also initiate formation of the premetastatic niche. In particular, MDSCs enhance tumor cell stemness, increase angiogenesis, and advance the metastatic process by promoting EMT through I6 secretion (44, 45). MDSCs also influence the TME (Fig. 1B) that further perpetuates their inherent immunosuppressive functions. For example, HIF-1α, a key player in the hypoxic tumor microenvironment, aids in MDSC differentiation to tumor-promoting TAMs (46). Also,
factors in the TME can alter the metabolism of MDSCs toward fatty acid oxidation, prompting an upregulation of Arg1 and NOX2 production (47). The critical role of MDSCs in tumorigenesis, growth, the establishment of the premetastatic niche, and metastatic outgrowth warrants the need to effectively target them by depletion or blockade. Although their critical role in the survival and advancement of tumors is well known, there are currently no FDA-approved drugs or therapies that directly target MDSCs.

Natural killer cells and natural killer T cells

NK cells are circulatory, innate lymphoid cells recognized for their cytotoxic effector functions. Classically, there are two subsets of NKS defined by their expression of CD16 and CD56 levels: namely, CD56hi CD16lo and CD56lo CD16hi (48). CD56hi CD16lo NKS secrete inflammatory cytokines, whereas CD56lo CD16hi NKS specialize in cytotoxic functions and cell-mediated killing. Within the cancer framework, these cells are extremely efficient in eliminating malignant cells and limiting tumor metastases (49). Their significance in tumor surveillance is illustrated by a correlation between low NK-cell activity and increased cancer risk (50). NKS employ death receptor-mediated apoptosis and perforin/granzyme-mediated cytotoxicity to target tumor cells and limit primary tumor growth (51). While NKS characteristically destroy circulating tumor cells, they are much less efficient at cell killing within the TME. Tumors deploy many mechanisms to evade destruction by NKS, including coating themselves in collagen to engage inhibitory NK receptors and utilizing platelets as a shield to avoid NK attack (52). Within the TME, both NKS exhibit reduced inflammatory cytokine production and reduced or no cytotoxicity and both subsets will be referred to collectively as tumor-infiltrating natural killer cells (TINKs). Many cytokines commonly present in the TME diminish NK effector functions (Table 1). These cytokines can stunt the cytotoxicity of TINKs (Fig. 1B), which not only display diminished cytotoxicity, but also contribute to arresting the proliferation and expansion of T cells, enhancing their immune-suppressive properties (these cells are often referred to as NKregs as well). Future efforts for developing therapeutic approaches could consider augmentation of cytotoxic NKS and/or targeting of TINKs. It is tempting to speculate that administration of NKS may enable a cancer-preventative approach, or at the very least a metastasis-preventative approach as NKS are extremely efficient at targeting circulating cancer cells.

Also prevalent in the TME are natural killer T cells (NKTs), which are CD41d restricted, innate-like T lymphocytes that, like T cells, possess a T-cell receptor, and like NKS, respond quickly to antigenic exposure (53). Also, like T cells, overstimulation of NKTs can render them anergic. There are two major types of NKTs, type 1 NKTs (NKT1) and type II (NKTII) cells, which are characterized by their distinct T-cell repertoires. While NKTs express the Vα14Jα18 invariant TCR alpha chain, the T-cell repertoire of NKTII is less defined (54). Both types can be dissected into further subsets that reflect the T-cell subsets that play inflammatory or immune-suppressive roles in the context of the TME. Specifically, NKTs can be divided into Th1-like, Th2-like, Th17-like, Treg-like, and T follicular helper (TFH)-like NKTs; and NKTII can be divided into Th1-like and Th2-like NKTs. Furthermore, NKTs are reported to switch back and forth between inflammatory and immune-suppressive subsets in response to their environment. In particular, NKTIs are typically antitumor, whereas NKTIIIs are predominantly protumoral. NKTIs have been reported to prevent metastatic breast cancer (55) in mouse models. However, NKTIIIs have been reported to support MDSCs in a B-cell lymphoma mouse model (54, 56). As such, targeting NKTIIIs and supplementation with NKTIs may provide an exciting therapeutic approach.

