Monocytes in tumor: The perspectives of single-cell analysis

Xin Fu and Mingzhu Yin*

Department of Dermatology, Huan Engineering Research Center of Skin Health and Disease, Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China

Abstract

Infiltration of mononuclear phagocytes into the tumor microenvironment (TME) is known to orchestrate the tumor progression and is generally considered to interfere with the efficacy of immune checkpoint blockade therapies. For a more precise definition of monocytes, we review some recent advances in the functions of monocytes and macrophages in TME based on single-cell analysis. We also summarize the details of the different subpopulations of monocytes and macrophages involved in immunotherapy and their potential applications in clinical studies. In this review, we briefly introduce the developmental trajectory of mononuclear phagocytes, including monocytes, tumor-associated macrophages, dendritic cells and myeloid-derived suppressive cells, and their functions in TME. In this review, the potential of monocytes and their derived cells as diagnostic and therapeutic targets, with regard to the interaction between monocytes and immune checkpoint therapies, is also summarized.

Keywords: Monocytes; Single-cell RNA sequencing analysis; Tumor microenvironment; Tumor-associated macrophages; Dendritic cells

1. Introduction

Monocytes and macrophages are major components of the mononuclear phagocyte system (MPS), which consists of a wide range of phagocytic cells derived from bone marrow and yolk sac progenitors[1]. Monocytes differentiate into macrophages and dendritic cells (DCs) in the tumor microenvironment (TME)[2]. Tumor-associated macrophages (TAMs) are abundant in tumor stroma both in mice and humans[3]. Some publications have also demonstrated different TAM subtypes and their diverse functions in tumor progression[4,5]. Previous studies have shown that monocytes/macrophages are important regulators of tumor progression.

As an important component of TME, monocytes are probably thought to be closely related to the efficacy of immune checkpoint blockade (ICB) immunotherapy. Several studies have revealed that monocytes express programmed cell death protein 1 (PD-1), and that CD14+CD16+HLA-DRhi monocytes could be potential indicators for responsiveness to ICB therapy[6]. To study monocytes that infiltrate into tumor tissue, a universal nomenclature and classification system is needed to ease the demonstration of further related findings about monocytes. There are a few reliable markers on the cell
Monocytes from single-cell analysis

In late monopoiesis, the bone marrow-derived monocytes can be divided into two subpopulations—"classical" Ly6C\(^{−}\)CX3CR1\(^{hi}\) (CCR2\(^{−}\)CD62L\(^{−}\)) and "nonclassical" Ly6C\(^{+}\)CX3CR1\(^{lo}\) (CCR2\(^{+}\)CD62L\(^{+}\)) monocytes\(^{[19]}\). In their subsequent work, researchers demonstrated an intermediate cell state, which is Ly6C\(^{hi}\)CD43\(^{hi}\)CX3CR1\(^{hi}\) in mice and CD14\(^{+}\)CD16\(^{+}\) in humans\(^{[20]}\). The difference between the two subtypes is that Ly6\(^{+}\) monocytes circulate in blood\(^{[21]}\), while classical inflammatory monocytes (CCR2\(^{+}\)-Ly6C\(^{−}\) in mice and CCR2\(^{+}\)CD14\(^{+}\)CD16\(^{+}\) in humans) are recruited to tumor sites where they increase the macrophage content and promote tumor growth and metastasis\(^{[22]}\). However, little is known about whether this kind of classification corresponds to the functional specialization of distinct subsets. Ly6C\(^{−}\) monocytes can be recruited and differentiated into macrophages or DCs in tissue, while Ly6C\(^{+}\) monocytes circulate in the blood and play a role as scavengers\(^{[23]}\).

Single-cell analysis is critical to providing new insights for studying human cancer. For example, researchers can study the cell components in the complex microenvironment of acute myeloid leukemia through scRNA-seq to investigate tumor progression\(^{[24]}\). Zilionis \textit{et al}. recently uncovered 25 tumor-infiltrating myeloid cells (TIMs) in non-small cell lung cancer patients using single-cell transcriptomics methods, and also profiled TIMs in mice. Compared to TIMs across species, they identified an almost congruence of population structures among DCs and monocytes, and found conserved neutrophil subsets and species differences among macrophages\(^{[25]}\). Moreover, Puram \textit{et al}. applied single-cell transcriptomic analysis of nearly 6000 single cells from 18 head and neck squamous cell carcinoma patients, refined the subtypes of tumors, and gave new insights into the ecosystem\(^{[26]}\).

Cell subpopulations are traditionally defined according to a combination of morphology, localization, functions, ontogeny, and expression of a restricted set of molecular markers. The aforementioned classification of three types of monocytes (classical, nonclassical, and intermediate) was first conducted in the late 1980s by using two-color flow cytometric detection of CD14 and CD16 antigens on human peripheral blood mononuclear cells (PBMCs)\(^{[27]}\). Single-cell transcriptional profiling has challenged our understanding of heterogeneity in monocytes and other well-established immune cell populations\(^{[28]}\). Single-cell RNA sequencing can identify these cells easily and quickly in an unbiased and precise way.

Recent technological advances that allow single-cell analysis of phenotypic and transcriptional states have revealed a vast heterogeneity of tumor-associated monocytes/macrophages. Villani \textit{et al}. revealed four
### Table 1. Origin, fate, and function of monocyte

