Tumor promoting capacity of polymorphonuclear myeloid-derived suppressor cells and their neutralization

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Funding information
Bundesministerium für Bildung und Forschung, SERPENTINE/ERA PerMed network; Deutsche Forschungsgemeinschaft, Grant/Award Number: 259332240/RTG 2099; Deutsches Krebsforschungszentrum, Grant/Award Number: CA181

Abstract
Myeloid-derived suppressor cells (MDSC) represent a highly immunosuppressive population that expands in tumor bearing hosts and inhibits both T and NK cell antitumor effector functions. Among MDSC subpopulations, the polymorphonuclear (PMN) one is gaining increasing interest since it is a predominant MDSC subset in most cancer entities and inherits unique properties to facilitate metastatic spread. In addition, further improvement in distinguishing PMN-MDSC from neutrophils has contributed to the design of novel therapeutic approaches. In this review, we summarize the current view on the origin of PMN-MDSC and their relation to classical neutrophils. Furthermore, we outline the metastasis promoting features of these cells and promising strategies of their targeting to improve the efficacy of cancer immunotherapy.

KEYWORDS
immunosuppression, immunotherapy, metastasis, neutrophils, PMN-MDSC

1 | INTRODUCTION

One of the hallmarks of cancer is its capability to avoid recognition and destruction by the immune system.1 Cancer cells achieve this aim, in particular, through the acquisition of mutations in the antigen-presenting machinery, the down-regulation of MHC molecules and the establishment of an immunosuppressive environment.2,3 Immunosuppression can be mediated both by tumor cells...
and host cells in the tumor microenvironment (TME). Tumor-infiltrating immune cells such as tumor-associated macrophages (TAM), regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC) possess potent capacities to suppress T cell and natural killer cell (NK) function and antigen presentation by dendritic cells (DC), contributing thereby to primary tumor progression, metastasis and therapy resistance. 3 MDSC consist of two main subsets: monocytic (M) and polymorphonuclear (PMN)-MDSC. 4 In contrast to M-MDSC, the study of PMN-MDSC is challenging due to their more fragile nature under ex vivo conditions. In recent years, researchers identified tumor-infiltrating PMN-MDSC as a negative prognostic factor in renal cell carcinoma, 5 melanoma, 6 colorectal cancer, 7 hepatocellular carcinoma, 8 head and neck cancer 9 and non-small cell lung cancer (NSCLC). 10 In addition, this MDSC subset has been shown to not only elicit immunosuppressive mechanisms comparable to M-MDSC but also unique tumor and metastasis promoting properties. Since the immunosuppressive function of these cells has been already described in detail elsewhere, 11,12 in this review, we focus on the current understanding of the origin and characteristics of PMN-MDSC, their metastasis promoting properties and their role as a therapeutic target.

2 CHARACTERIZATION OF POLYMORPHONUCLEAR-MYELOID-DERIVED SUPPRESSOR CELL

MDSC represent a highly heterogeneous population of myeloid cells that accumulate under chronic inflammatory conditions typical for cancer. 13 In mice, MDSC are characterized as Gr1−CD11b+ cells. The myeloid differentiation antigen Gr1, which is a glycosylphosphatidylinositol linked protein, consists of two subsets, Ly6G and Ly6C, allowing a further differentiation into Ly6G+Ly6C+ PMN-MDSC and Ly6GlowLy6C+ M-MDSC. 4 Since human cells lack the expression of Gr1, human PMN-MDSC and M-MDSC are defined as CD11b+CD14+CD15+ or CD11b+CD14+CD66b+, and CD11b+CD14+ human leukocyte antigen (HLA)-DR−/lowCD15− cells, respectively. 4 A third population, containing immature myeloid cells without immunosuppressive activity, is termed early-stage MDSC and defined as Lin− (including CD3, CD14, CD15, CD19 and CD56) HLA-DR−CD33− cells. 4 In most solid malignancies PMN-MDSC, resembling neutrophils, represent a dominant subpopulation of tumor-infiltrating and circulating MDSC. 14 In addition, the numbers of PMN-MDSC in vivo are assumed to be underestimated since experimental procedures, including freezing and thawing of samples can lead to degradation of these cells. 15 Furthermore, a high neutrophil to lymphocyte ratio has been shown to be a negative prognostic factor, indicating a pivotal role of pathologically activated neutrophils in malignant progression. 16 Despite intensive investigation of PMN cells, their nomenclature in tumor bearing mice and patients is often confusing. For instance, PMN-MDSC could be also termed immunosuppressive neutrophils, N2 neutrophils, tumor-associated neutrophils (TAN) or pathologically activated neutrophils.

