Models for Monocytic Cells in the Tumor Microenvironment

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Abstract

Monocytes (Mos) are immune cells that critically regulate cancer, enabling tumor growth and modulating metastasis. Mos can give rise to tumor-associated macrophages (TAMs) and Mo-derived dendritic cells (moDCs), all of which shape the tumor microenvironment (TME). Thus, understanding their roles in the TME is key for improved immunotherapy. Concurrently, various biological and mechanical factors including changes in local cytokines, extracellular matrix production, and metabolic changes in the TME affect the roles of monocytic cells. As such, relevant TME models are critical to achieve meaningful insight on the precise functions, mechanisms, and effects of monocytic cells. Notably, murine models have yielded significant insight into human Mo biology. However, many of these results have yet to be confirmed in humans, reinforcing the need for improved in vitro human TME models for the development of cancer interventions. Thus, this chapter (1) summarizes current insight on the tumor biology of Mos, TAMs, and moDCs, (2) highlights key therapeutic applications relevant to these cells, and (3) discusses various TME models to study their TME-related activity. We conclude with a perspective on the future research trajectory of this topic.

Keywords

Monocytes · Macrophages · Monocyte-derived dendritic cells · Ontogeny · Differentiation and commitment · Heterogeneity · Cancer · 2D versus 3D ·

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7.1 Introduction

Monocytes (Mos) traffic through vasculature to tissues during steady state and at increased rates during inflammation from cancer [1]. Upon entering cancer-associated tissue, Mos can give rise to tumor-associated macrophages (TAMs) and Mo-derived dendritic cells (moDCs) [1]. Subpopulations of all these cells shape the tumor microenvironment (TME) [1]. Thus, understanding Mo ontogeny and heterogeneity enables improved insight into their roles in the TME and the proper creation and interpretation of human models. Of note, we discuss Mo ontogeny and heterogeneity based on findings derived from human and murine models while recognizing that most murine-derived findings are yet to be validated in humans.

7.1.1 Monocyte Ontogeny

In children and adults, Mos derive from hematopoietic stem cells (HSCs) in the bone marrow [2]. Monopoiesis, a series of differentiation and commitment steps, drives their development [3, 4] and involves intermediary lineage-committed cells including common Mo progenitors (cMoPs), granulocyte-macrophage progenitors (GMPs), and macrophage-dendritic cell progenitors (MDPs) [2]. The sequential transcription of PU.1 and then IRF8 and KLF4 governs monopoiesis [5–7]. GMPs comprise multiple progenitors that differentiate along a spectrum of macrophage (MΦ) or dendritic cell (DC) lineage phenotypes [8]. However, GMPs phenotypically overlap with cMoPs and MDPs [9], suggesting that current definitions oversimplify ontogeny complexities.

Recently, advanced techniques in RNA sequencing (RNA-seq), epigenetic profiling, and fate mapping strategies have facilitated more in-depth understanding of the development hierarchy of Mo ontogeny in normal [10, 11] and cancer [12] settings. Also, the fate of TME-associated Mos is heavily influenced by TME-related cues such as cytokines (M-CSF, GM-CSF, and IL-13) [13–15] and extracellular matrix (ECM) [16], which may differ across cancer types [17, 18]. Thus, TME models must account for these parameters and complexities while remaining adaptable to new discoveries regarding the effect of cancer cells on the differentiation and commitment of cells of monocytic origin.

7.1.2 Monocyte Heterogeneity

Early studies established classical (Cla) ($CD14^+CD16^-$), non-classical (NC) ($CD14^{lo}CD16^+$), and intermediate (Int) ($CD14^+CD16^+$) [19] Mo subsets within the peripheral blood of humans. A developmental relationship, triggered by M-CSF [20], has been observed from the Cla, through Int, to NC subsets [1, 21, 22]. Although gradual transitions across subsets blur their distinctions, the CD14/CD16 nomenclature has proven useful in many studies. Subsets based on differential expression of CX3CR1 [23], CCR2 [24, 25], or 6-sulfo $LacNAc^+$ and $Fc\varepsilon RI^+$ [26–30] were later identified, pointing toward a growing appreciation of diverse Mo subpopulations in humans.

Subset definitions inevitably shift, particularly for Int Mos [31]. Indeed, advanced techniques such as high-dimensional mass cytometry (CyTOF) that allows multiplexed analysis of >40 protein markers in single cells revealed that CCR2, CD11c, CD36, and HLADR can improve the gating purity of Int Mos [32]. Single-cell transcriptional (scRNA-seq) profiling also identified Int Mo sub-clusters, of which 70% are Cla (Mono1) and NC (Mono2) clusters and 30% are Mono3 and Mono4 that, respectively, regulate cell cycle/trafficking and expression of NK/T cell activation genes [33]. In fact, colorectal cancer patients have increased Int Mo percentages, with these being higher in patients with localized disease versus (vs.) those with advanced metastasis [34]. Future studies must thus validate the existence and functions of Mo subpopulations both in healthy and cancer conditions.

Mos also differ by their tissue localization, including their retention within the vasculature of multiple organs [23, 35]. In steady-state conditions, Cla Mos are recruited to tissues where they can differentiate into MΦs or moDCs [11, 36]. On the other hand, NC Mos mainly patrol vasculature [37] through LFA-1 and CX3CR1 [38], scavenging cellular debris and flagging damaged endothelial cells (ECs) for disposal by neutrophils [39]. In inflamed conditions, both Mo subsets increase their trafficking to tissues [40–42]. Here, NC Mos traffic more slowly than Cla Mos [42] and can also give rise to MΦs that secrete inflammatory cytokines [38, 43, 44]. Specifically in cancer, patrolling/non-patrolling Mos can differently modulate primary tumor growth, cancer cell extravasation, and metastatic seeding, with these subsets commonly associated with having pro-tumor or anti-tumor effects, respectively [45, 46].

For the purposes of this review, we adopt the Cla/NC subset nomenclature and further identify Mos by patrolling/non-patrolling classes. The functional term "proinflammatory"/"inflammatory" is avoided as it disregards anti-inflammatory properties of an alleged "(pro)inflammatory" cell [47] and prematurely ascribes cells with ex vivo characterized functions, while they often remain to be validated in vivo. Mos are distinguished from MΦs/DCs as far as evidence is clear. However, where classifications are unclear, we refer to cells of monocytic origin to avoid confusion [42]. Finally, although there is evidence that some human Mo subsets are corollary to murine subsets, there is growing evidence of the heterogeneity between human and murine Mo subpopulations, particularly with regard to cell function [48]. Importantly, human-relevant models are required to fully clarify if murine-derived findings necessarily translate to humans.

7.2 Monocyte Functions in Cancer

Mos have an extensive role repertoire where environmental cues such as cytokines activate distinct transcriptional programs to direct their specific activities in the TME [49]. Here, we discuss current evidence of these various roles (Fig. 7.1) and present outstanding areas that remain to be clarified.

7.2.1 Recruitment to Tumors

Mos are recruited throughout the tumor lifespan, from the early stage of primary tumor growth [50, 51] to late-onset metastases [45, 52, 53]. CCL2/CCR2 signals chiefly recruit Mo to tumors [50, 53], with CCL2 expression correlating with the presence or amount of neoplasia [54]. Many studies in Mo recruitment also implicate modulation by CXCL8/IL-8, CCL5/RANTES, and vascular endothelial growth factor (VEGF) signaling [55, 56], as well as tumor microvasculature upregulation of angiopoietin-2 (Ang-2), CX3CL1, ICAM-1, selectins, and VCAM-1 [57, 58]. Moreover, Cla Mo recruitment could be evolutionarily conserved across tumors as adoptively transferred human Mos traffic to murine tumors [53]. Mos deploy to primary tumors primarily from the bone marrow [59], but the precise mechanism of their trafficking to tissues could differ for different anatomical locations and cancer types [42]. Such features and processes should be considered when modeling the human TME.