<table>
<thead>
<tr>
<th>Origin</th>
<th>Development</th>
<th>Cell types</th>
<th>Subsets</th>
<th>Surface markers</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow (and yolk sac</td>
<td>Bone marrow stem cell—Granulocyte—Monocyte</td>
<td>Monocytes</td>
<td>Classical</td>
<td>Ly6C&lt;sup&gt;+&lt;/sup&gt;CX3CR1&lt;sup&gt;+&lt;/sup&gt; (CCR2&lt;sup&gt;−&lt;/sup&gt;CD62L&lt;sup&gt;−&lt;/sup&gt;) / CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;−&lt;/sup&gt;</td>
<td>A classical monocyte frequency 19.38% before anti-PD-1 therapy initiation means better responsiveness to anti-PD-1 therapy and longer survival for melanoma patients. The responsiveness might depend on the number of CD14&lt;sup&gt;+&lt;/sup&gt;CD16 HLA-DR&lt;sup&gt;+&lt;/sup&gt; monocytes. Produce VEGF and facilitate angiogenesis via NF-κB and IL-1-IL-1R axis.</td>
<td>[6]</td>
</tr>
<tr>
<td>marrow (and yolk sac progenitors)</td>
<td>progenitor cells—Pro-monocytes—Mature monocytes (Ly6C&lt;sup&gt;+&lt;/sup&gt;inflammatory monocytes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>Ly6C&lt;sup&gt;+&lt;/sup&gt;CD43&lt;sup&gt;+&lt;/sup&gt;CX3CR1&lt;sup&gt;hi&lt;/sup&gt; in mouse/CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;−&lt;/sup&gt; in human (CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;−&lt;/sup&gt;)</td>
<td>Undefined</td>
<td></td>
<td></td>
<td></td>
<td>[29]</td>
</tr>
<tr>
<td>Nonclassical</td>
<td>Ly6C&lt;sup&gt;+&lt;/sup&gt;CD43&lt;sup&gt;+&lt;/sup&gt;CX3CR1&lt;sup&gt;hi&lt;/sup&gt; (CCR2&lt;sup&gt;−&lt;/sup&gt;CD62L&lt;sup&gt;−&lt;/sup&gt;) / CD14&lt;sup&gt;−&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt;</td>
<td>CD16&lt;sup&gt;+&lt;/sup&gt;monocytes (or their progeny) release VEGF, then generates a positive feedback loop that recruits more pro-angiogenic monocyte-derived cells.</td>
<td>[81]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEMs</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD16&lt;sup&gt;+&lt;/sup&gt;Tie2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>TEMs are pro-angiogenic for hepatocellular carcinoma</td>
<td>[85]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages TAMs</td>
<td>F4/80&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Efficiently cross-present tumor antigens to CD8&lt;sup&gt;+&lt;/sup&gt;T cells</td>
<td>[40]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F4/80&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>M (IL-4), M (Ic), M (IL-10), M (GC+TGF-β), M (GC), M (LPS), M (LPS+IFN-γ), and M (IFN-γ)</td>
<td>Anti-tumor and promote immune responses</td>
<td>[103]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>M2a, M2b</td>
<td>Expression of the CXCL9 by TAMs regulates the recruitment and positioning of CXCRC3-expressing stem-like CD8&lt;sup&gt;+&lt;/sup&gt;T.</td>
<td>[134]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIE2+macrophages</td>
<td>Benefit vasculature formation and then promote tumor metastasis.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[86]</td>
</tr>
<tr>
<td>Yolk sac progenitors</td>
<td>Common dendritic progenitor (CDP)—Pre-plasmacytoid DCs—Pre-conventional DCs (pDCs)—Pre-conventional DCs (cDCs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloid-derived suppressive cells (MDSC)</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;DC1</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;DC1</td>
<td>CD103&lt;sup&gt;+&lt;/sup&gt;DC2</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD33&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;+&lt;/sup&gt; (CD11b&lt;sup&gt;+&lt;/sup&gt;Gr-1&lt;sup&gt;+&lt;/sup&gt;Ly6C&lt;sup&gt;+&lt;/sup&gt;Ly6G&lt;sup&gt;−&lt;/sup&gt; cells in mice)</td>
<td>Suppress T cell function.</td>
<td>[115]</td>
</tr>
<tr>
<td>Monocytic DCs (moDCs)</td>
<td>CD103&lt;sup&gt;+&lt;/sup&gt;DC2</td>
<td>Antigen processing and presentation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytic (M-MDSC)</td>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;VEGFR1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Facilitate tumor growth.</td>
<td></td>
<td></td>
<td></td>
<td>[137]</td>
</tr>
<tr>
<td>Granulocytic (G-MDSC)</td>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;-&lt;/sup&gt;low</td>
<td>In melanoma and hepatocarcinoma patients.</td>
<td></td>
<td></td>
<td></td>
<td>[47; 50]</td>
</tr>
<tr>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;CD14&lt;sup&gt;+&lt;/sup&gt;HLA-DR&lt;sup&gt;-&lt;/sup&gt;, CD14&lt;sup&gt;+&lt;/sup&gt;PD-L1&lt;sup&gt;+&lt;/sup&gt;, CD15&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Frequencies of MDSC monocytes are helpful for identifying advanced melanoma patients and make therapeutical choices.</td>
<td></td>
<td></td>
<td></td>
<td>[134]</td>
<td></td>
</tr>
<tr>
<td>CD11b&lt;sup&gt;+&lt;/sup&gt;VEGFR1&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Promote tumor angiogenesis</td>
<td></td>
<td></td>
<td></td>
<td>[84]</td>
<td></td>
</tr>
</tbody>
</table>

VEGF, vascular endothelial growth factor; NK-kB, nuclear factor kappa B; IL, interleukin; IL-1R, Interleukin-1 receptor; TEMs, TIE2-expressing monocytes/macrophages; TAMs, tumor-associated macrophages; TGF-β, transforming growth factor beta; LPS, lipopolysaccharide; IFN-γ, interferon gamma; CXCL9, chemokine CXC motif ligand 9; CXCR3, CXC motif chemokine receptor 3; moDCs, monocyte-derived dendritic cells.
monocyte types after performing a single-cell RNA sequencing of ~2400 cells isolated from healthy blood donors and enrichment for HLA DR+ lineage cells[29]. Among them, Mono1 and Mono2, as the two largest clusters, contain the previously defined "classical" (CD14+CD16−) and "nonclassical" subtypes (CD14+CD16+). Besides, these two subtypes also contain 88 of the 124 cells derived from the "intermediate" monocyte gate (CD14+CD16+). Mono3 and Mono4, as the two additional clusters, include 40 of the 124 "intermediate" cells and express some of the Mono1 (classical monocyte) signature genes associated with cell cycle, differentiation, and activation of NK and T cells. The "intermediate" (CD14+CD16+) monocytes are usually distributed across the four types of monocytes. However, it should be noted that more studies are needed to validate the existence and function of the two new clusters. For this heterogenous intermediate state, scRNA-seq helps uncover a part of potential mechanisms related to the differentiation, that is, CCAAT-enhancer-binding protein beta (C/EBPβ), that activates a survival factor of monocyte and thus promotes the differentiation of Ly6C+ cells into Ly6C− cells.[33].

Both classical and non-classical monocytes can exhibit pro-tumoral or anti-tumoral functions in tumors. Of note, classical monocytes differentiate into pro-tumoral TAMs[30,31], inhibit function of T cells[32] and contribute to angiogenesis[33], while monocytes can also be cytotoxic to tumor cells[33] and facilitate antigen presentation[34]. On the other hand, non-classical monocytes mainly show anti-tumoral function, such as phagocytosis of tumor material, prevention of tumor metastasis,[35] and inhibition of Tregs.[36]. However, Tie-2+ monocytes can also promote angiogenesis in several tumors[37,38].

2.2. Origins and fates of monocytes

2.2.1. Macrophages

Macrophages develop from bone marrow stem cells, and then go through their cell cycle as granulocyte-monocyte progenitor cells, pro-monocytes, and mature monocytes[39]. Ly6C+ monocytes that patrol in the peripheral blood are responsible for detecting pathogens and maintaining vascular integrity, while Ly6C− monocytes are recruited to different tissues during infection or injury to mediate extravascular inflammatory responses. After entering various tissues, Ly6C+ inflammatory monocytes differentiate into macrophages[40,41]. Macrophages in adults derive from at least three origins, that is, yolk sac, fetal liver, and bone marrow. The progenitors in yolk sac populate all tissues and form the F4/80hi macrophage subtype in diverse tissues. In addition, resident macrophages are mainly regulated by colony stimulating factor 1 (CSF-1).

From the bone marrow, the progenitors give rise to circulating monocytes and their progeny, such as F4/80hi macrophages and DCs. The generation of macrophages from fetal liver is unclear; however, these macrophages may contribute to the evolution of Langerhans cells in adults[40].

2.2.2. DCs

In mice, DCs and monocytes arise from a macrophage/dendritic progenitor[42], which produces monocytes and a common dendritic progenitor (CDP) that is restricted to the DC fate. The CDP produces plasmacytoid DCs (pDCs) and conventional DCs (cDCs), the latter of which leaves the bone marrow and circulates in the blood before entering tissues and developing into different DC subsets[43]. The previously identified subset of Ly6C+ monocytes expressed DC-related genes that encode CD209a and MHC Class II (MHCII), and granulocyte-macrophage CSF (GM-CSF) stimulates the differentiation of Ly6C+ monocytes into DCs[44].

