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# Myeloid-Derived Suppressor Cells in Hematologic Diseases: Promising Biomarkers and Treatment Targets

Nikoleta Bizymi<sup>1,2</sup>, Sunčica Bjelica<sup>3</sup>, Astrid Olsnes Kittang<sup>4,5</sup>, Slavko Mojsilovic<sup>6</sup>, Maria Velegraki<sup>1,7</sup>, Charalampos Pontikoglou<sup>1</sup>, Mikael Roussel<sup>8,9,10</sup>, Elisabeth Ersvær<sup>11</sup>, Juan Francisco Santibañez<sup>3,12</sup>, Marie Lipoldová<sup>13</sup>, Helen A. Papadaki<sup>1</sup>

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## Abstract

Myeloid-derived suppressor cells (MDSC) are a heterogeneous group of immature myeloid cells that exist at very low numbers in healthy subjects but can expand significantly in malignant, infectious, and chronic inflammatory diseases. These cells are characterized as early-MDSCs, monocytic-MDSCs, and polymorphonuclear-MDSCs and can be studied on the basis of their immunophenotypic characteristics and their functional properties to suppress T-cell activation and proliferation. MDSCs have emerged as important contributors to tumor expansion and chronic inflammation progression by inducing immunosuppressive mechanisms, angiogenesis and drug resistance. Most experimental and clinical studies concerning MDSCs have been mainly focused on solid tumors. In recent years, however, the implication of MDSCs in the immune dysregulation associated with hematologic malignancies, immune-mediated cytopenias and allogeneic hemopoietic stem cell transplantation has been documented and the potential role of these cells as biomarkers and therapeutic targets has started to attract a particular interest in hematology. The elucidation of the molecular and signaling pathways associated with the generation, expansion and function of MDSCs in malignant and immune-mediated hematologic diseases and the clarification of mechanisms related to the circulation and the crosstalk of MDSCs with malignant cells and other components of the immune system are anticipated to lead to novel therapeutic strategies. This review summarizes all available evidence on the implication of MDSCs in hematologic diseases highlighting the challenges and perspectives arising from this novel field of research.

## Introduction

References to cells of myeloid origin that promote tumor progression through immune evasion mechanisms while also induce inflammatory and hemopoietic responses, go back to the

1970s.<sup>1</sup> These myeloid cells display immunosuppressive properties and expand particularly in neoplastic, infectious, and inflammatory diseases; they were initially characterized as *natural suppressor* or *veto* or *null cells* because of the absence of surface

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markers of T-cells, B-cells, natural killer (NK) cells or macrophages and later as *immature myeloid cells* or *myeloid suppressor cells* to denote their main biologic properties.<sup>1</sup> In 2007, the term myeloid derived suppressor cells (MDSCs) was introduced as the best to reflect the origin and functional trait of these cells despite the heterogeneity in their phenotypic, genomic and biochemical characteristics.<sup>2</sup> In recent years, MDSCs have been recognized as important immune regulators, potential biomarkers and even therapeutic targets in cancer and other diseases associated with chronic inflammation including infectious diseases, autoimmune diseases and trauma, among others.<sup>3,4</sup>

In humans, MDSCs are identified as CD11b<sup>+</sup>CD33<sup>+</sup>HLA-DR<sup>-low</sup> cells.<sup>5</sup> They can be further divided into 2 distinct populations with the main difference being the expression of CD14 (monocytic—MDSCs, M-MDSCs) or CD15 (polymorphonuclear—MDSCs, PMN-MDSCs) surface molecules. M-MDSCs are morphologically identical to conventional monocytes from which they can be distinguished on the basis of HLA-DR expression. PMN-MDSCs can be distinguished from conventional PMN based on their low-density properties following centrifugation over density gradient as well as on the expression of the lectin type oxidized LDL receptor 1 (LOX-1).<sup>3,6</sup> A third, minor population of MDSCs has been recognized, the early-stage MDSCs (e-MDSCs), which express neither CD15 nor CD14; these cells are characterized as Lin<sup>-</sup> (CD3, CD14, CD15, CD19, CD56)HLA-DR<sup>-</sup>CD33<sup>+</sup> and comprise immature progenitor and precursor cells with myeloid colony-forming activity.<sup>5</sup> In mice, MDSCs are characterized by the expression of Gr1 and CD11b and can also be divided into PMN-MDSCs (CD11b<sup>+</sup>Ly6G<sup>+</sup>Ly6C<sup>low</sup> cells), M-MDSCs (CD11b<sup>+</sup>Ly6G<sup>+</sup>Ly6C<sup>high</sup>), and non-PMN-MDSCs/non-M-MDSCs (CD11b<sup>+</sup>Ly6G<sup>med</sup>Ly6C<sup>med</sup> cells).<sup>5,7</sup> Notably, the term granulocytic-MDSCs (G-MDSCs) has previously been used for the definition of PMN-MDSCs in both human and mice.