7.2.2 Tumoricidal Activity

Mos elicit antitumor activity using multiple pathways. For example, Mos expressing SIRPα can directly phagocytose tumor cells expressing low levels of CD47, which normally provides cells with a protective "don't eat me" signal against phagocytotic cells [60, 61]. Notably, Cla Mos are viewed as the most phagocytic subclass, whereas NC Mos chiefly patrol the vasculature and scavenge cell debris [45, 62]. Growing evidence suggests that monocytic cells can also contribute to cancer cell death by cell contact-mediated antibody (Ab)-dependent cellular cytotoxicity (ADCC) and apoptosis [63–67]. For example, granzyme B expression is induced in human Mos that are treated with TLR8 agonists, leading to

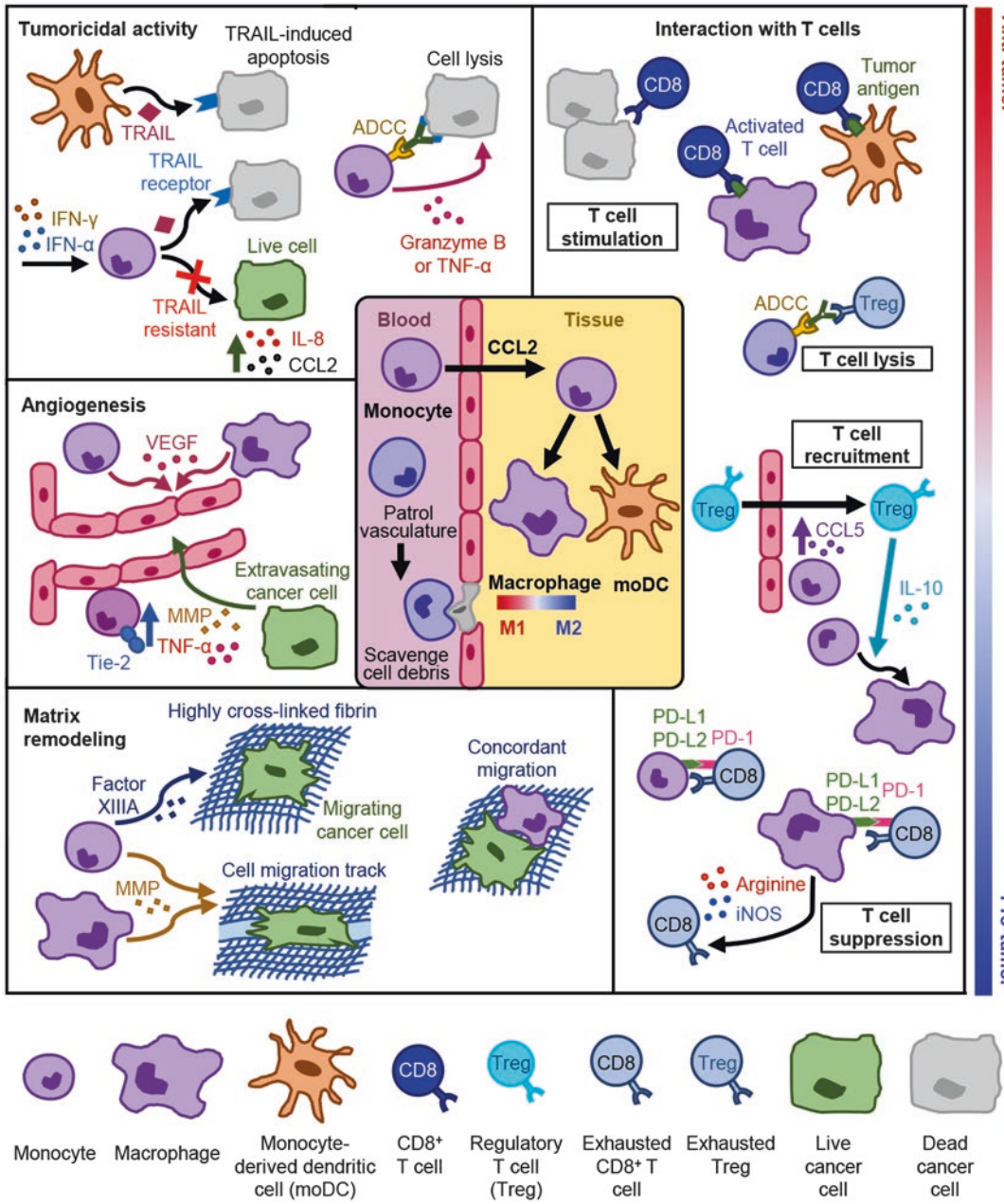

Fig. 7.1 Role of monocytic cells in the tumor microenvironment. Monocytes circulate in vasculature or egress into tissue and differentiate into macrophages/monocyte-derived dendritic cells. These cells display phenotypes along an anti-tumor-to-pro-tumor spectrum. Their roles include the lysis of cancer cells or immunosuppressive regulatory T cells (Tregs), T cell stimulation through Ag presentation, T cell recruitment and immunosuppression, matrix remodeling, and angiogenesis support. (*ADCC* antibody-dependent cellular cytotoxicity, *iNOS* inducible nitric oxide synthase, *MMP* matrix metallopeptidases, *PD-(L)1/2* programmed death-ligand 1/2, *TRAIL* TNF-related apoptosis-inducing ligand)

Mo-mediated ADCC of Ab-coated breast cancer cells [66]. CD16$^+$ Mos engage with Abs bound to cancer cells, inducing Mo secretion of TNF-α and subsequent TNF-α-mediated tumor cell lysis [64]. Mos exposed to IFN-γ and IFN-α can also produce TNF-related apoptosis-inducing ligand (TRAIL) that results in TRAIL-induced cancer cell apoptosis in vitro [67].

Tumor cells alter multiple pathways to evade the tumoricidal activity of Mo-derived cells. To combat the phagocytic functions of SIRPα-expressing Mo-derived cells, solid [60, 61] and hematologic cancer cells [68, 69] upregulate CD47 expression to increase SIRPα inhibitory signaling. Cancers can also be TRAIL-resistant, where a study observed that TRAIL stimulation induces cancer cell lines to secrete cytokines such as IL-8 and CCL2, contributing toward a tumor-supportive TME characterized by heightened accumulation of Mos and increased polarization of myeloid cells toward pro-tumor myeloid-derived suppressor cells (MDSCs) and M2-like MΦs [70]. Additionally, Mos phagocytose tumor-derived microparticles and exosomes, and this suppresses their inflammatory activities [71, 72] and gives rise to immunosuppressive MDSCs [73]. Indeed, many studies in established tumors concur that Mos display only weak or transient tumoricidal activity and, instead, predominantly display (as below-described) pro-tumor functions [74, 75].

7.2.3 Differentiation into TAMs and moDCs

Mos differentiate into TAMs or moDCs depending on the environmental cues of the TME [1, 11, 76]. For example, in the primary tumor, this differentiation process is driven by the exposure of Mos to IL-10 from $CD4^+$ T cells, tumor-synthesized factors including CSF1 and TGFβ, as well as hypoxia due to the poor supply of blood by leaky tumor vessels [76, 77]. scRNA-seq of Mo-derived cells within the TME shows transcriptional profiles or clusters that suggest a transition from blood to intratumoral Mos and then moDCs and TAMs [78]. Notably, although Mos differ from TAMs/moDCs transcriptionally, their phenotypes significantly overlap, and this has led to confusion. For instance, some researchers define $CD11c^+$ Mo-derived cells in the intestines as DCs [79, 80], while others classify these cells to be MΦs [81]. Such findings reinforce that heterogenous populations exist and further study is required to firmly establish unique phenotypes and functions for Mo-derived populations [11].