2.2.3. Myeloid-derived suppressive cells (MDSCs)

The concept of MDSCs was first suggested by Gabrilovich[22]. In cancer patients, MDSCs are typically CD11b+CD33−CD14+HLA DR+ cells and their expression of CD15 and other markers are different between subtypes[45]. Of note, a new subpopulation of MDSC, CD14+HLA DR+−/lo, identified in melanoma and hepatocarcinoma patients is emerging. This indicates that different subtypes of MDSC in human tumors are similar to those in mice[45,50]. Monocytes may develop into M-MDSCs but monocytes cannot be distinguished from M-MDSCs through markers. Increased G-MDSCs but not M-MDSCs can be detected in many cancer types[52]. Inflammation promotes the accumulation of MDSCs that down-regulates immune surveillance and antitumor immunity, thereby facilitating tumor growth[37]. C-C chemokine receptor type 2 (CCR2) drives monocyte polarization to MDSCs and M2-like macrophages, thereby facilitating tumor growth in patients with lung adenocarcinoma[53].

Understanding the origin and fate of monocytes will help in understanding the function of monocytes in tumors. The monocytes circulating in peripheral blood are developed from common monocyte progenitor (cMoP), a lineage-committed bone marrow progenitor[44]. In both mouse and human, cMoPs express stem cell marker
CD117, C-type lectin CLEC12A and CD64. Besides, in contrast to mouse cMoPs, human cMoPs express CD135, a cytokine receptor and early hematopoietic marker[55].

Using single-cell transcriptome profiling on the re-granulocyte-macrophage progenitor (pre-GM), Drissen et al. identified different differentiation pathways of myeloid cells[56]. To be specific, they found that mast cells, eosinophils, megakaryocytes, and erythroid cells are generated through a pathway expressing the gene encoding the transcription factor GATA-1, while monocytes along with neutrophils and lymphocytes are generated through a pathway lacking expression of that gene. This indicates a new pattern of an early hematopoietic-lineage bifurcation that separates the myeloid lineages.

CSF-1 tightly correlates with tissue morphogenesis[57]. Soluble CSF-1 in TME makes macrophages trophic to tumor cells, and CSF-1 and interleukin (IL)-6 impede the maturation of DCs together. CSF-1 expressed on the surface of tumor cells along with IL-4, IL-12, IL-13, and GM-CSF promotes maturation of DCs. IL-4, IL-10, IL-13, and other cytokines in the TME can activate TAMs and promote different immune responses[58]. Taken together, the molecular cytokines in the microenvironment fundamentally dictate whether TAMs promote or inhibit tumor progression[59]. M1 macrophages can be activated in response to microbial products, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and GM-CSF, followed by activation of Toll-like receptor signaling pathways[60]. A single-cell transcriptomic study that analyzed DCs and monocytes of 333 individuals suffering from single lymph node melanoma metastasis revealed that IFN-γ signature and 227-gene signature are highly positively correlated. They also found that different immune mononuclear phagocytes shared a conserved steady-state program during differentiation and entry into healthy tissue[60].

Cytokines including IL-4, IL-13, IL-10, IL-33, and IL-21, transforming growth factor factor beta (TGF-β), M-CSF/CSF-1, and glucocorticoids induce M2 activation programs[61,62,63,64]. Michielon et al. had established an in vitro three-dimensional (3D) organotypic human melanoma-in-skin model and revealed that IL-10 is partly responsible for the transformation of monocytes into M2-like macrophages (defined as CD163+ PD-L1+)[65]. Furthermore, monocytes orchestrated GM-CSF and IL-4 stimulation, resulting in a mode- and time-dependent differentiation relying on nuclear receptor corepressor 2 (NCoR2), a transcriptional regulator[62]. IL-1β also regulates the TAM/macrophage polarization by regulating expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in glioblastoma[66].

As mentioned previously, macrophages undergo a continuum of functional states that cannot be simply explained by polarization theory. M2 macrophages are classified into four subsets and are activated through different mechanisms. On the other side, another report may contradict previous findings. Franklin et al. found that RBPJ (an important transcriptional regulator of the Notch signaling pathway) was fundamental for the differentiation of inflammatory monocytes into TAM[67]. TAMs are phenotypically and functionally different from the traditional M2 TAMs (defined as MHCII−CD11b+ cells) in the mouse model of mammary cancer. This is unexpected because IL-4 produced by other immune cells in the TME is thought to be fundamental for polarization into M2[68]. In addition, they found that IL4−/− MMTV-PyMT mouse model of mammary tumor had normal proportions of CD11b+Vcam1+ TAMs. Taken together, these results indicate that TAMs might not be M2 and not be generated secondary to the adaptive immune response against tumors.

In addition, contrary to our traditional knowledge about polarized macrophage subsets, a recent study has found that macrophages, which were generated from monocytes in vitro and underwent classical (LPS-IFN-γ) or alternative (IL-4) activation, are not equivalent to M1 and M2 macrophages, respectively. The discrepancy might explain why most surface markers identified on macrophages in vitro do not translate into in vivo situations. Hence, more valid markers of in vivo M1/M2 are needed[69].

The reason that monocytes preferentially differentiate into immunosuppressive TAMs rather than immunostimulatory DCs is a subject of investigation. The unclear mechanisms underlying the preferential differentiation pathway have become a barrier for immunotherapies, especially in solid tumor treatment. Retinoic acid (RA) has been identified as a signal that induces tissue-specific localization and functional polarization of peritoneal macrophages through the reversible induction of transcription factor GATA6[70]. In 2016, a study showed that an in vitro treatment of sarcoma-bearing mice with all-trans RA (ATRA) eliminated monocytic MDSCs and dampened the immune-suppressive role of MDSCs. This indicates that ATRA synergizing with disialoganglioside-chimeric antigen receptor (CAR) T cells would be more effective in fighting against sarcoma xenografts[60]. In 2020, Devalaraja et al. revealed that TME induced tumor cells to secrete RA, which skews monocyte differentiation toward macrophages rather than DCs. This also highlights the potential of the combination of RA signaling blockade and anti-PD-1 therapy in enhancing the treatment efficacy[68].

The verification of some of the above-mentioned mechanisms by single-cell analysis yielded new findings to help with our understanding of the origin, function and related signaling pathways for differentiation of TAMs.
and other monocytes-derived cells, thereby providing new clues for developing strategies for cancer immunotherapy.

### 3. Function of monocytes in tumors

#### 3.1. Recruitment to tumors and direct tumoricidal function

C-C motif chemokine ligand 2 (CCL2) has been reported to be a crucial mediator of monocyte recruitment during tumorigenesis and metastasis in some cancer models, including colorectal cancer (CRC)[69], breast cancer[50], and prostate cancer[70]. CCL2 is also reportedly the main driver of classical monocyte recruitment in the mouse model of bone metastasis of breast cancer. Instead of resident macrophages, the recruited monocytes have been found to facilitate tumor growth[71]. Despite the great competence of migration displayed by the bone marrow monocytes, the spleen has its own group of monocytes. The spleen has also been recently identified as a reservoir of monocytes. The removal of the spleen, either before or after tumor initiation, significantly reduced TAM responses and delayed tumor growth, indicating that the spleen was able to maintain its reservoir capacity throughout the course of tumor progression[72]. Monocytes can kill tumor cells through cytokine-mediated cell death or phagocytosis. IFN-stimulated cells can produce TNF-related apoptosis-inducing ligand (TRAIL), which results in the apoptosis of some sensitive tumor cells, while other tumor cells can secrete more CCL2 and IL-8, thereby promoting tumor development instead[53]. Monocytes are also capable of antibody-dependent cytolysis and phagocytosis. For instance, such function of CD16+ monocytes is induced by the TNF-α signaling pathway and direct contact with tumor cells[73].