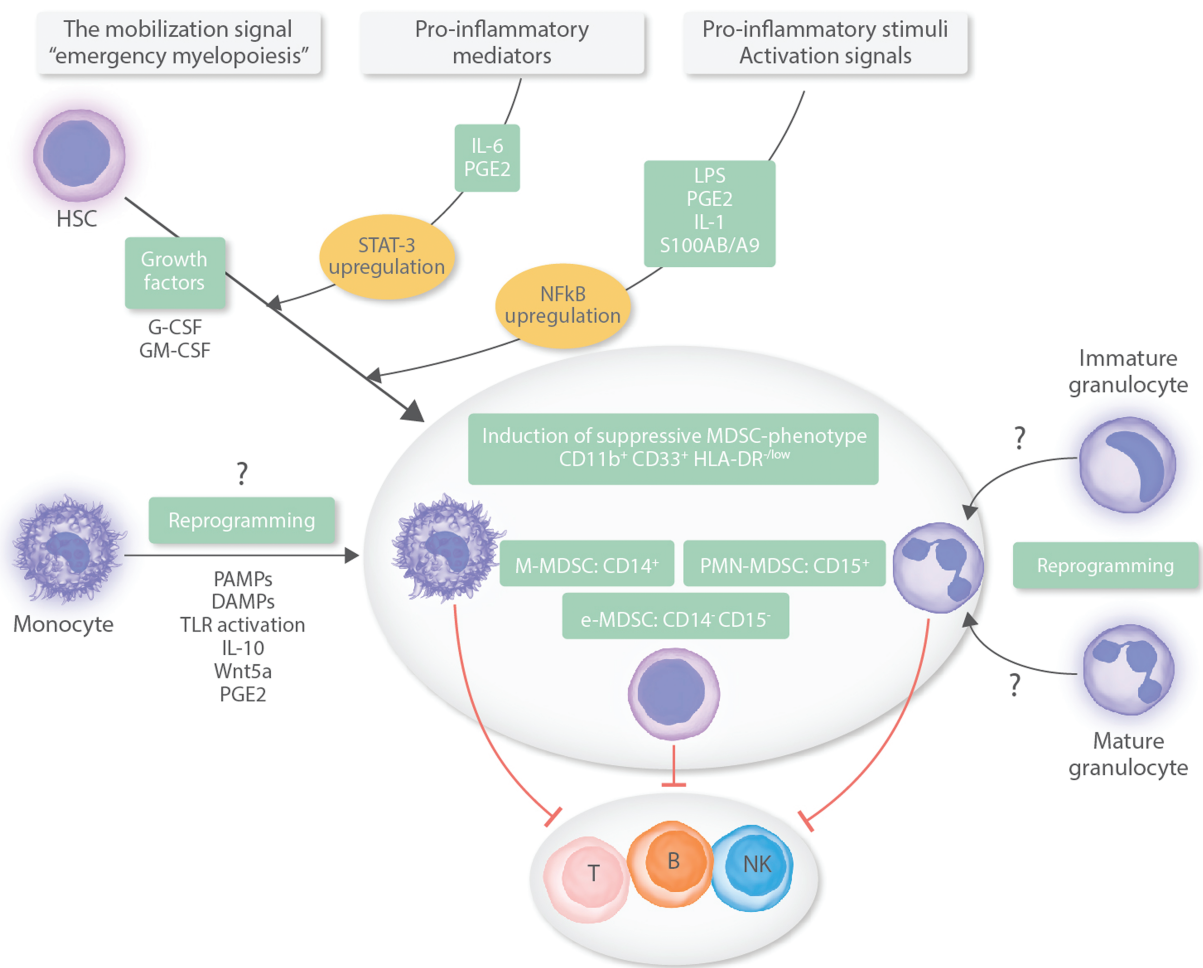
The precise mechanisms underlying the generation of MDSCs remain largely unknown. MDSCs are likely to arise under inflammatory conditions when there is an increased demand for myeloid cells (emergency myelopoiesis); they then expand as immature cells in the bone marrow (BM) or even extramedullary (mainly in the spleen) and migrate into the peripheral blood (PB) where their terminal differentiation is blocked finally transforming into functionally active MDSCs. According to this model, 2 signals are required for MDSCs' generation; the expansion/mobilization signal mediated mainly through growth factors such as granulocyte and granulocyte/monocyte colony stimulating factors (G-CSF and GM-CSF, respectively) and proinflammatory mediators such as interleukin-6 (IL-6) and prostaglandin E2 (PGE2) resulting in upregulation of the signal transducer and activator of transcription (STAT)-3 in myeloid progenitor cells; and the activation signal mediated through proinflammatory stimuli such as lipopolysaccharides (LPS), PGE2, IL-1 and S100A8/A9 resulting in NF-κB upregulation and induction of the suppressive MDSC phenotype. Recent evidence suggests that M-MDSCs may also arise by reprogramming of monocytes through pathogen- or danger-associated molecular patterns (PAMPs or DAMPs, respectively) and Toll-like receptor (TLR) activation as well as through certain cytokines and mediators such as IL-10, Wnt5a, and PGE2.<sup>8</sup> Another hypothesis, although still controversial, indicates that PMN-MDSCs may represent an activation stage of PMNs derived from immature or mature granulocytes<sup>8</sup> (Fig. 1).