TAMs are highly abundant within the TME [82] and are viewed to arise from recruited Mos (mostly from the Cla subset and less from NC Mos) [50–53] or from tissue-resident MΦs [11]. However, as seen from the large spectrum of monocytic populations in breast cancer patients [78], there is a need for further studies to better understand the origin of TAMs in the TME. Some studies suggest that TAMs can proliferate [50] and both $CCR2^+$ Mos and resident MΦs contribute to TAM numbers [83]. More recent evidence shows that in some tumor models, $CCR2^-$ mice do not have fewer TAMs [50], suggesting that while CCR2 is fundamental for recruiting Mos to TMEs [50, 53], it is not crucial for amassing TAMs. Such findings also support the notion that TAMs primarily derive from tissue-resident MΦs that are believed to be seeded during waves of embryonic hematopoiesis and to self-renew independently of bone marrow-derived cells during adulthood [11]. Additionally, Mo-TAM differentiation is not fully understood. Cla Mos can differentiate into two populations in the TME that either upregulate DC markers (CD11c and MHCII) or upregulate VCAM-1 plus the murine MΦ marker F4/80 [50, 84]. The differentiation process may further depend on spatiotemporal factors as Mos first localize in deeper regions of the TME but are later found in perivascular sites using sequential CCR2 and CXCR4 signaling pathways in Mo-derived TAMs, respectively [85, 86]. We can speculate that stromal cells secrete factors that drive early recruitment and differentiation of Mos, but cues from the vasculature provide signals that retain monocytic cells within the TME.

TAMs are described with some anti-tumor roles [50, 53] but are generally believed to predominantly play an immunosuppressive role within the TME [87–91]. Higher TAM density at the tumor front correlates with better patient survival [92], suggesting that TAM position in the TME shapes their functions [76, 82, 93]. TAMs are customarily believed to be M2 polarized based on the simplified M1 (IL-1β, IL-6, TNF-α, and CCL3)/M2 (CD163 and CD206) anti-/pro-tumor axis [94, 95], but the M1/M2 dichotomy overlooks how many factors define a MΦ's state [91, 96]. Studies have shown that

M1/M2-like MΦs can co-exist [97, 98] and display mixed M1/M2 functions [99–101], suggesting that the TME supports the emergence of both populations and reinforcing that the M1/M2 concept is likely to be an inaccurate description of Mo-derived cells in the TME. Importantly, this simplistic M1/M2 concept, originally proposed by Mills et al. [102], may stem from how poorly we currently understand polarization cues in the TME because many early studies relied on in vitro protocols that used simple cytokine cocktails or tumor-conditioned media in 2D culture. Also, multiple unique MΦ transcription profiles suggest that specific Mo/MΦ subtypes exist [103] and that TAMs should be grouped or clustered based on high-dimensional analysis (such as scRNA-seq and CyTOF) to account for the complexity in phenotypes, with future studies focused on the function of these subtypes within specific cancers [104, 105].

moDCs form a small fraction of the TME infiltrate [106–108] and often display a phenotype intermediary of Mos and the DC family [109]. In addition to moDCs, other DC subpopulations in the TME include conventional DCs (cDCs) (further categorized by CD103/CD11b expression) and plasmacytoid DCs (pDCs), all of which are efficient cross-presenters of cell-associated antigens (Ags) that can either support or inhibit T cell anti-tumor cytotoxicity in the TME [110]. Specifically for moDCs, these cells show their Mo origins via CD64 and FcγR1 [111, 112] and co-express DC markers (MHCII, CD11b/c) but are viewed as DCs if they have higher MHCII/CD11c expression or dendrite morphology [11]. One study on human breast cancer found that total DCs from the TME ranged from 0% up to 28% of $CD45^+$ leukocytes and clustered closely with Mo/MΦ subsets, supporting the notion that TAMs and DCs are distinct but closely related myeloid subsets in the TME [113].

Polarization within the TME is thought to elicit anti-tumoral effector function through type I IFNs which rapidly mature Mos into tumoricidal moDCs that either produce increased levels of IL-15 to support anti-tumor T helper cell type I responses [114] or express TRAIL to mediate tumor cell apoptosis [115]. Loss of moDCs in tumor-bearing mice can lead to poor chemotherapeutic response [116], and adoptive transfer of Mos [107]/cMoPs [116] can delay tumor growth rates through Ag presentation and drive anti-tumor cytotoxic T cell responses. moDCs are also akin to DCs producing M1-like effector proteins TNF-α and inducible nitric oxide synthase (iNOS) [117] or "inflammatory DCs" [118, 119]. Finally, current understanding of human moDCs is mostly based on studies of in vitro differentiated bone marrow-derived Mos [118]. However, studies in mice show that the specific differentiation cocktail used can result in strikingly different DC subtypes; GM-CSF with IL-4, compared to Flt3L-only differentiation, gives rise to a subtype that more closely resembles in vivo moDCs, whereas the latter gives rise to a phenotype that is typical of cDCs [120]. Thus, consideration should be given to the specific protocols used both in literature and future studies for an improved understanding of moDCs in the TME.

7.2.4 Interaction with Tumor Microenvironment (TME) Matrix

The highly disorganized TME matrix promotes metastasis [121]. The composition of the ECM provides specific biophysical and biochemical cues that influence Mo polarization and activation state [121, 122]. One study showed that THP-1 cells (monocytic cell line) can display spontaneous polarization toward a pro-tumor M2-like phenotype when they are cultured within a 3D in vitro ECM that is rich in hyaluronic acid (HA) [123], an ECM component that is abundant within the TME [124]. Cla Mos can remodel the TME matrix via release of factor XIIIA which cross-links fibrin and provides a scaffold for tumor cells to migrate [125]. In lung cancer patients, densely cross-linked fibrin correlates with $CD14^+$ cells and poor prognosis [125]. $CCR2^+$ Mos that differentiate into MΦs remodel ECM [126] through matrix metallopeptidases (MMP) which degrade collagen and create tracks for cell migration [127]. Moreover, MΦs migrate

concordantly with tumor cells [128, 129], and this contributes to metastasis [128–132]. Interestingly, matrix remodeling is more extensively explored for MΦs [133], due to the short lifespan of Mos in tissue where they promptly differentiate into MΦs [104]. Tumors may also hijack the wound-healing functions of M2-type MΦs that encourage connective tissue cells to reform the ECM to thus shape a pro-tumorigenic TME [134, 135]. Other studies report that Mos give rise to matrix-remodeling programs associated with synthesizing and assembling collagen type I/VI/XIV, which mainly constitute TME ECM [136]. Finally, it would be interesting to understand if undifferentiated Mos can autonomously influence their differentiation into MΦs or M1/M2 polarization by MMP-dependent digestion of the TME ECM, since activated Mos highly produce MMP [49, 137, 138].