3 ORIGIN OF POLYMORPHONUCLEAR-MYELOID-DERIVED SUPPRESSOR CELL

Neutrophil production occurs primarily in the bone marrow where hematopoietic progenitor cells (HPC) differentiate into mature neutrophils that are released into the bloodstream. 17 The most prominent change in morphology during this process is the segmentation of the initially banded nucleus, which allows the identification of different stages of neutrophil maturation. 18 The accumulation of immature and mature granulocytes in cancer is due to their increased production in the bone marrow, primarily mediated by increased concentrations of the granulocyte-colony stimulating factor (G-CSF), which is regarded as the master regulator of neutrophil differentiation and proliferation. 19 Under physiological conditions, neutrophils are retained in the bone marrow until their maturation process is completed. Proliferated neutrophils that express the CXC chemokine receptor (CXCR)-4 remain in the bone marrow mediated by a constitutive production of its ligand, chemokine CXCL12 by osteoblasts and bone marrow stromal cells. 20 Another mechanism that mediates neutrophil retention is the expression of α4β1 integrin (VLA-4) on neutrophils, which is lost during maturation and the production of its ligand VCAM-1 by bone marrow stromal cells. 21 In tumor bearing individuals, this process is impaired by tumor-derived factors, in particular, by G-CSF. 22 It reduces the production of CXCL12 by bone marrow stromal cells and the expression of its receptor CXCR4 on neutrophils, leading to an excessive release of neutrophils into the circulation 23,24 and their migration to the tumor site mediated mainly by CXCR2 and CXCR4 ligands. 25 Furthermore, G-CSF and GM-CSF have been shown to act as chemoattractants for neutrophils. 26 Factors, inducing neutrophil recruitment originate from both cancer and tumor infiltrating immune cells. 27 It has been reported that G-CSF production could be stimulated by IL-17, produced mainly by γδ T cells. 28 Blocking of IL-17 or G-CSF or the depletion of γδ T cells diminished neutrophil accumulation in breast cancer metastases. 29 Activated T cells have been also demonstrated to produce GM-CSF, CXCL1 and CXCL2, however, a specific contribution of different T cell subsets to neutrophil recruitment needs further investigation. 27 In addition to its effect on neutrophil expansion and migration, G-CSF is considered a potent inducer of tumor promoting properties of neutrophils. 29

Another important factor involved in the acquisition of immunosuppressive features by neutrophils is transforming growth factor (TGF)-β. Blocking TGF-β resulted in an increased influx of classical neutrophils into murine tumors. 30 Most importantly, upon TGF-β blockade, these neutrophils demonstrated antitumor cytotoxicity, which suggests TGF-β as a major factor which induces neutrophil polarization. This assumption is further supported by studies that showed impaired cytotoxicity of TGF-β treated neutrophils and the retention of an antitumor activity of neutrophils lacking the TGF-β receptor. 31,32 In contrast, interferon β was shown to block tumor supporting functions of neutrophils by suppressing the production of proangiogenic factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP) 9. 33
4 | POLYMORPHONUCLEAR-MYELOID-DERIVED SUPPRESSOR CELL VERSUS NORMAL NEUTROPHILS

In the course of neutrophil differentiation and proliferation, the density of neutrophils rises due to increased granularity and reduced cell size resulting from subsequent cell divisions.34 Stimulation of granulopoiesis due to cancer-derived factors leads to the increased level of circulating neutrophils and to the release of not terminally differentiated cells into the periphery. Several studies therefore examined the differences in the frequency and immunosuppressive capacity between circulating high density neutrophils (HDN) and low density neutrophils (LDN) in cancer patients.35-37 Circulating LDN are mostly absent in healthy donors but expanded in cancer patients; they are unable to induce T cell activation, possess a strong immunosuppressive effect on effector cells of the immune system in vitro and show proangiogenic properties. Therefore, they can be described as a population of PMN-MDSC.35-37 However, considering only a low density or an immature phenotype of neutrophils as a sufficient indicator for their immunosuppressive activity is, probably, an over-simplified view since the LDN fraction has been found to consist of both cells with banded, and segmented nuclei.37 In addition, a study including patients with head and neck and urological cancers identified a circulating neutrophil population with a high expression of the maturation markers CD11b and CD16 as cells with the highest immunosuppressive potential.38