The fundamental functional characteristic of MDSCs is the capacity to suppress immune cells, predominantly T-cells and to a lesser degree B-cells and NK-cells.<sup>1,3,9</sup> Main transcription factors involved in the suppressive function of MDSCs include STAT3, hypoxia inducible factor 1a (HIF-1a) and CCAAT/enhancer binding protein b (C/EBPb).<sup>1,3,9</sup> Effector molecules produced by MDSCs include arginase-1, which induces L-arginine deprivation and causes nitrosylation and down-regulation of the CD3z part of the T-cell receptor complex; cyclooxygenase (COX)-2 and indoleamine 2,3-dioxygenase (IDO); inducible nitric oxide synthase (iNOS), which leads to the production of NO, induction of T-cell apoptosis and suppression of T-cell proliferation; NADPH oxidase 2 (NOX2), which inhibits the proliferation of T-cells through production of reactive oxygen species (ROS) and nitration of CD3z and major histocompatibility complex (MHC)-I; heme oxygenase 1 (HO-1) which also inhibits T-cell proliferation through carbon monoxide (CO) production; mediators reducing cysteine provision to T-cells by antigen presenting cells; membrane-bound transforming growth factor β1 (TGFβ1), which promotes the anergy of NK-cells and the development of regulatory T cells (Treg); IL-10, which leads to Th2 deviation and type 2 polarization of macrophages; and ADAM metalloproteinase domain 17 (ADAM17) which cleaves L-selectin (CD62L) from T-cells leading to their homing in lymph nodes and sites of inflammation.<sup>1,3,9</sup> In addition to their immune-suppressive properties, MDSCs promote tumor progression and metastasis by affecting the remodeling of the tumor microenvironment and tumor angiogenesis via production of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and matrix metalloproteinase-9 (MMP9).<sup>1,3,9</sup>

The immune system displays a prominent role in the pathogenesis, pathophysiology and response to treatment of patients with hematologic malignancies, BM failure syndromes and autoimmune disorders<sup>10,11</sup>; therefore, immune modulating agents (i.e., lenalidomide, monoclonal antibodies, hypomethylating drugs, signal transduction inhibitors among others) have significantly improved the outcome of these patients.<sup>10,11</sup> As expected, the possible implication of MDSCs in the immune dysregulation associated with these disease entities and their potential role as biomarkers and therapeutic targets has started to attract a particular interest in hematology.<sup>10,11</sup> This is further triggered by the fact that MDSCs in addition to their immunosuppressive properties on T-cells can also interact with the mesenchymal stromal cells (MSCs) in the BM through shared molecules and mechanisms and this interplay may alter the immunoregulatory properties of the BM microenvironment and consequently, the disease pathophysiology and response to treatment.<sup>12</sup> This review summarizes all available evidence on the implication of MDSCs in hematologic malignancies and immune-mediated BM failure syndromes and cytopenias highlighting the challenges and perspectives arising from this novel field of research.