7.2.5 Pro-angiogenic Effects

Angiogenesis allows tumors to meet their metabolic needs [139], recruit pro-tumorigenic cell types such as Mos [140], and, in metastatic disease, allows tumor cells to intravasate into tumor vessels to then disseminate from the primary TME [141]. Mos support angiogenesis via VEGF family members, such as VEGF-A, coercing tissue-resident ECs and VEGFR2/CD34$^+$ circulating endothelial progenitor cells to form angiogenic sprouts [142–145]. In vitro, Mos from renal cell carcinoma (RCC) patients are observed to produce more VEGF and better support angiogenesis compared to normal Mos [146]. Studies have also identified a cluster of Mos around tumor blood vessels that express higher levels of Tie-2 than Mos residing elsewhere in the TME or that remain in circulation [147]. Tie-2$^+$ Mos are often studied for their pro-angiogenic role in human cancers as their frequency correlates with tumor vessel density, tumor grade, lymph node status, and frequency of metastasis (TNM stage) [148–150]. Ang-2, overexpressed by tumor vasculature [151], is believed to recruit Tie-2$^+$ CD16$^+$ Mos and augment their production of pro-angiogenic enzymes such as cathepsin B [140, 152]. Tie-2$^+$ Mos secrete other pro-angiogenic factors (MMP and TNF-α) [140, 147, 153] and mediate tumor release of VEGF to recruit other pro-angiogenic Mo-derived cells [154]. MΦs expressing Tie-2 also associate with increased vessel maturation [85], where their depletion by clodronate is linked to the anti-angiogenic effects that was observed in mice [155]. However, future studies should clarify if Tie-2$^+$ MΦs represent polarized tissue-resident MΦs or differentiated Tie-2$^+$ Mos, so that anti-angiogenic therapies can target specific monocytic cell types that mainly drive angiogenesis in the TME. One study provides evidence that Tie-2$^+$CD14$^+$CD45$^+$ MΦ-like cells are specifically found in the blood circulation of cancer patients, and not healthy individuals, suggesting that Tie-2$^+$ cells are bone marrow-derived [156].

7.2.6 Establishing the Pre-metastatic Niche

Beyond their roles in the primary TME, monocytic cells have an important role in establishing the pre-metastatic niche (Pre-MN), distant sites from the primary tumor within the body which enhance the homing of circulating tumor cells (CTCs) in the process of metastasis [157, 158]. Studies of lung metastasis show that CTCs arrest in target tissue vessels [159], enabling tumor-secreted CCL2 to generate a chemoattractive gradient that recruits CCR2$^+$ Mos [53, 160]. These Mos enhance CTC extravasation in part by VEGF secretion, which elevates vascular permeability [161]. This study also found that the genetic or chemical inhibition of CCR2$^+$ VEGFR1$^+$ MΦ (derived from recruited Mos) inhibits metastatic seeding [53, 161]. Other studies have identified a population of metastasis-associated MΦs (MAMs) which promote the extravasation and survival of metastasizing cancer cells by suppressing CD8$^+$ T cell cytotoxicity through superoxide production, thus supporting the establishment of Pre-MNs [52]. Growing evidence further suggests that the primary TME influences the formation of a unique population of MDSCs from particular Mo subsets within the bone

marrow through the systemic release of GM-CSF, G-CSF, IL-6, or tumor-derived extracellular vesicles [162]. These same factors drive systemic monocytosis in cancer and also increase Mo-derived MDSCs which have been shown to suppress anti-tumor T cell responses by anti-inflammatory cytokines and reactive oxygen species (ROS) production [162]. Furthermore, MDSCs can act on distant stromal cells to generate the Pre-MN via cytokine release, pro-angiogenic signaling, and metabolic reprogramming [163, 164].

7.2.7 Interaction with T Cells

Monocytic cells profoundly interact with T cells which directly kill malignant cells [51, 97, 165]. In melanoma, Cla Mos give rise to immunosuppressive Mo-derived cells which produce immunosuppressive iNOS and arginase (Arg), inhibiting the infiltration of effector T cells into the TME [166]. In mice which lacked $CD8^+$ T cells [167], inhibiting CCR2 did not change tumor growth, supporting that effector T cells are downstream targets of Cla Mos which can either suppress or activate T cell functions. Mos and TAMs also express immune checkpoints (proteins that place a "break" on the immune system to keep host immunity in check) such as programmed death-ligand 1/2 (PD-L1/2) that bind to PD-1 on $CD8^+$ T cells to impair T cell proliferation and anti-tumor cytotoxicity [87, 88, 168, 169].

Monocytic cells also present Ags in the context of surface MHCI/II [37], in conjunction with their secretion of T cell-activating cytokines (TNF-α, IL-2, IL-15) or metabolites (iNOS, Arg-1), for homeostasis and response to infection [40, 170]. However, their individual presentations of tumor-associated Ags (TAAs) are less studied. One murine study suggests that $F4/80^{hi}$ Mos can present TAAs to $CD8^+$ T cells as efficiently as MΦ/moDCs [107, 171]. Moreover, TAMs, and not pDCs, which are activated to phagocytose tumor cells by addition of CD47-blocking Abs, can activate $CD8^+$ T cells to induce tumor cell lysis [172]. Also, MHCII-restricted interaction of MΦs (pulsed with OVA-specific peptides) and peptide-specific $CD4^+$ T helper cells can instruct M2-M1 polarization of MΦs, thereby facilitating anti-tumor immune attack [173].

TAA presentation mainly occurs at the primary tumor or lymph nodes, but a recent study shows that $MHCII^+$ Mos also present TAAs to $CD4^+$ T cells within inflamed vasculature of renal glomeruli [174], but future studies are needed to confirm the implications of these findings in other cancer models. Additionally, growing evidence suggests that tissue Mos can retain their monocytic profile without becoming MΦs or moDCs and can patrol for Ags presented across tumor vasculature to transport to draining lymph nodes [37, 40]. Such results support the possible notion that circulating Mos can patrol the vasculature for TAAs and present these to effector T cells to prime them for TAA-specific anti-tumor responses. Thus, future studies should better understand the TAA-presenting capabilities of monocytic cells and where such processes occur with respect to the TME.

Mo-derived cells can also regulate recruitment of effector T cells to the TME. Tumor recruitment of Mos correlates inversely with $CD8^+$ T cell numbers, suggesting that the predominant role of Mos in murine tumor models is in restricting T cell entry into the TME. In murine tumors, CCR2-/CSF1R-based reductions of monocytic cells can increase infiltrating $CD8^+$ T cells and reduce tumorigenesis [167, 175]. Pancreatic cancer patients with lower $CCL2^+$ and higher $CD8^+$ cells display improved survival [176]. Monocytic cells can also secrete CCL5 that recruits regulatory T cells (Tregs) [177] which produce cytokines such as IL-10, differentiating Cla Mos into immunosuppressive TAMs [77, 178]. Interestingly, melanoma patients who responded to immune checkpoint therapy (ICT) that blocked cytotoxic T lymphocyte-associated protein 4 (CTLA-4) have more NC Mos in blood and less intratumoral Tregs [179]. Here, NC Mos induced FcRγ-dependent Treg lysis in vitro [179], suggesting that NC Mos possibly compete against pro-tumor Cla Mos.

7.3 Therapeutic Applications Related to Cells of Monocytic Origin

7.3.1 Biomarkers for Prognosis

Monocytic cells have emerged as biomarkers for early cancer diagnosis. Absolute Mo frequency in blood is associated with improved survival in locally advanced cervical cancer [180]. Improved diagnostic power may be gained from discerning between Mo subsets given their distinct roles as pro- or anti-tumorigenic cells. For instance, lower blood frequencies of Cla Mos and their increase in bone marrow are correlated with improved pancreatic cancer patient survival [176]. Higher levels of myeloid marker CCR2 (primarily recruits Mos to tumors) in metastatic tissues compared to primary prostate tumors is linked to TNM pathologic stage [181]. Finally, PD-L1$^+$ Mo-derived cells are a prognostic factor for patient responders to anti-PD-1/PD-L1 therapy in melanoma [182, 183], head and neck squamous cell carcinoma [184], RCC [185], and colorectal [185] and non-small cell lung cancer [184, 186]. These findings support the capability of monocytic cells to determine disease progression and survival at the clinical level and provide the rationale for future studies aimed at better defining subtype phenotypes and numbers to develop improved therapies and outcomes.