Mature neutrophils have been found to suppress T cell activity upon stimulation through the secretion of reactive oxygen species (ROS).39 In this setting, inhibition of T cells was dependent on CD11b activation on neutrophils. In contrast to these findings, PMN-MDSC from renal cell carcinoma patients demonstrated a decreased expression of CD16 and CD62L associated with an increased CD66b expression.40 Furthermore, PMN-MDSC with low CD16 expression correlated with the resistance to capecitabine in colorectal cancer patients.41

The expression of Fc receptors on neutrophils is of interest in this context since they can exert antibody-dependent cell-mediated cytotoxicity (ADCC).42 The lower expression of the Fc receptors CD16 and CD32 on PMN-MDSC detected in some studies might result in a decreased cytotoxic potential. However, since recent studies also suggested a role of CD16 as a decoy receptor that can reduce ADCC towards cancer cells, interpretation of Fc receptor expression is complicated.42

It is quite difficult to distinguish immunosuppressive PMN-MDSC from nonimmunosuppressive neutrophils based only on the expression of surface markers. The hallmarks of both cell populations are summarized in Table 1. Recent studies suggest a different metabolic program between normal neutrophils and PMN-MDSC. While neutrophils rely mostly on glycolysis to meet their energy demand, PMN-MDSC demonstrate a higher flexibility in their response to the glucose availability.37,43 PMN-MDSC are known to generate high amounts of ROS, which can inhibit antitumor activity of effector immune cells. ROS could be an important inducer of the endoplasmatic reticulum (ER) stress response which is strongly upregulated in PMN-MDSC.44 Together with the upregulation of the ER stress response, PMN-MDSC acquire an immunosuppressive pattern and express the lectin-type oxidized low-density lipoprotein receptor 1 (LOX-1), which is absent on neutrophils from healthy donors.45 LOX-1 upregulation by ER stress has also been demonstrated in macrophages, although it was not investigated in the context of immunosuppression.46

LOX-1 upregulation in response to ER stress has been found to be mediated by the transcription factor X-box binding protein (XBP) 1 in lung adenocarcinoma cell culture models.47 Additional studies have demonstrated that it could serve as a promising marker for immunosuppressive cells in glioblastoma.48 Moreover, a recent publication demonstrated an increase in circulating LOX-1+ PMN-MDSC in NSCLC patients that were not responding to anti-PD1 therapy.49 This suggests that LOX-1 identifies a subpopulation of PMN-MDSC that exerts immunosuppression. Although LOX-1 was described as a marker for immunosuppressive neutrophils in patients, it could not be used to distinguish PMN-MDSC from nonsuppressive PMN cells in mice.45

Due to their generally more immature phenotype, PMN-MDSC were described to display a decreased granularity, which also impairs their cytotoxicity.30 Transcriptome analysis of bone marrow neutrophils

| Characteristics | Physiological neutrophils | PMN-MDSC |
|-----------------|--------------------------|----------|
| Surface marker in mice | CD11b^Lin^Ly6G^- | CD11b^int^Ly6G^- |
| Surface marker in humans | CD15^-CD66b^-CD16^-CD14^- | CD15^-CD66b^-CD16^-CD14^-CD11b^-CD33^-HLA-DR^-Lox1^- |
| Maturity | Mature, segmented nuclei,17 High CD16 expression,38 High density,36 | Immature, banded nucleus,40 Intermediate CD16 expression,39 Low density,36 |
| Immunosuppressive potential | Can induce T cell activation,35-37 Nonimmunosuppressive35-37 | Unable to induce T cell activation,13 Suppress T and NK cell function35-37 |
| Metabolism | Glycolysis as a main source of energy production,43 Regular ER stress response44 | Higher metabolic flexibility,37 Increased fatty acid oxidation,96 Higher PEG2 production,98 High ER stress response,44 |
| Cytotoxic potential | High cytotoxicity36 | Low cytotoxicity36 |
| Angiogenic potential | Low angiogenic potential117 | High angiogenic potential117 |
| Life span | 6-8 hours in circulation, up to several days in tissues118 | Shorter life span due to increased apoptosis119 |
from mesothelioma bearing mice showed a decrease in genes associated with phagosome assembly and antigen processing, suggesting a reduced potential for phagocytosis and T cell stimulation. A recent study supported the view that PMN-MDSC represented a distinguished subset of PMN neutrophils. The authors identified three distinct PMN neutrophil populations in cancer bearing mice: classical PMN neutrophils, PMN-MDSC and activated PMN-MDSC. The latter were found only in the TME during early tumor development, were highly immunosuppressive and could be identified based on the expression of CD14. In patients, the authors described classical PMN neutrophils and tumor infiltrating PMN-MDSC that showed a gene expression profile similar to activated murine PMN-MDSC.