#### 3.2. Interactions with lymphocytes

##### 3.2.1. Recruitment of lymphocyte

As aforementioned, MDSCs can suppress the activation of CD4+ and CD8+ T cells. Here, we would like to summarize several main mechanisms associated with its suppressive role. On the one hand, the uptake of arginine and high intracellular levels of arginase lead to depletion of arginase, which is a crucial amino acid for T cell activation[74,75]. On the other hand, MDSCs also suppress the activation of CD4+ and CD8+ T cells by downregulating the T cell receptor-associated ζ-chain; this phenomenon is observed in most cancer patients[76] and is caused by inflammation[74,75]. Both antigen recognition and signal transduction are crucial initial steps of antigen-specific immune responses. Without the ζ-chain, T cells are unable to transmit the required signals for activation.

However, the states of MDSC are not clearly identified and many superficial markers and functions overlapped, thus whether M-MDSCs represent a terminally differentiated cell type rather than a cell state induced by cancer and other pathologies requires further exploration. Monocytes and monocyte-derived cells interact with adaptive immunity by regulating the recruitment and function of lymphocytes within the TME through paracrine signaling, as well as by serving as antigen-presenting cells (APCs)[77].

##### 3.2.2. Antigen-presenting function

MPS-lineage cells, including monocytes, TAMs, and DCs, are thought to have the potential for antigen presentation due to their phagocytic capacity. While monocytes cannot function as APCs they may at least act as precursors of TAMs and DCs in normal and dysfunctional tissues. Past studies have revealed their antigen presentation function during homeostasis and infection, while the role of tumor-derived antigen presentation remains unclear[77].

Besides direct tumoricidal function, monocyte-derived DCs (moDCs) can also serve as APCs in the tumor context. In a recent study, anthracyclin chemotherapy induced CD11c+CD11b+Ly6C− cells at the tumor site by an ATP- and CCR2/CCL2-dependent mechanism[78]. Monocytes transferred into mice depleted of CD11c+ cells were sufficient to rescue CD8+ T cell priming in the lymph node and delayed tumor growth[79]. Similarly, a population of F4/80+ monocyte-derived cells (with Mϕ phenotype but functional DC features) can efficiently cross-present tumor antigens to CD8+ T cells[80]. The underlying association between monocytes and T lymphocytes could be an interesting research direction. Whether these interactions exist in the vasculature of tumors or even in the peripheral circulation requires further exploration.

#### 3.3. Interactions with other components in TME

##### 3.3.1. Angiogenesis

Since the blood vessel delivers oxygen and nutrition to the neoplasm, angiogenesis is a necessary step for tumor progression. Pro-angiogenic monocytes and TAMs are reported to regulate different processes of angiogenesis in gliomas, which are characterized by extensive neoangiogenesis[80]. The formation of new vasculature requires hypoxia-inducible factor (HIF)-mediated release of chemokines and growth factors. In renal cell carcinoma patients, peripheral blood CD14+ monocytes produce vascular endothelial growth factor (VEGF) and facilitate angiogenesis through the NF-κB and IL-1-IL-1R axis[81]. CD16+ monocytes (or their progeny) release VEGF, and then generates a positive feedback loop that recruits more pro-angiogenic monocyte-derived cells.

In mouse models, a circulating monocyte subset expressing angiopoietin receptor TIE2 shows a pro-
angiogenic role in solid tumors[82]. It is proposed that CD11b+Gr1+ myeloid cells[83] and CD11b+VEGFR1+ MDSC[84] might also promote tumor angiogenesis. However, human TIE2+ monocytes have been reported to belong to CD16+ subsets and to exert proangiogenic activity in solid tumors in vivo[88]. A study enrolled 168 hepatitis C virus (HCV)-infected patients including 89 with hepatocellular carcinoma (HCC) and examined the level of TIE2-expressing monocytes/macrophages (TEMs) had defined TEMs as CD14+CD16+TIE2+ cells in the peripheral blood and liver[86].

Of note, we are still facing some challenges. First, whether the pro-angiogenic function depends on monocytes or differentiated TAMs is unclear. Second, the relationship between circulating TIE-2+ monocytes and intratumoral pro-angiogenic TAMs (including TIE-2+ macrophages) has not been directly unveiled as TIE-2+ macrophages also facilitate angiogenesis, which promotes tumor metastasis[86].

3.3.2. Remodeling of extracellular matrix (ECM)

ECM is comprised of collagens, glycoproteins, and proteoglycans, which nourish the tumor cellular ecosystem. The tumoral ECM exerts its pro-tumor function by providing critical biomechanical and biochemical cues that facilitate tumor cell growth, survival, invasion, and metastasis as well as by regulating angiogenesis and immune function. Besides, the tumoral ECM is an outcome of aberrantly modified structural proteins and remodeling events regulated by specific proteolytic and protein cross-linking enzymes[87,88]. In tumor ECM, the physical barriers between cells are highly proteolytically degraded, thus permitting the invasion of malignant and endothelial cells and promoting the activation and production of cryptic proteins, which are responsible for tumor cell survival, proliferation, motility, and the neoangiogenic switch[89,90].

In an orthotopic CRC model, monocyte-derived TAMs advance tumor development by remodeling its ECM composition and structure. To be specific, unbiased transcriptomic and proteomic analyses defined a distinct TAM-induced ECM molecular signature composed of an ensemble of matricellular proteins and remodeling enzymes that they provided to the TME. The synthesis and assembly of collagen types I, VI, and XIV are upregulated[91]. Macrophages derived from CCR2+ monocytes can directly degrade the ECM within tumors by endocytosing deposited collagen[92]. Collagen introduced into the dermis of mice undergoes cellular endocytosis through partial MMP-dependent manner and is degraded completely in lysosomes. Collagen uptake was predominantly executed by a minor population of M2-like macrophages[93].

3.4. Differentiation into TAMs and moDCs

In tumor tissue, monocytes can be differentiated into TAMs or moDCs. Nevertheless, proper classification of TAMs and DCs, especially in inflamed or tumor tissue is still lacking[94].

3.4.1. TAMs

TAMs comprise macrophages derived from both embryo and hematopoietic stem cells (HSCs)[95]. Multiple studies have shown that the embryonic TAMs are responsible for wound healing and tissue remodeling, while the HSC-derived TAMs are mainly engaged in immune inhibition and antigen-presentation[96]. Tumor cells produce chemoattractants, including CSF-1, CCL2 (MCP-1), CCL3, and CCL5. Then, monocytes in the circulating blood are recruited into tumor tissue and differentiate into TAMs[97].

Recent studies showed that TAMs promote spheroid formation and tumor growth at the early stages of transcoelomic metastasis in an established mouse model of epithelial ovarian cancer. To be specific, epidermal growth factor (EGF) secreted by M2 macrophage-like TAMs upregulated VEGF/VEGFR signaling in surrounding tumor cells, thus promoting their proliferation and migration[89].