## Myeloproliferative neoplasms and acute leukemia

Myeloproliferative neoplasms (MPNs) are a group of hemopoietic stem cell disorders characterized by clonal proliferation of myeloid-lineage cells and chronic inflammation.<sup>13</sup> Classic MPNs include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis



**Figure 1. Proposed signals for MDSC generation.** In humans, MDSCs are identified as CD11b<sup>+</sup>CD33<sup>+</sup>HLA-DR<sup>low</sup> cells and are classified by the expression of CD14 as monocytic-MDSCs (M-MDSCs) or CD15 as polymorphonuclear-MDSCs (PMN-MDSCs). A minor population of MDSCs, the early stage MDSCs (e-MDSCs), expresses neither CD15 nor CD14. The fundamental functional characteristic of MDSCs is the capacity to suppress immune cells, predominantly T-cells and to a lesser degree B-cells and NK-cells. MDSCs arise under inflammatory conditions due to an increased demand for myeloid cells (emergency myelopoiesis); they expand from the hematopoietic stem cell (HSC) as immature cells in the bone marrow (BM) or extramedullary, and migrate into the peripheral blood (PB) where their terminal differentiation is blocked transforming into functionally active MDSCs. Two types of signals are required for MDSCs' generation: the expansion/mobilization signal through growth factors such as granulocyte and granulocyte/monocyte colony stimulating factors (G-CSF and GM-CSF, respectively) and proinflammatory mediators such as interleukin-6 (IL-6) and prostaglandin E2 (PGE2) resulting in upregulation of STAT3 in myeloid progenitor cells; and the activation signal mediated through proinflammatory stimuli such as lipopolysaccharides (LPS), PGE2, IL-1, and S100A8/A9 resulting in NF-κB upregulation and induction of the suppressive MDSC phenotype. M-MDSCs may also arise by reprogramming of monocytes through pathogen- or danger-associated molecular patterns (PAMPs or DAMPs, respectively) and Toll-like receptor (TLR) activation as well as through certain cytokines and mediators such as IL-10, Wnt5a, and PGE2. PMN-MDSCs may also represent an activation stage of PMNs derived from immature or mature granulocytes.

(PMF). Studies on MDSCs in CML have demonstrated increased frequency of PMN-MDSC subsets in Sokal high-risk patients, expressing high levels of programmed death receptor ligand-1/programmed death receptor-1 (PD-L1/PD-1) and arginase-1.<sup>14,15</sup> Increased PMN-MDSC and M-MDSC subsets in CML patients at diagnosis have been shown to return to normal levels after treatment with the tyrosine kinase inhibitor (TKI) imatinib and M-MDSCs frequency has been proposed as a prognostic factor in CML patients receiving the TKI dasatinib.<sup>16</sup> Overall, CML patients appropriately responding to TKI therapies (imatinib, nilotinib, dasatinib), parallel to the reduction of abl-bcr transcripts display a decrease in M-MDSC frequency and immunosuppressive activity and restoration of T-cell and NK-cell immune functions.<sup>17</sup> Recent evidence also suggests that MDSCs may have a critical role in CML malignant cell immune escape. In particular, it has been shown that the malignant cell

expansion in CML is maintained by a small subset of CD34<sup>+</sup>/CD38<sup>-</sup> leukemic stem cells that may escape immune cell surveillance within immunosuppressive BM niches consisting of populations of MSCs and PMN-MDSCs with T-cell suppressive capacity.<sup>18</sup> It is therefore reasonable to hypothesize that targeting of MDSCs in CML may restore the T-cell mediated leukemia surveillance and improve further patients' long-term outcome.