5 | METASTASIS PROMOTING PROPERTIES OF POLYMORPHONUCLEAR-MYELOID-DERIVED SUPPRESSOR CELL

PMN-MDSC have been shown to support all steps of the metastatic process. These include dissemination of tumor cells from the primary tumor, their migration through endothelial barriers, reaching the lymphatic or blood circulation and seeding at the distant organs that offer an environment, supporting metastasis (Figure 1).

PMN-MDSC have been demonstrated to secrete IL-17a which induces a downregulation of E-cadherin in gastric cancers cells. This loss of epithelial markers together with an increase in the mesenchymal markers vimentin and ZEB1 induced a higher motility in gastric cancer cells and enabled their dissemination from the primary tumor. As immunosuppressive cells, PMN-MDSC can inhibit NK cells, which promotes the survival and metastatic capacity of circulating tumor cells (CTC) in the luminal space of microvessels. In addition, PMN-MDSC have also been shown to directly interact with CTC. Co-injection of MDA-MB-231 breast cancer cells and melanoma or breast cancer patient-derived CTC together with PMN-MDSC led to increased metastases formation in mice. In this context, ROS derived from PMN-MDSC induced the expression of Notch1 in CTC, promoting thereby their survival.

Extravasation of tumor cells is supported by the secretion of MMP8 and 9 by PMN-MDSC, increasing vessel permeability. Tumor cells that already disseminated from injected mammary tumors showed an increased potential to form macroscopic distant metastases when PMN-MDSC frequencies in target organs were elevated. In addition, co-injection of 4T1 breast cancer cells in the tail vein of mice with PMN-MDSC (but not with M-MDSC) increased their potential to form lung metastases. Another important factor that influences metastasis is neutrophil elastase (NE) that affects tumor cell dissemination, extravasation and seeding in the premetastatic niche. NE is a serine protease that is primarily produced by neutrophils and other immune cells, including macrophages and lymphocytes, and that is accumulated in the serum of cancer patients. In primary tumors from breast cancer patients, increased NE levels were associated with metastasis and poor prognosis. In addition, high NE concentration correlated with poor response to tamoxifen and trastuzumab treatment in breast cancer patients. Metastases of subcutaneous Lewis lung carcinomas were decreased in mice deficient for NE. This observation was found to be dependent on the diminished degradation of insulin receptor substrate 1 (IRS-1), which acted as a negative regulator of the PI3K pathway, reducing thereby tumor cell proliferation.

NE together with myeloperoxidase (MPO) represent essential components of neutrophil extracellular traps (NET). The process of NET formation (NETosis), during which neutrophils release high amounts of DNA and proteases was first described as a mechanism for defense against bacteria. Although NETosis was initially considered as a form of cell death, it has become clear that both vital and lytic forms can occur. In infections, vital NETosis is predominant while in sterile injuries, primarily lytic NETosis is occurring. If there is a preference of a particular form of NETosis in cancer patients and if this influences tumor progression needs further investigation. In recent years, NETosis has been associated with different pathological conditions, including primary tumor growth and metastasis. PMN-MDSC derived NETs have been shown to trap CTC both in vitro and in vivo, which was associated with increased metastasis formation. Furthermore, NETs act as a physical shield for tumor cells, protecting them from the cytotoxic effect of immune effector cells. Studies with PMN-MDSC isolated from peripheral blood of cancer patients demonstrated that tumor-derived CXCR1 and CXCR2 agonists, especially IL-8, could be main inducers of NETosis. NE and MMP9 released into the extracellular space during NETosis have been shown to sequentially cleave the basal membrane component laminin. Proteolytically modified laminin could bind to αvβ1 integrin on dormant tumor cells, inducing thereby their proliferation and the outgrowth of metastases. It is important to mention that the capacity to induce NETosis differs among various mediators. In addition, studies demonstrated a high variability among neutrophil donors in this regard. In the same study, induction of NETosis through G-CSF and phorbol 12-myristate 13-acetate (PMA) in neutrophils from healthy donors and cancer patients did not show any differences. In contrast, a significantly higher induction of NETosis by these stimuli was observed in neutrophils from tumor bearing mice as compared to healthy animals. This indicates that data generated in murine models cannot be directly transferred to the human situation.