It has been verified in the mouse mammary tumor model that the tumor-monocyte pool almost exclusively consisted of Ly6ChCX3CR1low monocytes, which were enriched in tumor tissue and replenished M2-like TAMs. In addition, these TAMs were suggested to promote angiogenesis and inhibit T cells activation[22]. Nevertheless, studies on identifying spatially and functionally distinct TAM subpopulations are currently lacking. In healthy tissue, blood monocytes are recruited to different tissues and then, they differentiate into monocyte-derived macrophages and moDCs to maintain homeostasis. While in tumors, monocytes can be divided into immunosuppressive TAM and monocyte MDSCs, promoting tumor progression and immune evasion[99].

TAMs are abundant in tumor stroma both in mice and in humans[9]. Microenvironmental stimuli drive the formation of a macrophage either toward the “classic” (M1) or the “alternative” (M2) activation state[100]. It is known that fully polarized M1 and M2 (or alternatively activated) macrophages are the extremes of a series of consecutive states[100]. M1 macrophages are considered to be anti-tumor and promote immune responses. In contrast, TAMs, which display an M2-like phenotype and have high scavenging ability, promote tissue repair and angiogenesis[9] and facilitate tumor progression[5,102]. However, Franklin et al. pointed out that TAMs did not express M2 markers, such as Ym1, Fizz1, and Mrc1 in mammary cancer. Instead,
mammary tissue macrophages bear close resemblance to M2 macrophages[64].

Murray et al. have proposed a common framework for macrophage activation nomenclature. TAMs are divided into eight subtypes, including M (IL-4), M (Ic), M (IL-10), M (GC+TGF-β), M (GC), M (LPS), M (LPS+IFN-γ), and M (IFN-γ). This nomenclature expanded the M1–M2 definitions considering different activation scenarios (M2a, M2b, etc.)[100].

Of note, there is a discrepancy between mouse and human models of alternative activation[100]. In vitro macrophages, generated from monocytes after classical (LPS+IFN-γ) or alternative (IL-4) activation. In in vivo situations, these macrophages preferentially induce the inducible nitric oxide synthase (iNOS) or arginase and are called M1 and M2. These two concepts are used interchangeably. However, a recent study pointed out that M1(M2) in vivo was not equivalent to classically (alternatively) activated macrophages in vitro[60]. Thus, reliable markers of macrophage in vivo remain to be identified.

It is becoming clearer that the dichotomic polarization of pro-inflammatory M1 and anti-inflammatory M2 cannot reflect the complexity of TME[104]. Actually, the application of single cell transcriptome analysis helped determine that the states of all cell components (including cancer cells, lymphocytes, and TAMs) in the TME may span two extreme states[105]. Some findings challenged the common dogma of the M1/M2 classification of TAM. Through scRNA-seq, Elham et al. found that the gene expression signatures of M1 and M2 macrophages were positively related to the myeloid populations, and that the macrophages differentiated in the same trajectory[104]. They found that TAMs might coexpress M1 and M2 markers instead of representing a mixture of M1 and M2 subsets, indicating that human TAMs cannot be divided into M1 and M2 in a conventional sense[106]. This finding, however, can controvert a previous theory that only polarization of M1 and M2 exist. Recently, the heterogeneity of TAMs in different tissues has been reviewed[107]. Resident macrophages of TAMs can be divided into two subsets – resident macrophages derived from the embryo and those from the monocyte. This implies that monocyte-derived macrophage replaced the original models (rapid in gut and dermis while slow in heart, lung, and pancreas). Except for ontogeny of TAMs, the TME also induced some shared signatures of them[107]. In brain tumors, TAMs comprised microglial cells and monocytes, which were also validated through scRNA-seq[108]. The two subtypes can be distinguished from each other by CD49d[109]. Thus, we may possibly identify and target these immunosuppressive macrophages derived from blood monocytes from other TAMs.

TAM can be defined using MHCII, CD11c, and macrophage markers, and clustered with macrophages on transcriptional basis[64]. Inflammatory cells and cytokines could play an important role in tumor progression and immunosuppression of some therapies[17]. Fibiger et al. pointed out that chronic inflammatory irritation could trigger cancer development[110]. The functions of macrophages have been well documented; macrophages produce angiogenic factors and growth factors that promote tumor invasion and metastasis. In tumors, Ly6C<sup>hi</sup> monocytes exist as highly suppressive monocytic myeloid-derived suppressor cells (M-MDSC)[111] and can differentiate into TAMs and inflammatory DCs[112].

### 3.4.2. Monocytic DCs (moDCs)

DCs arise from a common bone marrow progenitor — the common DCs progenitor (CDP) — and then differentiate into pDCs and precursors for cDCs. Here, we chiefly discuss the moDCs that originate from Ly6C<sup>hi</sup> monocytes under the scenario of tumor or inflammation. Since TAMs are the predominant component of TME, moDCs just make up a minor fraction of tumor-infiltrated myeloid cells. MoDCs play an anti-tumorigenic role mediated by some cytokines, including iNOS, TNF-α, IL-6, and IL-10, which impede T cells proliferation in vitro[113]. However, they can also efficiently engulf and process antigens and then activate CD8<sup>+</sup> T cell in several tumor models[114]. In breast cancer models, DC populations were divided into “CD11b<sup>+</sup> DC1” and “CD14<sup>+</sup>CD33<sup>-</sup>HLA-DR<sup>-</sup> expression (CD11b<sup>+</sup>Gr-1<sup>+</sup>Ly6C<sup>+</sup>Ly6G<sup>+</sup> cells in mice)” through an 11-color flow cytometry panel and progressive gating strategy. CD103<sup>+</sup> DC2 showed unique antigen processing and presentation capabilities[115] (summarized in Table 1).

### 4. Targeting monocytes during clinical treatment for cancer

#### 4.1. Monocytes as diagnostic marker

Monocytes, TAMs, and moDCs may be promising diagnostic markers for early diagnosis of some malignant tumors. Angiogenesis is a critical step in the development of HCC. As mentioned previously, TEMs are pro-angiogenic. The levels of circulating TEMs were observed to be significantly higher in HCC than in non-HCC patients. This implies that the level of circulating TEMs can be used as a diagnostic marker for HCC[65]. This has also been proven in a study that the TEMs percentage in peripheral blood monocytes of 84 HCC patients who received hepatectomy was significantly increased. The study showed that the level of TEMs in peripheral blood
may be applied as a biomarker for identifying HBV-related HCC\textsuperscript{116}.

4.2. Monocytes as therapeutic targets

At present, the use of monoclonal antibodies small-molecule inhibitors, and RNA interference techniques targeting CCL2 and CSF-1 signaling pathways, which are responsible for recruitment of TAMs from circulating blood into the TME, is being studied\textsuperscript{117}. The clinical trial of the combination of CSF-1R and PD-1 blockade therapy in pancreatic cancer patients is currently at phase II\textsuperscript{118}. CCL2 blockade combination therapies led to elevated responsiveness in pancreatic cancer patients\textsuperscript{119}. However, this may result in an infiltration of an abundance of monocytes into the TME from bone marrow, which has been found to increase metastasis in a breast cancer mouse model\textsuperscript{120}. Besides, alternative strategies could directly target immune suppressive TAM effector molecules, such as Arg1 inhibitors\textsuperscript{121}. Strategies to deplete pro-tumorigenic TAMs or to convert TAMs are needed. In addition, exploring specific markers can also enhance the efficacy of therapy. Therapies can target the process of MDSC formation in the bone marrow\textsuperscript{122}, their recruitment to tumor site\textsuperscript{123}, and some immune suppressive activities induced by MDSCs\textsuperscript{124}.