In addition, higher lymphocyte-to-Mo ratio (LMoR) positively correlates with improved prognosis in colorectal [187], lung [188], and ovarian cancer [189]. However, a recent study observed that there is a significant variability in the ratio of T cell to MΦ infiltration across different TMEs and that human tumors are vastly heterogenous [190]. The study clearly shows that patient prognosis must consider multiple factors, such as the extent of neoantigen load and the expression of immunomodulatory genes, both across and within immune cell subtypes [191]. Such findings also emphasize the complexity and intricacies of the human TME that must be modeled precisely to represent the tumor immune milieu in specific cancer contexts.

7.3.2 Combinational Therapeutic Strategies

7.3.2.1 Monocyte-Associated Strategies

Many studies in mice show the potential therapeutic advantage of combining strategies to exploit Mo functions. For example, the combined use of anti-CCL2 Abs and cancer vaccines can lead to reduced Mo accumulation in the TME, enhanced T cell effector functions, and reduced tumor volumes [192]. Vascular density can also be effectively reduced by combining anti-VEGF anti-angiogenesis therapy with the inhibition of Mo activity in the TME via anti-Gr1 Abs, as shown in mice [193]. Also, co-administration of Mos and immunostimulatory IFN-α2a/IFN-γ into xenograft murine models gives rise to reduced tumor growth and prolonged survival [194]. Linehan et al.'s work is one of few human-based studies that demonstrates that co-treatment with a CCR2 agonist (inhibits Mo recruitment) and chemotherapeutic drugs can improve overall survival of pancreatic cancer patients [195]. Notably, the majority of murine-derived findings must be clarified more extensively in humans, reinforcing the need for improved in vitro human TME models.

7.3.2.2 TAM- or moDC-Associated Strategies

TAM-based anti-cancer strategies [76, 196] are broadly classified by limiting their recruitment and localization in the TME [197–201], directly depleting TAMs [202, 203], or reprogramming TAM activities [204–212]. For example, targeting CSF1 can reduce CSF1R$^+$CD163$^+$ MΦs in tumor tissues, translating into positive clinical objective responses in diffuse-type giant cell tumor patients [198]. The chemotherapeutic agent trabectedin can deplete TAMs via apoptosis to give rise to reduced tumor vessel density in patient tumor biopsies [203]. Finally, low-dose gamma irradiation can program the differentiation of iNOS$^+$ MΦs, fostering enhanced infiltration and anti-tumor T cell cytotoxicity [206]. TAMs can also be targeted to achieve anti-tumor

effects alongside other cancer or immune cells such as T cells. For instance, CSF1R blockade can enhance MΦ Ag presentation, but potent tumor regression is only elicited when CTLA-4/PD-1 on $CD8^+$ T cells is also blocked [213]. In vitro or ex vivo tumor Ag-loaded DCs are widely used as cancer vaccines, where they stimulate $CD8^+$ T cells and $CD4^+$ T helper cells to elicit anti-tumor immunity [214]. Further, in vivo tumoricidal activity can be achieved by combining DC vaccination with chemotherapy [215, 216] or phototherapy (therapy using light of specific wavelengths to kill tumor cells) [217]. Other combinational strategies are well reviewed elsewhere [218]. These findings suggest that combinational therapies may be required to block multiple immune evasion strategies that tumors utilize to survive within the TME.

7.3.3 Autologous Monocytic Cell Therapy

Autologous cell therapy (ACT) involves harvesting cells from patients, cell manipulation ex vivo, and re-infusion into patients. This approach provides patients with an adequate supply of highly activated Mos [219], tumoricidal effector MΦs [220], and efficient Ag-presenting moDCs [221]. Overall, clinical studies show that ACT is well tolerated in patients without significant toxicity and decreases cancer relapse frequencies for Mos [222, 223], MΦs [224–226], and moDCs [227, 228]. For example, in melanoma patients, moDC ACT induces cell-mediated anticancer immunity [227, 229] and is also proven safe and potentially effective when combined with chemotherapy [230].

7.3.4 Nano-immunotherapy

Nanoparticles (NPs), particles in the size range of 1–1000 nm, can be engineered to regulate Mo and TAM functions [231, 232]. Lipid and cationic NPs encapsulating siRNA against CCR2 have been developed to interrupt the CCL2-CCR2 axis, disabling Mo recruitment to tumor tissues [201, 233]. Glycocalyx-mimicking NPs (GNPs) can bind to lectin receptors on TAMs, increasing TAM secretion of immunostimulatory IL-12 and decreasing secretion of immunosuppressive IL-10/Arg-1/CCL22. Also, the co-administration of GNPs and anti-PD-L1 Abs can synergistically reduce tumor burden in mice [234]. Lipidoid NPs (LNPs) containing PD-L1 siRNA (siLNPs) can silence PD-L1 in liver-resident MΦs and enhance $CD8^+$ T cell cytotoxicity [235], with similar results being observed for moDCs [236]. Mo/MΦs can also serve as cellular "Trojan horses" that deliver therapeutic cargo due to their tumor-homing capabilities. One study shows that Mos which phagocytized gold nanoshells (Au-NS) can accumulate in breast tumors to result in tumor cell death through photoablation of Au-NS-loaded Mo/MΦs [237, 238]. Also, Mos attached with NP "backpacks" of therapeutic cargo can accumulate more in inflamed organs compared to "free backpacks" [239, 240]. Alternatively, Mos can be loaded with a NP complex of cytotoxic mertansine conjugated to a protease-sensitive peptide [241] and, upon entering lung metastases, differentiate into MΦs that upregulate protease and initiate the on-demand release of mertansine into the TME. Figure 7.2 summarizes the above therapeutic applications.

7.4 Experimental Cancer Models for Studying Monocytes

TME models that study Mos may consider different steps of their activity, from trafficking through vasculature, differentiation, and polarization, to effector functions (including phagocytosis, cytokine secretion, and Ag presentation) and interactions with ECM or TME-specific cells (including cancer-associated fibroblasts (CAFs), tumor, and T cells). These models can mimic the primary tumor or Pre-MN and help to better study mechanisms of human cancer and identify unique human markers. Such models should allow for infusion of whole blood or media containing elements from immune subpopulations while mimicking the physiological shear forces experienced