In addition, PMN-MDSC infiltration into various organs was shown to be a prerequisite for metastasis formation. These myeloid cells have been shown to secrete the proteins Bv8, S100A8 and S100A9, which can act as chemoattractant for both PMN-MDSC and tumor cells.

6 | THERAPIES TARGETING POLYMORPHONUCLEAR-MYELOID-DERIVED SUPPRESSOR CELL

Due to their immunosuppressive nature, PMN-MDSC are considered as one of the major contributors to cancer resistance to chemo- or...
immunotherapies. To counteract their effects, several strategies are pursued, including the inhibition of PMN-MDSC migration to the tumor site, depletion of PMN-MDSC or induction of their differentiation into mature neutrophils and the blockade of their immunosuppressive function (Figure 2).

The most prominent mediators of neutrophil migration in cancer and other pathological conditions are CXCR2 ligands, namely the chemokines CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8. In humans, CXCR2 forms homodimers or heterodimers together with CXCR1 to facilitate signaling. Therefore, treatment...
approaches aim at inhibiting the expression or function of these molecules to abrogate pathologic neutrophil recruitment. In a spontaneous model of murine pancreatic cancer, genetic deletion of CXCR2 led to reduced formation of metastases. In addition, pharmacological inhibition of CXCR2 increased the influx of tumor-reactive T cells and synergized with anti-PD1 therapy. Our recent investigation also demonstrated a beneficial effect of adjuvant anti-CXCR2 treatment on the occurrence of distant metastases in melanoma bearing mice, which was presumably attributed to increased NK cell function.

It is important to mention that CXCR2 has been shown to play other roles besides inducing neutrophil migration. In vitro and in vivo analysis of breast cancer cell lines demonstrated a lower apoptotic rate, higher chemoresistance and stronger capacity to undergo EMT of CXCR2 expressing cells. Stronger capacity to undergo EMT was attributed to a lower expression of E-cadherin and β-catenin. These observations were supported by clinical data, where high CXCR2 expression was associated with the induction of cyclooxygenase 2 (Cox-2) and predicted poor overall survival of breast cancer patients. In addition, CXCR2 mediated augmented survival of prostate cancer cells. Activation of hypoxia-inducible factor (HIF)-1 and nuclear factor-κB (NF-κB) resulted in an increased expression of CXCR2, leading to partial resistance against etoposide. This protective effect was abrogated by treating cells with CXCR2 targeting siRNAs. Furthermore, SX-682, an allosteric inhibitor of both CXCR1 and CXCR2, was demonstrated to reduce the infiltration of oral and lung carcinomas with PMN-MDSC and thereby increasing the number of both endogenous and adoptively transferred T cells in preclinical tumor models.

The CXCR2 ligands belong to a group of chemokines characterized by a Glu-Leu-Arg sequence (ELR-motif), which enables them to promote angiogenesis by binding to CXCR2+ endothelial cells. Concordantly, the inhibition of CXCR2 signaling also influences tumor vascularization. Therefore, beneficial therapeutic effects observed under anti-CXCR2 therapy may not only be caused by decreased PMN-MDSC recruitment.

An inhibitor of CXCR2, AZD5069, is currently testing in a phase I/II clinical trial in combination with enzalutamide in patients with metastatic castration resistant prostate cancer (NCT03177187) and several other inflammatory conditions including asthma and chronic obstructive pulmonary disease (COPD).