Innate lymphoid cells

Another crucial component of the TME is the ILCs that have characteristics similar to those of NK cells. ILCs share a common lymphoid progenitor with B and T cells, but lack B- and T-cell receptors and are thus classified as innate immune cells (57). ILCs contribute to T-cell polarization through antigen presentation and cytokine secretion (58). There are three types of ILCs (ILC1, ILC2, and ILC3) classified on the basis of their production of Th1, Th2, and Th17-based cytokines and distinct transcription factors (59). ILC1s tend to exhibit antitumor functions through cytokine production (mainly IFNγ). Furthermore, ILC1s can be divided into NK ILC1s and non-NK ILC1s based on their expression or lack thereof of the NK-specific transcription factor, Eomesoderm. Importantly, NK ILCs can be distinguished from conventional NKS by differences in transcriptional regulation, phenotype, and localization as described by Seillet and colleagues (60). While ILC2s can functionally either promote or antagonize tumor growth depending on the tumor type (Fig. 1), ILC3s are classically protumorigenic. ILC polarization is determined by the composition of each specific TME (Table 1). As such, ILCs are differentially associated with different tumor types, likely because different tumor types have distinct TME compositions; for example, ILC2s are typically found in the TME of breast and colon cancer, ILC3s are implicated in colorectal cancer (61, 62), and ILC1s prevent melanoma growth through the production of inflammatory cytokines (63, 64). ILC3s may differentiate into ILC1s upon IL12 stimulation, and ILC1s may differentiate into ILC3s upon stimulation by retinoic acid and IL23 (62). The conversion of ILC1 to ILC3 stunts their ability to aggressively target the tumor. This plasticity offers an attractive opportunity for therapeutically reprogramming ILC3s to ILC1s.

Immune cells and other components of the microenvironment

While the importance of direct interactions between tumor cells and immune cells is clear, it is also noteworthy to mention that immune cell interactions with other components in the TME can impact cancer progression. For example, it has been reported that the extracellular matrix can play both supportive and inhibitory roles to the adaptive immune response by providing migratory pathways that allow T cells to invade the tissue or by directly inhibiting T-cell proliferation, respectively (65). Also, lymphatic vessels can regulate the immune microenvironment. Lymphatic vessels have been linked to providing nutrients to tumors through increased angiogenesis. They may also serve as migratory highways for immune cells (66), and lymphatic endothelial cells have also been reported to directly regulate DC activation (67). Immune cells also interact with stromal cells, including cancer-associated fibroblasts (CAFs). CAFs exhibit wound-healing properties and have been implicated as contributors to tumor proliferation, invasion, and metastasis. CAFs may secrete immune-suppressive cytokines that polarize Mps to the M2 phenotype and contribute to CD8+ T-cell exhaustion and deletion (68).

These observations indicate a complex series of interactions between immune cell types and nontumor cells within the TME that clearly impact tumor progression, invasion, and metastasis. Therefore, not only should therapy designs consider tumor-
immune cell cross-talk and tumor–stromal cross-talk, but also stromal–immune cell cross-talk as it contributes significantly to tumor development.

**Current and future therapeutics**

The tumor masterfully controls its surrounding environment to promote its establishment, growth, survival, and spread. One of the chief ways it does this is through reprogramming innate immune cells to foster tumor growth and survival, leaving the patient with a weakened defense and often a worse prognosis. This is a potential Achilles heel of the tumor; as such, reprogramming the innate immune system is a potentially important approach to improve patient outcomes.