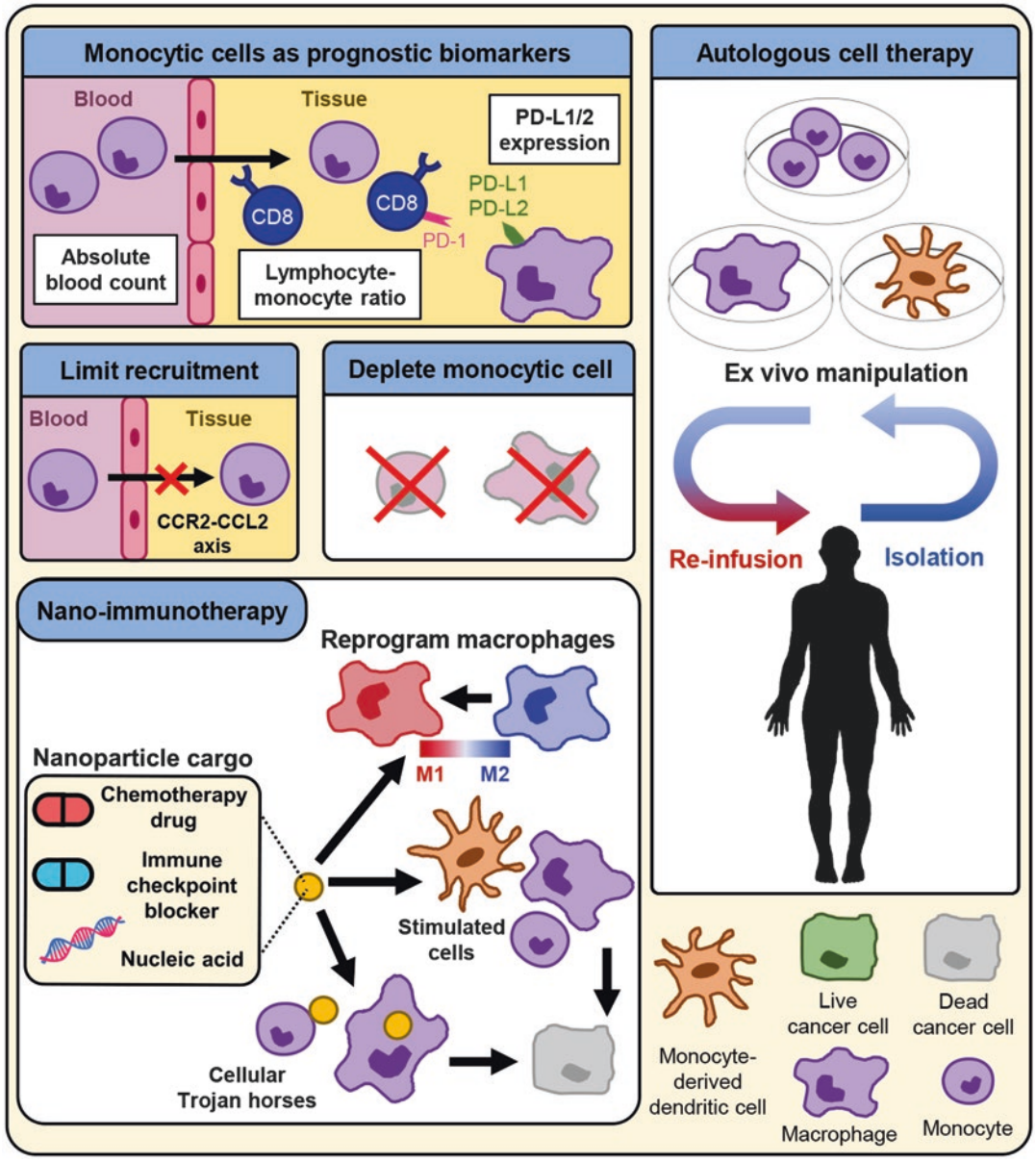

Fig. 7.2 Therapeutic applications and combinational immunotherapy targeting multiple roles of monocytic cells. Monocytes and PD-L1/2^{+} macrophages may be used as prognostic biomarkers for early diagnosis and positive clinical objective response, respectively. Anticancer therapy may be achieved by combinational strategies that limit monocyte recruitment, deplete or reprogram monocytic cells, or employ nanoparticles (encapsulating therapeutic cargo) that exploit the functions of monocytic cells. Monocytic cells can also be used to deliver drug-encapsulating nanoparticles to target sites. Autologous monocytic cells can be manipulated ex vivo and re-infused into patients for anticancer cell therapy

by circulating Mos. Importantly, models should enable the development of human-relevant interventions while complementing preclinical animal models. Here, we describe the progress toward developing such models, existing limitations, and potential solutions.

7.4.1 Conventional 2D In Vitro Cancer Models

Suitable TME models are needed to study the roles and therapeutic applications linked with monocytic cells. Traditionally, these models utilize two-dimensional (2D) cultures of cells in contact with neighboring cells, the culture vessel (made of rigid plastic), and chemically defined medium. These are advantageous in terms of their simple setup and low cost. They also lay important foundations of cancer immunology and TAA discoveries [242]. However, 2D models do not mimic natural tissue structures and fail to recapitulate 3D in vivo cell events [243, 244] which are responsible for cell processes such as differentiation, gene/protein expressions, and others [245–248]. For example, cells in monolayers have relatively free access to signaling molecules and nutrients, which contrasts in vivo environments where barriers to transport, including variations in blood supply, vascular permeability, interstitial fluid flow, and complex matrix interactions that limit diffusion and dynamic cellular consumption rates, generate chemical gradients and unique signaling outcomes that are better recapitulated using 3D culture systems [245, 248]. Alternatively, there are transwell models where cells can be cultured in 2D or 3D settings and which allow for simple cell migration measurements across a filter membrane between upper and lower chambers [249, 250]. However, 2D models often present endpoint readouts that can be confounded by in vitro artifacts such as the non-physiologic constraints of structural materials (such as polycarbonate, polystyrene, or polyester), the lack of mechanical stimuli such as fluid shear stress or mechanical forces, and the absence of cellular, tissue, or ECM heterogeneity seen in patient TMEs. Thus, 3D in vivo and emerging in vitro models in hydrogels or scaffolds can better represent the physical, architectural, and biochemical cues of the in vivo TME.

7.4.2 Conventional 3D Cancer Models

Murine in vivo models are the gold standard of 3D cancer models and, due to their complex nature and feasibility of genetic manipulation, are responsible for many of our recent advances in understanding the TME, particularly in tumor immunology [251]. Such models also facilitate in vivo evaluation of drug pharmacokinetics and enable studies of drug uptake and biodistribution in specific organs [251, 252]. However, murine models raise ethical issues and are costly and time-consuming, and the relevance of results from murine models has been questioned due in part to low conservation between murine vs human tumors and immune systems [253, 254]. Also, despite successful preclinical testing in mice, more than 80% of drug trials in patients fail in early phases, and only 50% of those that pass phase III are approved clinically [255]. Therefore, 3D in vitro models may be improved representations of human cancer and include suspension cultures in non-adherent plates and cultures in scaffold or in gel-like matrix within well plates [256]. Multicellular aggregates/spheroids are a common feature of these models by virtue of their ability to mimic metabolic/chemical gradients, hypoxic conditions, and cell-cell/cell-matrix interactions [247, 257–259]. Aggregates also enable functional studies of monocytic cells in terms of their infiltration of a 3D tumor mass or support of cancer invasion into the 3D TME ECM [260–263].

7.4.3 Comparative Studies of 2D Versus 3D In Vitro Cancer Models

Clearly, 2D and 3D models offer distinct experimental advantages, with the former being more easily adapted for higher-throughput studies and the latter being generally more representative of

in vivo TMEs. However, 2D vs. 3D comparisons reveal disparities in their evaluation of cell immunotherapies [88] and drug sensitivity for both single drugs [264–266] and drug combinations [267]. Other 2D-3D differences include the reduced expression of TAAs and HLA type I by cells grown as a 3D spheroid compared to a 2D monolayer [268]. Cells also differentially express protein(s) when they migrate through a 3D matrix or 2D substrate [244, 250, 269, 270]. For instance, FAK is crucial in 3D, but in 2D, FAK-null cells compensate for migration defects by overexpressing other migration machineries [270]. Morphological [271] and quantitative [269] differences arise between 2D and 3D migration. Loss of diverse phenotypes [272] also results from 2D culturing, and this is especially relevant for monocytic cells which in vivo have a broad spectrum of functional responses to environment cues of the TME. Thus, a 3D environment will more accurately predict in vivo drug responses for different pathways. In the example of the aforementioned FAK pathway that is under-represented in a 2D culture of tumor cells, drugs targeting these pathways may be falsely deemed to be negative in 2D studies. Conversely, 2D studies might yield drugs that are ineffective in clinical studies because compensatory pathways can also emerge under more physiologic 3D settings. Finally, although more studies are needed to confirm that 3D cultures better indicate clinical outcome [252], a 3D model should be strongly considered over simplistic 2D cell monolayers so that experimental conclusions have improved physiological relevance.