Another important receptor, regulating neutrophil migration, is CXCR4, which mediates retention of neutrophils in the bone marrow under physiological conditions. Concordantly, inhibition of CXCR4 is an approved treatment for systemic neutropenia. Interestingly, it has been shown that in cancer, CXCR4 ligands are upregulated in the tumor microenvironment and are able to recruit CXCR4+ PMN-MDSC to the tumor. This indicates the CXCR4/CXCR4 ligand axis as a potential therapeutic target to inhibit PMN-MDSC migration. Recent preclinical studies aimed at inhibiting PMN-MDSC migration by targeting 5-lipoxygenase that is essential for the synthesis of leukotriene A4, a strong chemoattractant for neutrophils. Here, the inhibitor of 5-lipoxygenase was entrapped in nanoparticles that contained
5-hydroxytryptamine (5-HT) on their surface, which mediated their binding to cells, expressing high levels of myeloperoxidase such as neutrophils in inflamed tissue. Using this approach in a xenograft breast cancer model, tumor growth and metastasis were significantly reduced.\textsuperscript{84}

Depletion of PMN-MDSC in preclinical studies is usually done by the application of monoclonal antibodies against either the Gr1 antigen or its Ly6G subunit, both of which are predominantly expressed by PMN-MDSC. While binding to Gr1 on neutrophils has been shown to induce complement-mediated membrane complex formation and, therefore, cell lysis, antibody against Ly6G stimulated phagocytosis of neutrophils by macrophages, resulting in a better depletion efficacy.\textsuperscript{85} In addition, targeting Ly6G offers a higher specificity over targeting Gr1 in depleting granulocytes.\textsuperscript{85} Although both approaches demonstrated a significant reduction of tumor weight and an improved mouse survival, their effect was highly dependent on the cancer entity, mouse strain as well as dosage and duration of the depletion approach.\textsuperscript{86} Depletion efficacy is also influenced by the degree of extramedullary granulopoiesis in the spleen, low bioavailability of the depleting antibodies, compensatory granulopoiesis in the bone marrow and the production of host antibodies against the anti-Ly6G or anti-Gr1 depleting antibodies. To optimize depletion efficacy, Boivin et al.\textsuperscript{87} proposed a promising double antibody-based depletion approach.

Importantly, one of the major side effects of chemotherapy is myelotoxicity, suggesting that PMN-MDSC could be affected by chemotherapeutics. Indeed, a decrease in circulating PMN-MDSC in human pancreatic cancer patients under gemcitabine treatment has been demonstrated.\textsuperscript{88} Interestingly, only PMN-MDSC were affected in this setting, while level of circulating M-MDSC remained stable. Data from several studies conducted with 5-fluorouracil (5-FU) indicated a reduction of MDSC numbers not only in the tumor but also in the circulation.\textsuperscript{89} In addition, 5-FU has been shown to decrease MDSC level to a higher degree than gemcitabine and without affecting antitumor immune cells in both patients and preclinical mouse models.\textsuperscript{90,91} It is important to mention that the efficacy of MDSC depletion is dependent on the used chemotherapeutic agent, treatment duration, dosage and tumor type.\textsuperscript{91}

In addition to the depletion of MDSC, chemotherapeutics are capable of inducing MDSC differentiation. Treatment of melanoma bearing mice with low-dose paclitaxel significantly reduced the frequency of intratumoral MDSC associated with an elevation of DC numbers.\textsuperscript{92}

PMN-MDSC could be also depleted through the targeting of tumor-derived mechanisms and factors that promote excessive granulopoiesis. PGE2-secreting breast cancer cells were shown to induce microRNA (miR)-10a in MDSC, which resulted in the activation of the AMP-activated protein kinase (AMPK) pathway and MDSC expansion.\textsuperscript{93} In line with this finding, inhibition of miR-10a abrogated this effect and increased survival of tumor-bearing mice.\textsuperscript{93} In addition, PGE2 has been shown to promote both CXCL12 and CXCR4 expression in a mouse model of prostate cancer, leading to an increased migration of these cells to the tumor site.\textsuperscript{94} PGE2 increased the immunosuppressive capacity of PMN-MDSC by inducing arginase-1 expression.\textsuperscript{95} A recent study described an upregulation of the fatty acid transporter (FATP)2 in PMN-MDSC in contrast to normal neutrophils in both humans and mice in a GM-CSF/signal transducer and activator of transcription (STAT)5-dependent manner.\textsuperscript{96} Increased expression of this receptor facilitated enhanced uptake of arachidonic acid, increasing thereby PGE2 synthesis. An inhibition of this transporter with lipoferrna restored the normal neutrophil phenotype and reduced tumor formation in mice, suggesting this receptor as a potential therapeutic target.\textsuperscript{96}