4.3. Monocytes and immune checkpoint therapy

4.3.1. Function of monocytes in immune checkpoint therapy

Current efforts focus on the combination therapies of blocking immune inhibitory pathways and immune stimulatory co-receptors to improve the responsiveness to these two kinds of ICB therapies\textsuperscript{125}. Tumors exploit multiple mechanisms, including local immune suppression, tolerance, and systemic T cell dysfunction states, to evade intrinsic immune system or to resist immunotherapies\textsuperscript{126}. Blocking T cell co-inhibitory molecules, including cytotoxic T lymphocyte-associated protein 4 (CTLA-4), PD-1 or its ligand (PD-L1), have displayed durable anti-tumor efficacy and relatively longer clinical remissions in patients with either solid or hematological tumors\textsuperscript{127}.

PD-1 is an inhibitory receptor expressed on the surface of T cells, and PD-L1 is expressed on the surface of various other cell types, including tumor cells and myeloid cells\textsuperscript{128}. Anti-PD-1 therapy aims to dampen the interaction between tumor-reactive T cells and tumor cells by blocking PD-L1 and/or PD-L2/PD-1 signaling pathways\textsuperscript{129}. To be exact, the inhibitory checkpoint receptor PD-1 was found to impede cytotoxic CD8\textsuperscript{+} T cells in TME\textsuperscript{130}. In addition, tumor cells frequently overexpress PD-L1, helping itself to escape from the surveillance of the immune system. Blocking PD-1/PD-L1 through monoclonal antibodies has shown outstanding clinical efficacy in patients with various tumors\textsuperscript{131-133}. It has been reported that the testing of anti-PD-1 antibody lambrolizumab in patients with advanced melanoma led to a high rate of durable tumor regression\textsuperscript{134}. However, Topalian et al. pointed out that the objective responses produced by anti-PD-1 antibody (BMS-936558) occurred in approximately one out of four to five patients with non-small-cell lung cancer, melanoma, or renal cell cancer\textsuperscript{12}.

Das et al. introduced a cancer-specific prognostic immune score model based on the analysis of immune function-related genes from four single-cell RNA-Seq data sets and 20 bulk tumor RNA-Seq data sets. They revealed that the immune score models can predict disease-free and overall survival for 20 types of cancers. Besides, the models can also predict response to immunotherapies in some specific cancers\textsuperscript{135}.

Although immune-checkpoint cancer therapy (ICT) shows encouraging results in many types of cancers, its effect is still limited. The reason why the efficacy of immunotherapy varies among different tumor types is probably due to heterogeneity of the immune cell composition in individual tumors\textsuperscript{136}. It is universally known that TAM is an important component of TME. Devalaraja et al. found that monocytes and TAMs are a major part of intratumoral leukocytes in the three sarcoma models\textsuperscript{137}. The effects of immunotherapy, to some degree, are impeded by immunosuppressive TME. Cytokines in the TME promote the differentiation of suppressive TAMs and inhibit DCs, hampering the function of cytotoxic anti-tumor T cells\textsuperscript{138}. As mentioned before, tumor-educated TAMs facilitate tumor progression and metastasis through cytokines in the TME\textsuperscript{9}. In return, TME is likely assumed to be altered by intratumoral monocytes and TAM, which is the main component of tumor stroma.

4.3.2. Monocytes as a predictive marker for response to ICT therapy

(A) Level of monocyte predicts responsiveness to anti-PD-1 therapy

Due to the variable efficacy of ICT and the extremely high costs, it is critical to assess the condition of individuals and avoid wasting time in treatment. Therefore, many researchers are devoted to finding reliable predictive biomarkers, which can be used to filter patients who have good responsiveness to anti-PD-1 therapy before application and to predict progression-free survival and reduce the chance of relapse.

Krieg et al. studied the PBMCs isolated from 20 patients with melanoma and ten healthy people, which were
collected from baseline and after 12 weeks of anti-PD-1 therapy. After single-cell mass cytometry along with clustering analyses, researchers characterized superficial molecular markers and intrinsic gene expression between responders and non-responders, and ultimately found that the level of CD14+CD16 HLA-DRhi monocytes was a strong predictor of progression-free survival in response to anti-PD-1 immunotherapy. By analyzing 53 standard clinical parameters and the levels of classical monocytes via Cox proportional-hazards model, they found that immature granulocytes and classical monocytes are the only variables tightly associated with progression-free survival.

FlowSOM can be used to identify CD14+(CD11b+HLA-DRhi) myeloid cells and CD14+(CD11b+HLA-DRhi) immature granulocytes. According to the comparison of cluster frequencies in healthy donors, non-responders, and responders from 60 data sets, researchers observed a higher frequency of CD14+ myeloid cells in responders than in non-responders. Besides, CD14+ myeloid cells were abundant in cancer patients, but there was no significant difference between responders and non-responders. With the application of the machine-learning algorithm CellCnn, they further detected the core signature of this subpopulation, that is, CD14+CD33+HLA-DR+ICAM-1+CD64+CD141+CD86+CD11c+CD38+PD-L1+CD11b+ monocytes, which showed a significant overlap with the CD14+CD16 HLA-DRhi cluster characterized by FlowSOM. More specifically, the level of classical monocyte >19.38% before the initiation of anti-PD-1 therapy indicates a better responsiveness to anti-PD-1 therapy and a longer survival for melanoma patients. However, RNA sequencing showed no notable difference in this monocyte cluster between the responders and non-responders. This indicates that the responsiveness might depend on the number of CD14+CD16 HLA-DRhi monocytes.

Emerging data suggest that the expression of the chemokine CXC motif ligand 9 (CXCL9) by TAMs regulates the recruitment and positioning of CXC motif chemokine receptor 3 (CXCR3)-expressing stem-like CD8 T cells (Tstem and antitumor immunity). The CXCL9 expression of TAM should be considered in the determination of the efficacy of therapies that enhance anti-PD-L1 response rates. In another word, enhancing the level and function of CXCL9-expressing TAMs can be synergistic with anti-PD-(L)1 therapy to inhibit tumor growth. Except for Tstem recruitment, the potential functions of these cells deserve further exploration.

MDSC is thought to be the cornerstone of cancer-related immunosuppression as it can influence the response to therapy and the disease outcomes in melanoma patients. Patients with melanoma whose frequency is above the cutoff values of myeloid index score (MIS) are stratified according to their prognosis. In addition to having been confirmed in the validation set, the MIS was negatively related to survival, independent of the type of therapy, and was not interfered with by the clinical prognostic factors. MIS hazard ratio (HR) was remarkably superior to that of lactate dehydrogenase, tumor burden, and neutrophil-to-lymphocyte ratio. These results demonstrated that the levels of monocytes are helpful for identifying advanced melanoma patients and making therapeutical choices.