7.4.4 Microfluidic Cancer Models

Microfluidic models of the TME could represent an advantageous intermediate step that links the findings of 2D in vitro cell assays, preclinical animal studies, and clinical patient trials. Unlike conventional 3D models, microfluidic technologies capture immune cell processes through spatial compartmentalization [273] and the capability to mimic precise chemokine gradients [274], endothelial barrier function [275, 276], and flow conditions [277, 278]. These models can be built using gels of specific composition (e.g., collagen, fibrin, or various proteoglycans) that more closely mimic the ECM of cancer-specific TMEs. Moreover, because the culture of monocytic cells in a 3D matrix supports their de novo production of ECM [279], such systems yield more physiological 3D environments from an initial setup based on a simple gel. Their small dimensions also allow for experiments that require less reagents and cells [273, 280], making them ideal for testing precious patient specimens. Such systems can be incorporated with vasculature to mimic the transport of circulating immune and tumor cells and their intravasation into vasculature or extravasation into the surrounding matrix [275, 281, 282]. They also enable high-resolution imaging and real-time tracking of cell migration [274, 275, 282, 283], a procedure that may be feasible (e.g., by intravital two-photon imaging) but is technically demanding in animal models [273, 280].

7.4.4.1 Microfluidic Cancer Models to Study Monocytes

Studies have increasingly used microfluidic platforms to gain improved insight on the role of Mos [88, 275, 284–286], TAMs [99, 131, 243, 277, 287, 288], and DCs [289–291] (Table 7.1). Lee et al. revealed the differential capability of PD-L1$^+$ Mos to suppress the anti-tumor efficacy of retrovirally transduced vs. mRNA-electroporated T cells, results that were not shown through 2D cytotoxicity assays [88]. Otano et al. showed the therapeutic boost of anti-sense oligonucleotides against PD-1 to CD8$^+$ T cells that allow them to overcome PD-L1$^+$ Mo suppression [284]. Finally, a vascularized model revealed that Mos reduce cancer cell extravasation independently from their contact with cancer cells and Mos have little effect on cancer cell extravasation once they transmigrate across the microvasculature [275]. Importantly, microfluidic models of the human TME provide a system of improved physiological relevance to validate the above-discussed effects of Mos in 3D which to date have mostly been specific to murine systems, including their effect on the growth of tumor aggregates, ADCC-based tumoricidal activity, and their support toward developing the Pre-MN.

Table 7.1 Microfluidic cancer models for studying monocytes/macrophages/monocyte-derived dendritic cells

Research topic	Cells in co-culture	Description of model setup	Gel matrix	Key findings	Ref.
MΦ impairment of endothelial barrier and effect on cancer cell intravasation	1. RAW 264.7 cell line 2. HT1080 cell line 3. HUVECs (endothelial cells)	One central gel channel (MΦs and HT1080), flanked by two media channels (one has HUVEC monolayer). Cancer cell intravasation was assessed by confocal imaging	2.5 mg/mL rat tail collagen I	MΦs secrete TNF-α, resulting in endothelial barrier impairment, increasing intravasation of HT1080	[276]
MΦ glycolytic activity and support of cancer cell extravasation	1. Human primary Mos differentiated in vitro into MΦs via cancer-/normal cell line-conditioned media 2. Panc1, MiaPaCa2, and HPNE cell lines 3. HUVECs	One central gel channel flanked by two media channels (one has HUVEC monolayer). MΦs and cancer cells were added into the HUVEC-covered channel lumen, and extravasation across the HUVEC layer was tracked by live confocal imaging	2.5 mg/mL rat tail collagen I	Tumor-conditioned MΦs promote cancer cell extravasation compared to control MΦs	[99]
Mo extravasation across vasculature and support of cancer cell extravasation	1. Human primary Mos 2. MDA-MB-231 and MDA-MB-435 cell lines 3. HUVECs 4. NHLFs (fibroblasts)	One central gel channel (vasculature self-assembled from a HUVEC/NHLF co-culture), flanked by media channels. Mos were perfused through vasculature with/without cancer cells, and extravasation across the vasculature was tracked by live confocal imaging	3 mg/mL fibrin	$CCR2^+$ Mos extravasate via actomyosin. Mos reduce cancer cell extravasation in a non-contact-dependent manner, with little impact on extravasation once Mos transmigrate across vasculature	[275]
Non-contact- vs. contact-dependent MΦ support of EMT and dispersion of A549 cell aggregates	1. Human primary Mos differentiated/polarized in vitro into M0, M1, M2a, M2b, and M2c subtypes 2. A549 cell line 3. HUVECs	Two inner gel channels (MΦs and A549 aggregates in one or separate channels), flanked by two media channels (one has HUVEC monolayer). Aggregate dispersion was tracked by live confocal imaging	2.5 mg/mL rat tail collagen I	M2a MΦ subset supports A549 aggregate dispersion via ICAM-1 and β2 integrin contact-dependent mechanisms	[131, 298]
MΦ-derived cytokines that support cancer cell migration	1. THP-1 cell line differentiated into M2-like MΦs using PMA 2. MRC-5 cell line 3. CL1-0 (fibroblasts)	Three multi-layer cell culture chambers (each with 1 cell type). Microvalves connected chambers and were opened/closed by pneumatic conduits embedded in the second layer. Migration was tracked by live differential interference contrast (DIC) imaging	–	MΦs enhance cancer cell migration but restrain the tumor-promoting effect of myofibroblasts through TNF-α	[299]
Mo PD-L1 expression and suppression of engineered T cells	1. Human primary Mos 2. HepG2 cell line 3. Retrovirally transduced (Tdx)/mRNA-electroporated (EP) T cells	One central gel channel (Mos and HepG2 aggregates), flanked by two media channels (T cells added in one media channel and migrated toward HepG2 targets). HepG2 death was tracked by live confocal imaging	2.5 mg/mL rat tail collagen I	$PD\text{-}L1^+$ Mos suppress Tdx but not EP T cells. PD-1 knockdown in Tdx T cells overcomes Mo suppression	[88, 284]