**Macrophage therapies**

Previous clinical trials targeting Mφs in the TME have been unsuccessful. Many prior trials involved the activation and injection of Mφs into patients with cancer using various activation methods such as IFNγ, mifamurtide, and LPS, but none of these methods were therapeutically efficacious (69–71). There have been some promising clinical trials utilizing anti-M-CSFR antibodies. One such example includes the administration of RG7155, an anti-M-CSFR antibody, to diffuse-type giant cell tumor (Dt-GCT) patients. This strategy led to decreased TAM infiltration and overall positive patient responses (72). It is noteworthy that anti-M-CSFR antibodies have yet to be successful in glioblastoma models, and there is still work to be done on this front. Ongoing clinical trials that target Mφ receptor, CSF-1R, and the CCL2–CCR2 signaling axis ablate tumor-infiltrating Mφs and show promise in advanced solid tumors (73). Moreover, the efficacy of CSF-1R inhibition is vastly improved when combined with receptor tyrosine kinase inhibitors. In addition to targeting CSF-1R and the CCL2–CCR2 signaling axis, there are ongoing clinical trials targeting CXCL12/CXCR4, CD40, and angiopoietin1/2 (74). Treatment with IFNα has yielded favorable outcomes in patients with melanoma. IFNα promotes an inflammatory environment, stimulates Mφs toward an M1 type, and has been demonstrated to reduce tumor growth and diminish metastasis (75).

**DC therapies**

Targeting DC activation via DC vaccination is another therapeutic option. An important consideration in using DC vaccinations as cancer treatment is the method of priming DCs with tumor antigen. Options including priming with whole tumor cells, tumor cell lysate, apoptotic bodies, exosomes, or DNA or RNA need to be considered when designing an effective DC vaccine (76–78). Thus far, whole-cell vaccines seem to be the most promising. Several DC vaccination trials are currently ongoing (clinicaltrials.gov). One trial (NCT01204684) involves enrichment of DCs from patients with glioma, pulsation with tumor lysate, and autologous intradermal injection. In their phase I clinical trial, Hus and colleagues primed DCs from patients with B-cell chronic lymphocytic lymphoma with tumor lysates and autologously vaccinated patients with these primed DCs (79). This strategy resulted in an increase in cytotoxic T-cell response. An example of a successful DC-based therapy for prostate cancer is Provenge. The regimen for Provenge therapy involves harvesting monocytes from prostate cancer patients, differentiating and activating them in vivo with PAP antigen, and introducing them back into the patient. This therapy has achieved significant success marked by diminished tumor burden in patients with prostate cancer. A new DC vaccine targeting glioblastoma is DCVac-L, that includes autologous DCs loaded with glioblastoma tumor lysate. This vaccine has been tested in a phase III clinical trial for glioblastoma, and overall patient survival was shown to increase by 6 months (80).

Despite success with DC vaccinations, there are challenges associated with them, including high cost, the absence of universal vaccine, the need for massive amounts of DCs, and issues with polarizing conventional DCs in vitro. Previous attempts at DC vaccinations focused on moDCs that are rare and do not functionally resemble cross-presenting DCs in vivo (81). It is now recognized that cDCs comprise the DC subtype that is most likely to come into contact with cancer cells in the TME and mount the ensuing immune response. While cDCs are challenging to isolate, a cDC vaccine for melanoma has been reported to elicit a cytotoxic T-cell response making them functionally more relevant (82). Further work is required to standardize methods to effectively isolate cDCs for antigen loading and DC vaccination. A new focus for DC therapy involves directly targeting them in vivo. In vivo delivery of antibodies to cDC1 receptors conjugated to tumor antigens results in better DC activity and a higher rate of primed T cells. This is expected to reduce treatment costs due to the universality of the therapy and improve therapeutic effectiveness because DCs in vivo are already at the tumor site (in contrast to direct tumor injections that are not always possible or effective depending on tumor type). Combining this approach with immune checkpoint inhibitor blockade therapy will allow for rapid, effective T-cell priming without T-cell exhaustion.