(B) Monocytes and its derived APCs are related to the efficacy of PD-1 checkpoint blockade

As a result of the expression of PD-1, the phagocytosis of tumor cells by TAM is inhibited, thereby increasing tumor progression both in mouse tumor models and in humans with cancer. Blockade of PD-1/PD-L1 in vivo benefits macrophage phagocytosis, restricts tumor growth, and prolongs survival in mouse cancer models through a macrophage-dependent fashion. Schetters et al. recently demonstrated that the moDCs in TME express abundant costimulatory molecules to interact with lymphocytes, which can only be found in responding mouse melanoma model after anti-PD-1 therapy. Besides, they used scRNA-seq and other techniques to investigate the two differentiation pathways of monocyte. moDC has been found to be significantly abundant in responding patients, while macrophage-prone monocytes infiltration was found in the nonresponsive patients. This indicates that macrophages may regulate other DCs or promote CD8+ T cells through other pathways. In addition, another study revealed the suppressive role of monocytes in HCC. The objective response rate was relatively high (about 40 – 50%) in patients with solid tumors such as melanoma and lung cancer compared to merely 17% in those with HCC. The authors found that M-MDSCs facilitate tumor growth. As for the intrinsic mechanism, human hepatic stellate cell (HSC) caused monocytes accumulation in the fibrotic microenvironment through p38 MAPK signaling pathway. Since the bromodomain and extraterminal domain (BET) decreased the M-MDSC filtration and enhanced the efficacy of ICB therapy, which also makes it rational to implement clinical trials of combination therapy of BET bromodomain and PD-L1 co-blockade. IFN gene therapy led to single-cell transcriptional reprogramming of tumor-associated myeloid cells, and this counteracted the expansion of immunosuppressive myeloid cells induced by leukemia, which were shown by bulk single-cell transcriptome analyses. Thus, it has been suggested that IFN gene therapy probably can enhance the efficacy.
of other immunotherapies\textsuperscript{139}. Taken together, monocytes and their derived APCs are critical to the response of anti-PD-1 therapy and could serve as a potential target for combination therapy.

### 4.3.3. ICB induces remolding of monocytes/macrophages

Devalaraja \textit{et al.} studied several solid tumor models and revealed that IL-13 in the TME induced cancer cells to produce RA, resulting in the differentiation of monocytes toward immunosuppressive TAMs rather than immunostimulatory DCs\textsuperscript{68}. In addition, they generated Raldh1/3 double knockout (DKO) mice which showed >80% reduction in Raldh1 and Raldh3 transcripts, and found that F4/80\textsuperscript{+} TAMs from DKO tumors were significantly less suppressive to T cell proliferation and activation \textit{ex vivo}. This indicates that the inhibitory role is probably confined to within the TME.

Although ICT is designed to target lymphoid cells, it is also related to the remodeling of the TME. Monocytes/macrophages that infiltrated into the TME constitute the largest group in the scRNA-seq tSNE plots and undergo a remarkable remodeling during the ICT. Using scRNA-seq and CyTOF, Gubin \textit{et al.} discovered macrophage remodeling during the ICT; these macrophages, which were derived from the T3 tumor mouse model, could be divided into five and eight subpopulations, respectively, according to the technique used. In addition, they also studied the polarization process and found that 7 – 9 days after ICT or control antibody treatment showed a branch point. Then, they conducted a parallel cohort of monoclonal antibody and ICT treatment along with the inhibition of IFN-γ. On day 7, the proportion of iNOS\textsuperscript{+} cells in CD45\textsuperscript{+} cells increased 11.4-fold from 1.2% at the baseline to 13.7%, compared to the group treated with anti-mouse IFN-γ monoclonal antibody increased 7.3-fold. This indicates one of the possible differentiation mechanisms, that is, the generation of an abundance of iNOS\textsuperscript{+} pro-inflammatory macrophages induced by the cytokine (IFN-γ) released by the reinvigorated T cells in ICT\textsuperscript{140}. They found increased CD206 expression in the monoclonal antibody-treated mice and increased iNOS expression in the ICT-treated mice. Taken together, ICT leads to a change in the macrophages that play an immunosuppressive role and favor tumor growth during ICT in a manner partially dependent on IFN-γ\textsuperscript{140}.

### 4.3.4. Potential application in other therapies

The function and interactions of monocytes in ICB reflect their important role in TME. Several studies have demonstrated the potency of targeting monocytes to inhibit tumor growth and metastasis. Inhibition of BET family proteins is widely studied for devising cancer treatment. Yin \textit{et al.} discovered a BET inhibitor (NHWD-870), which impedes the proliferation of TAMs in mouse models and has better potency and bioavailability than some BET inhibitors currently in clinical stage. One of the potential mechanisms is that NHWD-870 inhibits BRD4 and its target HIF1α, and then reduces the expression of macrophage CSF-1\textsuperscript{141}. Different subpopulations of monocytes possess different roles in tumor metastasis. Transferring of Ly6Clo monocytes into Nr4a1-deficient mice that lack Ly6Clo monocytes led to reduced tumor metastasis by nearly four-fold in a mouse model of melanoma metastasis to the lung\textsuperscript{35}. On the contrary, adoptive transfer of Ly6Chi monocytes led to almost doubled tumor metastasis, which is consistent with their pro-tumoral role. This indicates that intravascular adoptive transfer of anti-tumor monocytes might be a potential treatment strategy. However, CAR T cell therapy has received great success in the clinical treatment of some hematological malignancies\textsuperscript{142}, and the application of transferring monocytes has faced with the challenge that their proliferative capacity \textit{ex vivo} is limited, rendering gene editing difficult. Nevertheless, several clinical trials on patients with recurrent chemotherapy-resistant ovarian cancer are now underway\textsuperscript{143}. Results from this clinical trial will be informative for future directions in monocyte transfer therapy.

### 5. Challenges and prospects

This review briefly introduces the traditional monocyte states and new findings of the classification of monocytes and their derived mononuclear phagocytes, including macrophage, moDC and MDSC. These cells are reportedly crucial in tumor proliferation and metastasis due to their correlation with other components in TME. Single-cell analysis is used to identify the subsets and differentiation trajectories of monocytes through accurate profiling of each single cell and to verify previous assumptions.

Over the last decade, studies have gradually found out the heterogeneity of MPS and the diverse functions facing different stimuli. We now realize that monocytes are collectively a heterogenous population\textsuperscript{28}. However, the nomenclature of monocytes has always been a challenge for researchers. Despite the much improved techniques at the single-cell level that are used for the classification of system (MPS), there still exist many miscommunication and confusion due to the lack of unifying nomenclature. We consider a systematic classification system coupled with naming convention to be important because each single subset implies a functional specialization. Therefore, it is important for us to demarcate new subsets in the immune system and establish standardized system, which will be
used in different laboratories. Information pertaining to the surface biomarkers overlapping between subsets and a unifying classification of monocytes, TAMs and moDCs based on ontogeny, location, function, and phenotype are urgently needed. Full delineation of the origin and function of TAMs, along with new pathways for the differentiation of TAMs can provide new insights into strategies of tumor immunotherapy. Some concepts, such as TAM heterogeneity and the nature of the monocyteic TAM precursors, are speculative and difficult to be unified. Moreover, the ontogeny, transcriptional regulation, and heterogeneity of MPS remain largely unknown, which hampered their clinical application. Monocytes are thought to preferentially generate immunosuppressive TAMs in solid tumors, yet the basis for this is largely unknown. Moreover, monocytes can diversify into a continuum of states, which may promote or impede tumor growth. More evidence is needed to determine whether monocytes differentiate with intermediate modes of functioning. Conventional definitions of monocytes will likely be expanded, and new subpopulations of monocytes and intermediate subsets deserve further exploration and to be identified. The mechanisms of tissue-specific immunity and the basis of tumor immune escape still remain to be elucidated. In addition, the mechanisms of balancing immune protection and self-tolerance and their correlation with tumor surveillance and escape are still not well documented. The clinical studies and experiments in mouse models clearly indicate that TAMs show a protumoral phenotype that facilitates tumor cell invasion, motility, and intravasation. Therefore, it is not surprising that extensive TAM infiltration is positively associated with cancer metastasis and poor clinical prognosis in various human cancers. As for treatment for malignancy, early diagnosis and screening for high-responsive patients for immunotherapies are key to be part of prophylactic approach and effective treatment. One of the future directions is to unveil the underlying functions of mononuclear phagocytes and their potential as diagnostic and therapeutic targets. Besides, combination therapy targeting monocytes and immune checkpoint therapy may be promising.