Patient-specific screen of immune checkpoint blockade	1. Patient-derived/murine-derived organotypic tumor spheroids (PDOTS/MDOTS)	One central gel channel (PDOTS/MDOTS), flanked by two media channels. Spheroids were cultured with/without various immune checkpoint blocking Abs for downstream characterization	2.5 mg/mL rat tail collagen I	PD-L1/2$^+$ MΦs are seen in PDOTS/MDOTS. PDOTS exposed to dual PD-1/CTLA-4 blockade show increased M0 MΦs than single blockade	[286, 288]
MΦ migration toward cancer cells	1. RAW 264.7 cell line 2. MDA-MB-231 cell line	Three inner gel channels flanked by two media channels. Migration was tracked by live confocal imaging	4 mg/mL Matrigel (RAW 264.7) and rat tail collagen I (MBA-MB-231)	MΦs invade into neighboring gels containing MDA-MB-231 but not into empty gels	[285]
MΦ M2 polarization and migration toward cancer cells	1. RAW 264.7 cell line 2. T24 cell line 3. HUVECs 4. BJ-5Ta (fibroblasts)	Four innermost culture chambers linked by conduits to an outermost media chamber that could be linked to media perfusion equipment. Migration was assessed by live fluorescence imaging	2.5 mg/mL (approximated) Matrigel	MΦs express high Arg-1 in the quadruple cell culture and migrate toward cancer cells	[287]
MΦ M2 polarization and migration under interstitial flow (IF)	1. BMDM (bone marrow-derived MΦs) 2. RAW 264.7 cell line	One central gel channel (MΦs), flanked by two media channels. To generate IF, a media reservoir was added directly above the inlets of 1 fluidic channel. Migration was tracked by live phase-contrast imaging	2.5 mg/mL rat tail collagen I	IF supports MΦ M2 polarization via integrin/Src and STAT3/6 and enhances MΦ migration activity	[277]
DC contact with T cells under IF	1. Adherent murine DC cell lines (MutuDC) 2. CD4$^+$ and CD8$^+$ T cell hybridomas	One flow channel (DC monolayer) with two inlets/outlets that were connected to syringe pumps (to insert fluids and cells). Migration was tracked by confocal imaging	–	DCs interact more transiently with CD8$^+$ T cells vs. CD4$^+$ T cells and more strongly with specific vs. non-specific T cells	[290]
DC migration toward cancer cells	1. Human primary Mos treated (3 days) with M-CSF and IFN-α2b 2. SW620 cell line	One central gel chamber (DCs) linked by conduits to two chambers (tumor cells) and two outer media channels. DC migration was tracked by fluorescence imaging	3 mg/mL rat tail collagen I	IFN-α2b-treated DCs migrate toward and phagocytose tumor cells that were pre-treated with drugs	[289]
DC migration under precise chemokine gradients	1. Murine DCs matured from LPS-treated BM-derived cells	One central gel channel (DCs), flanked by two media channels (chemokines added to create a gradient across the central channel). Migration was tracked by live fluorescence imaging	1.5 mg/mL rat tail collagen I	DCs respond to small CCL19/CCL21 gradients, but CCL21 directs migration more potently than CCL19	[291]

7.4.4.2 Microfluidic Cancer Models to Study Monocyte-Derived Cells

Complex TME models have successfully captured the in vivo profile of Mo-derived cells in the TME. In one of the more cellularly complex TAM-associated models, MΦs upregulate Arg-1 in their quadruple cell culture with ECs, fibroblasts, and bladder cancer cells, analogous to their activation in vivo [287]. The same model capably screens for chemotherapy regimens. Other models characterize TAM supportive capabilities in cancer cell extravasation [99] or intravasation [276] across EC barriers. The impact of specific Mo-derived MΦ subsets can also be elucidated as shown by Bai et al., where a subset of M2-like MΦs (specifically, the M2a MΦ subset) show the capability to mediate contact-dependent epithelial-to-mesenchymal (EMT) transition of tumor aggregates [131].

Microfluidic models have been developed for the general DC population, but no study that specifically focused on moDCs has been performed. One model reveals that CXCR4 mediates the migration of IFN-α2b-conditioned DCs toward cancer cells that were treated with epigenetic drugs [289]. Other models provide insight on DC chemotaxis under precise CCL19/21 gradients [291], cell volume changes under hyperosmotic stress [292], and contact with T cells under different shear stresses [290]. Other areas such as the effect of DCs on T cell activation (via TAA presentation) or immunosuppression or DC trafficking between the TME and draining lymphatics should also be explored in microfluidic models of the human TME.

7.4.4.3 Patient-Derived Microfluidic Cancer Models

Microfluidic models have the great advantage of allowing the culture of patient-derived explants such as patient-derived organotypic tumor spheroids (PDOTS) that retain the relevant immune cell types found in tumor tissues. Jenkins et al. developed an ex vivo system that retains key features of patient-specific immune TMEs, showing the presence of $CD14^+$ monocytic cells and heterogenous PD-L1 expression which is reminiscent of in vivo Mo-derived cell profiles [286]. Further, Aref et al. demonstrated the capability of such platforms to screen ICT, a form of therapy which includes the use of Abs against complementary checkpoint proteins (e.g., between PD-L1 and PD-1) to inhibit checkpoint protein signaling. Through the platform, authors could observe an expansion of both $CD8^+$ T cells and naïve M0 MΦs within in vitro PDOTS that received dual checkpoint blockade against CTLA-4 and PD-1 [288]. Because such ex vivo models mimic the patient-specific TME, they have the potential to predict patient-specific responses to immunotherapies.

7.5 Future Directions

Despite recent progress, multiple areas remain to be clarified of monocytic cells in the TME, ranging from questions of their ontogeny, heterogeneity, and functions. At the same time, the versatility of these cells presents an opportunity to exploit combinational approaches to achieve superior cancer immunotherapy. For example, NPs are candidate therapies that can simultaneously modulate multiple roles of Mos, which include inhibiting their recruitment to tumors, differentiation into pro-tumor MΦs/moDCs, and potential tumoricidal activity in the TME. Current understanding has mostly derived from studies performed in murine models and remains to be validated in human settings. Therefore, improved physiologically relevant TME models are needed for investigating human-relevant monocytic cell biology and developing human-relevant therapeutic strategies.

While simplistic 2D and traditional 3D (transwell) in vitro cell cultures are scalable and robust, their relevance in vivo is limited by their lack of biological functionality. Conversely, animal models replicate function at both organ and multi-organ levels but are inherently flawed due to human-murine species differences. As such, we support that microfluidic human TME models combine the best features of both models by culturing human cells in tissue-specific conditions that are designed to mimic human-relevant biological and physical cues of the TME. To date, microfluidic human TME models have been developed to study Mos, TAMs, and moDCs

(Table 7.1), all of which are capable of mimicking relevant tumor-immune interactions in a controlled setting that is unique to the specific cancer. Future model developments can draw from the growing understanding of the biochemical and biophysical properties of the TME, such as the impact of tumor interstitial flow and the function of tumor lymphatics. Incorporating these elements in TME models would enhance the physiological accuracy of TME models and enable deeper characterizations of monocytic cells in the TME to design and screen immunotherapies. Moreover, by incorporating patient specimens, scientific understanding can be specific to the patient's pathology and can be applied for developing patient-specific treatments.

The emergence of microfluidic human TME models highly complements ongoing immunophenotypic studies that utilize advanced techniques such as CyTOF, RNA-seq, and single-cell analysis [113, 190, 293]. Gubin et al. observed multiple subpopulations of Mos/MΦs (distinguishable by markers such as CD206, CX3CR1, and CD1d) that evolve over the course of ICT. These findings further suggest that ICT contributes toward broader remodeling of the TME, supporting that circulatory Mos/early MΦs are more important than pre-polarized intratumoral MΦs in tumor progression [113]. Such findings also highlight the intricacies and complexity of the TME that must thus be meaningfully recapitulated through a precise and controlled mimic of environmental cues in human cancer-specific TMEs.

Notably, one can envision future organ-on-a-chip technology, for example, of the human brain [294], to be integrated with tumor spheroids to model primary or metastatic TMEs. Patient tumor samples, as well as patient-derived monocytic cells, can be incorporated into such models to explore patient-specific tumor progression and response to novel immunotherapies [295, 296]. Moreover, immunophenotyping of parallel devices at different time points can be utilized to capture, in detail, human responses to immunotherapy over time, an area of study that is currently not possible due to ethical concerns and practical limitations of repeat patient biopsies.

As such, research groups have increasingly focused on the development of culture reactors to extend the lifetime of in vitro and ex vivo cultures and on the design of high-throughput and automated systems toward the aim of establishing standardized platforms for clinical precision medicine applications [252]. These microfluidic models could complement existing in vivo preclinical studies while reducing the economical and ethical burden of preclinical investigations. Further, by developing several organ-specific TME models and connecting these using appropriate perfusion conduits [297], a comprehensive model of the human system can be built to study the dynamic functions of Mos across different cancer stages and cancer-specific TMEs.

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