**Neutrophil therapies**

There are ongoing efforts to target neutrophils in the TME. Preclinical models have yielded optimistic success in reducing neutrophil number by squelching G-MCSF from the TME. Reparixin is a noncompetitive allosteric inhibitor of CXCR1 and CXCR2 (83) and targets neutrophil maturation to inhibit the immunosuppressive impact of tumor-induced N2 neutrophils. Reparixin is currently in one phase I and two phase II clinical trials for metastatic breast cancer. Targeting neutrophil polarization is another enticing therapeutic option through TGFβ inhibitors (84). While there are currently many clinical trials that use TGFβ inhibitors, off-target effects, and cytotoxicity have been reported (85).

**MDSC therapies**

There are currently several ongoing clinical trials that target MDSCs in different cancer types including leukemia, melanoma, glioblastoma, and breast cancer (86). These trials utilize different mechanisms of indirectly impacting MDSC function, including targeting Arg1, iNOS, and STAT3 activities, metabolism through CD36, and trafficking through CXCR2 (86). MDSC depletion is another tested avenue for cancer therapeutics. There has been some success in triggering MDSC apoptosis with gemcitabine and 5-fluorouracil, correlating with diminished tumor growth. Docetaxel, doxorubicin, paclitaxel, and tyrosine kinase inhibitors have also been demonstrated to reduce the numbers and effectiveness of MDSCs in the TME (86). There also are therapies targeting MDSCs in combination with immune checkpoint inhibitors. A phase I/II clinical trial in patients with renal cell
carcinoma using atezolizumab and a histone deacetylase inhibitor shows promise (NCT03024437). Also, a phase II clinical trial in patients with melanoma combines ipilimumab and ATRA, which blocks retinoic acid signal transduction, leading to the differentiation of MDSCs into Mps and DCs (NCT02403787). ATRA alone also leads to a reduction of MDSC frequencies in small-cell lung cancer (87, 88). While these trials show moderate yet encouraging success, off-target effects of these drugs may contribute to diminished therapeutic efficacy.

NK-cell therapies

Multiple enduring clinical trials aim to stimulate the immune system with NK-cell therapy. For example, there is a phase I trial targeting advanced biliary tract cancer via allogeneic NK injection (NCT0358849). Yang and colleagues pioneered allogeneic NK-cell therapy by activating allogeneic NKS with IL2, followed by administration to patients with advanced lymphoma (89). The results revealed diminished T-reg and MDSC populations and increased expression of NKGD on cytotoxic T cells (90). NK-cell therapy in combination with chemotherapy for small-cell lung cancer (NCT03410368) is also an effective strategy (91). Also, the use of CAR-NK cells, genetically engineered cells that directly target tumor-specific antigens in an HLA-unrestricted manner, has shown favorable outcomes in preclinical studies for B-cell malignancies, ovarian, breast, prostate, and colon cancers (92). All of these approaches have exhibited varying degrees of positive outcomes, but they also are limited by toxicity and detrimental side effects, high cost, and low efficacy (51, 53). In contrast, there have been few successful clinical trials for ILC therapy in cancer.

Conclusion

Each of the therapeutic approaches discussed in this review has focused on targeting one aspect of the immune system. While some of these treatments yield positive outcomes, a more definitive and likely more effective approach involves altering multiple facets of the TME through a strong inflammatory response by promoting the inflammatory innate immune cells. There are multiple strategies that target immune-suppressive cells, but unfortunately many of these responses are important for self-tolerance mechanisms and aid in protection against autoimmunity. Targeting immune-suppressive cells cannot focus on a global depletion of all innate cells in the TME as this could cause dire effects in the host. The solution must be an intricate combination that involves selective inhibition or depletion of robust tumor-suppressive cytokines and cell types in addition to bolstering the inflammatory phenotype of immune cells.

Disclosure of Potential Conflicts of Interest

L.A. Shevde is a consultant/advisory board member for NIH and CDMRP. No potential conflicts of interest were disclosed by the other author.

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