Most patients do not show durable responses to immunotherapy, especially ICT, despite a dramatic increase in progression-free survival. Therefore, more accurate biomarkers of the clinical response to ICT are urgently needed. In addition, it is still not clear how these APCs develop and function before and during anti-PD-1 ICB or how they are associated with tumor rejection. The functions of monocytes in ICB therapy and other immunotherapies require further exploration because they are quite promising targets for the diagnosis and treatment of human cancers. In addition, the benefits, limitations, and potential side effects of these therapeutic approaches are also important for future clinical applications.

### Funding
This study was supported by grants from General Program, The National Natural Science Foundation of China (No. 81874138, 82073020 and 81903222).

### Conflict of interest
The authors declare no conflict of interest.

### Author contribution
Conceptualization: Mingzhu Yin
Data curation: Xin Fu
Writing - original draft: Xin Fu, Mingzhu Yin
Writing - review & editing: Xin Fu, Mingzhu Yin.

### References


https://doi.org/10.1136/gutjnl-2015-310514

https://doi.org/10.1186/1475-2867-3-25

https://doi.org/10.1016/j.celrep.2017.10.024

https://doi.org/10.1126/science.aac9407

https://doi.org/10.1073/pnas.1417320112

https://doi.org/10.1016/j.ccr.2005.08.002

https://doi.org/10.1182/blood-2006-10-053504

https://doi.org/10.1038/s41392-021-00506-6

https://doi.org/10.1038/nature12034

https://doi.org/10.1038/nri3671

https://doi.org/10.1126/science.1117729

https://doi.org/10.1084/jem.20141442

https://doi.org/10.1016/j.immuni.2016.12.001

https://doi.org/10.1158/0008-5472.CAN-06-3037

https://doi.org/10.1038/s41590-017-0022-x

https://doi.org/10.1200/JCO.2006.08.5829

https://doi.org/10.1158/0008-5472.CAN-04-4505

https://doi.org/10.1158/0008-5472.CAN-06-3037

https://doi.org/10.1016/j.immuni.2016.12.001

https://doi.org/10.1084/jem.20141442

https://doi.org/10.1186/s40428-020-001223

53. Hartwig T, Montinaro A, von Karstedt S, et al., 2017, The TRAIL-induced cancer secretome promotes a tumor-
https://doi.org/10.1016/j.molcel.2017.01.021

https://doi.org/10.1038/ni.2638

https://doi.org/10.1016/j.immuni.2017.04.019

https://doi.org/10.1038/ni.3412

https://doi.org/10.1182/blood-2002-02-0569

https://doi.org/10.1146/annurev-immunol-032712-095906

https://doi.org/10.1016/j.cell.2017.06.016

https://doi.org/10.1158/2326-6066.CIR-15-0230

https://doi.org/10.1007/s00262-020-02626-4

https://doi.org/10.1016/j.immuni.2017.11.024

https://doi.org/10.1016/j.ejphar.2021.174216

https://doi.org/10.1126/science.1252510

https://doi.org/10.1126/science.1204351

https://doi.org/10.3389/fimmu.2019.01084

https://doi.org/10.1016/j.cell.2014.04.016

https://doi.org/10.1016/j.cell.2020.02.042

https://doi.org/10.1016/j.celrep.2015.06.024

https://doi.org/10.1016/j.cytogfr.2009.11.009

https://doi.org/10.1073/pnas.1113744109

https://doi.org/10.1038/srep34310


https://doi.org/10.1038/s41577-019-0127-6

https://doi.org/10.1016/j.immuni.2017.07.014

https://doi.org/10.1172/JCI31422

https://doi.org/10.1172/JCI87252

https://doi.org/10.1007/s12307-012-0123-x

https://doi.org/10.1146/annurev.immunol.021908.132532

https://doi.org/10.1016/s1471-4906(02)02302-5

https://doi.org/10.3390/cells9041062

https://doi.org/10.1016/j.immuni.2014.06.008

https://doi.org/10.1016/j.cell.2018.05.060

https://doi.org/10.1038/s41591-021-01233-9

https://doi.org/10.1126/scitranslmed.aat1500


https://doi.org/10.1016/j.celrep.2016.10.052

https://doi.org/10.1016/S0140-6736(00)04046-0

https://doi.org/10.1016/j.it.2016.01.004

https://doi.org/10.1016/j.immuni.2016.01.014

https://doi.org/10.1038/ncomms13720

https://doi.org/10.1016/j.coi.2017.01.002

https://doi.org/10.1016/j.ccc.2014.09.007

https://doi.org/10.1371/journal.pone.0143657

https://doi.org/10.3389/fimmu.2018.01977


https://doi.org/10.1016/S1470-2045(16)00078-4


https://doi.org/10.1038/nature13862


https://doi.org/10.1186/s40425-017-0308-4


https://doi.org/10.1158/0008-5472.CAN-07-2593


https://doi.org/10.1158/0008-5472.CAN-09-3690


https://doi.org/10.1016/j.ejca.2017.01.001


https://doi.org/10.1016/j.sci.2016.01.001


https://doi.org/10.1006/s0065-2776(06)90002-9


https://doi.org/10.1038/s41568-019-0235-4


https://doi.org/10.1126/science.aar4060


https://doi.org/10.1146/annurev.immunol.26.021607.090331


https://doi.org/10.1016/j.ccell.2015.03.001


https://doi.org/10.1016/j.cell.2016.02.065


https://doi.org/10.1056/NEJMoa1305133


https://doi.org/10.3390/cancers12092476


https://doi.org/10.1038/nature22396


https://doi.org/10.1126/scitranslmed.aad7118


https://doi.org/10.1136/gutjnl-2018-317257

https://doi.org/10.1016/j.cell.2018.09.030

https://doi.org/10.1038/s41467-020-15290-0

https://doi.org/10.1056/NEJMr1706169

143. Green DS, Nunes AT, David-Ocampo V, et al., 2018, A phase 1 trial of autologous monocytes stimulated *ex vivo* with sylatron (peginterferon alfa-2b) and actimmune (interferon gamma-1b) for intra-peritoneal administration in recurrent ovarian cancer. J Transl Med, 16(1): 196.

https://doi.org/10.1038/cmi.2014.83

https://doi.org/10.1016/j.immuni.2014.06.010