As regards to the Philadelphia negative MPNs, namely PV, ET, and PMF, a number of studies have documented abnormal reserves and function of immune cells including increase of monocyte/macrophages, altered regulatory T-cell frequency, NK-cell dysfunction, and expansion of MDSCs.<sup>19</sup> Recent evidence also suggests that although MDSCs are significantly elevated in MPNs, no differences can be identified in their frequency among different MPN categories and no correlations with JAK2 allele



burden can be made.<sup>20</sup> MPN-derived MDSCs have also been shown to display significantly elevated arginase-1 mRNA and T-cell suppressive activity.<sup>20</sup>

There are only a few studies on MDSCs in acute leukemias clearly showing the negative impact of their presence on the disease prognostic characteristics. A recent study has shown variable but significantly higher number of MDSCs, defined as CD14<sup>+</sup>HLA-DR<sup>+</sup>CD33<sup>+</sup>CD11b<sup>+</sup> cells, in PB of patients with acute myeloid leukemia (AML) compared to patients with acute lymphoblastic leukemia (ALL) and significant correlation of AML-MDSCs with conventional prognostic factors at diagnosis, namely WBC count, CD34 frequency and nucleophosmin (NPM1) and fms-like tyrosine kinase 3 (FLT3) gene mutations. It has also been shown that CD33<sup>+</sup>CD11b<sup>+</sup>HLA-DR<sup>-/low</sup> MDSCs accumulate in the BM of AML patients and their presence may have an impact on disease prognosis and patients' clinical course.<sup>21</sup> Furthermore, MDSCs levels in newly diagnosed AML patients have been reported to correlate with AML subtype, presence of chromosomal abnormalities and gene alterations, extramedullary involvement, and plasma D-dimer levels. After the induction therapy, MDSCs significantly decrease in patients with complete remission but not in patients with partial or no response while on follow-up and MDSCs' frequency correlate with minimal residual disease (MRD) levels and Wilms 1 (WT-1) gene detection.<sup>21</sup> Another study has shown expansion of MDSCs from PB mononuclear cells (PBMCs) following contact with AML cell lines or primary AML cells via tumor-derived extracellular vesicles; it has been postulated that the mucin 1 (MUC1) oncoprotein induces increased expression of c-myc in extracellular vesicles that are then taken-up by the myeloid progenitor cells resulting in downstream effects on cell cycle proteins and selective proliferation of MDSCs.<sup>22</sup> As regards to ALL, it has been shown that pediatric patients with B-cell ALL display increased number of PMN-MDSCs in PB and BM compared to age-matched healthy individuals and PMN-MDSC frequency correlate with prognostic markers such as MRD and CD20<sup>+</sup> blast cell counts as well as with response to therapy.<sup>23,24</sup> PMN-MDSCs from patients in remission have been reported to lose their suppressive function corroborating the effect of these cells in mediating immune evasion mechanisms.<sup>23,24</sup> All these observations and available evidence implicating MDSCs in both AML and ALL progression and outcome highlight the emerging role of these cells as independent biomarkers and promising targets for the development of novel therapeutic strategies.

## Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are clonal hemopoietic stem cell disorders characterized by PB cytopenias, dysplastic changes in one or more BM cell lineages and increased risk of AML.<sup>25</sup> Allogeneic hemopoietic stem cell transplantation (Allo-HSCT) with donor T-cell reactivity against the malignant cells is the only curative therapy for MDS. BM microenvironment in low-risk MDS is characterized by persistent inflammation and expansion of autoimmunity-associated T-helper 17 (Th17) cells whereas expansion of MDSCs and Tregs and reduced number of Th17 characterize high-risk MDS.<sup>26,27</sup> Lin<sup>−</sup>(CD3/CD14/CD16/CD19/CD20/CD56)HLA-DR<sup>−</sup>CD33<sup>+</sup> MDSCs in high-risk MDS are capable to induce apoptosis of erythroid precursor cells and their expansion is driven, at least in part, by the interaction of S100A9 protein with the CD33

cell surface antigen.<sup>28</sup> Immune phenotyping with mass cytometry and unsupervised viSNE analysis of 35-markers per cell of thawed MDS and AML BM samples has revealed that one of the strongest marker signals was the expression of S100A9. This protein is seen in multiple cell types including MDSCs and its expression was more common in MDS without excess of blasts. S100A9 mainly characterizes M-MDSCs and together with its binding partner, S100A8, is included in the DAMP molecules displaying intra- and extracellular functions and inflammatory, antiinflammatory and immune regulatory properties.<sup>29,30</sup> Varying S100A9 expression resulted in different MDSC functions, with more proinflammatory effect in low risk and immunosuppressive effect in high-risk MDS. S100A9 and CD33 ligand/receptor pair interactions recruit components to the immunoreceptor tyrosine-based inhibition motif (ITIM) finally resulting in secretion of suppressive IL-10 and TGFβ1.<sup>28</sup> Work based on fresh PB and BM samples from MDS patients confirmed the secretion of these 2 cytokines by MDSCs, but interestingly, this secretion was higher in PMN-MDSCs than in M-MDSCs.<sup>31</sup> In fact, PMN-MDSC subset dominated the MDSC-expansion in high-risk patients. The BM homing chemokine receptors (CXCR4, CX3CR1) were expressed at a higher level on M-MDSCs in high-risk MDS, and there was different expression of CX3CR1 between healthy donors, low-risk MDS and high-risk MDS patients.<sup>31</sup>