Since neutrophils have also antitumor properties, it seems plausible to reprogram PMN-MDSC into tumor attacking cells. Interestingly, promising results in skewing PMN-MDSC into tumor-reactive neutrophils were observed in the studies with cabozantinib, a tyrosine kinase inhibitor that targets c-MET, VEGFR2, RET and AXL.\textsuperscript{97} Treatment of mice bearing castration resistant prostate cancer with cabozantinib led to a strong influx of tumor-reactive neutrophils into tumors and to their eradication.\textsuperscript{97} Since antibody mediated depletion of granulocytes abolished the effects of cabozantinib, this inhibitor is likely to render PMN-MDSC into tumor reactive neutrophils in vivo.\textsuperscript{97} In the same model, treatment of cabozantinib synergized with immune-checkpoint blockade leading to reduced tumor growth.\textsuperscript{98} For the treatment with cabozantinib, c-MET seems to be especially important as a target since c-MET\textsuperscript+ neutrophils have been shown to mediate resistance against adoptive T cell therapy in murine melanoma.\textsuperscript{99} It was demonstrated that cabozantinib was able to reduce both PMN-MDSC survival and their immunosuppressive capacity presumably through the inhibition of the PI3K-Akt-mTOR pathway and the upregulation of IL-1 receptor antagonist.\textsuperscript{98,100}

Another major player in the induction of protumorigenic properties of PMN-MDSC is TGF-β.\textsuperscript{30,50} Currently, two inhibitors of TGF-β signaling are tested in phase II clinical trials. The treatment with monoclonal antibody fresolimumab, targeting all isoforms of TGF-β and with galusertinib, an inhibitor of TGF-β1, demonstrated acceptable safety and prolonged patient survival.\textsuperscript{101,102} In addition, galusertinib exerted synergistic effects when being applied together with anti-PD-L1 or anti-PD1 antibodies as well as with conventional chemotherapy.\textsuperscript{103-105}

Initially, an immature phenotype was considered a general characteristic of MDSC. However, it has recently been shown that mature neutrophils can also exert immunosuppressive function in cancer patients.\textsuperscript{106} Nevertheless, promotion of myeloid cell maturation has been proven to be beneficial for the treatment outcome.\textsuperscript{107} The most intensively studied approach to induce MDSC maturation is the application of the all trans retinoic acid (ATRA), which induces expression of genes containing a retinoic acid response element.\textsuperscript{108} Activation of these genes leads to the production of glutathione, which detoxifies ROS. In absence of ROS, MDSC have been shown to lose their immunosuppressive capacity and develop rapidly into mature macrophages and dendritic cells.\textsuperscript{109} In patients with small cell lung cancer, ATRA was able to increase the response to a dendritic cell-based vaccine by 2-fold.\textsuperscript{107} At the present time, ATRA is tested in combination with ipilimumab (NCT03200847) or pembrolizumab (NCT02403778) in melanoma patients, showing promising results.\textsuperscript{110}
According to many publications, PMN-MDSC rely to a large degree on the expression of arginase-1 to exert an immunosuppressive capacity.\textsuperscript{40,111-113} This enzyme reduces the concentration of L-arginine in the extracellular space, leading to the inhibition of T cell proliferation and contributing to the production of immunosuppressive polyamines.\textsuperscript{114} The treatment of cancer patients with L-arginine might, therefore, be helpful since such therapy showed beneficial effects in preclinical studies.\textsuperscript{115}

7 | CONCLUSION

Our understanding of PMN-MDSC biology is rapidly evolving and reveals these cells as major players in the progression of primary tumors and the formation of distant metastases. Since the majority of cancer deaths is attributed to metastatic process, a targeting of PMN-MDSC in this context could be considered as a promising strategy of tumor immunotherapy.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - Project number 259332240/RTG 2099 (to JU and VU) and TRR179 (TP07 to Adelheid Cerwenka), the Cooperation Program in Cancer Research of the DFKZ Heidelberg and Israel's Ministry of Science, Technology and Space (MOST, CA181 to VU) and the German Federal Ministry of Education and Research (SERPENTINE project in the ERA PerMed network to VU). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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