The importance of MDSCs in suppression of hemopoiesis in MDS has been demonstrated in 2 genetically manipulated animal models; the S100A9 transgenic mice, displaying BM accumulation of MDSC and progressive cytopenia and the mDia1/miR146-a double knockout mice, developing age-related inflammatory BM microenvironment and anemia.<sup>28,32</sup> S100A8/A9 activation of MDSC is through the NF-κB signaling pathway; therefore, we may hypothesize that by targeting this pathway we could reduce MDSCs levels.<sup>33</sup>

Drugs widely used for MDS have lately been shown to affect T-cell polarization, which may suggest effects on MDSCs activity. Azacitidine, the drug of choice in high-risk MDS, has been shown to affect T-cell polarization in the Th17/Treg-axis in high-risk MDS and to influence levels of BM CD57<sup>+</sup> T-cells, CD57<sup>+</sup> T-cell degranulation and CD34<sup>+</sup> BM cell directed cytotoxicity.<sup>34,35</sup> Specific MDSC-targeting in MDS has so far been aiming at CD33-expressing cells.<sup>36</sup> So far, BI 836858 (Fc-engineered anti-CD33 mAb) for antibody-dependent cell-mediated cytotoxicity by NK-cells is currently tested in MDS patients in an ongoing Phase I/II clinical trial (ClinicalTrials.gov Identifier: NCT02240706). Additionally, a clinical trial with CD16/IL-15/CD33 (161533) tri-specific killer cell engager for the treatment of CD33-expressing myeloid malignancies, including high-risk MDS, is not yet recruiting (NCT03214666). Overall, the implication and therapeutic targeting of MDSCs in MDS is an interesting, open field of research.

## Lymphomas

Different mice models have been used to study MDSC biology in lymphomas. For example, M-MDSC and PMN-MDSCs from EL4 and EG7 lymphoma models display immunosuppressive capacity associated with increased production of NO and nitrotyrosine (M-MDSCs) and ROS (PMN-MDSCs).<sup>37,38</sup> Furthermore, MDSCs from A20 B-cell lymphoma model, operate as tolerogenic antigen presenting cells capable of antigen uptake and presentation to tumor-specific Tregs.<sup>39</sup> In humans, the number of circulating MDSCs has been correlated with poor prognosis in

diffuse large B-cell lymphoma (DLBCL),<sup>40,41</sup> indolent lymphoma,<sup>42</sup> chronic lymphocytic leukemia,<sup>43,44</sup> and Hodgkin lymphoma (HL).<sup>40,45</sup> However, only few of the suppressive mechanisms involved in MDSC biology in these disease entities have been elucidated.<sup>46</sup> In DLBCLs, an increase of immunosuppressive PMN-MDSC numbers expressing arginase-1 has been noticed, however correlation with clinical outcome was not been documented in all studies.<sup>40,41,47</sup> Similarly, increased numbers of M-MDSCs (CD14<sup>+</sup>HLA-DR<sup>low</sup>) have been detected in PB from DLBCL patients in various studies.<sup>41,42,48,49</sup> M-MDSCs from DLBCL patients have been found to overexpress genes involved in MDSC biology such as IL4-R, IL6-R, RELB, STAT3, NFkB, CEBP $\beta$ , AIM2, TNFR2, and NOX2. Additionally, the T-cell suppressive effect of MDSCs was mediated by a release of IL-10 and S100A12 and an increase in PD-L1 expression.<sup>41</sup> Increased circulating PMN-MDSC (CD66b<sup>+</sup>CD33<sup>dim</sup>HLA-DR<sup>-</sup>) numbers compared to healthy donors with elevated arginase-1 activity has been observed in a cohort of 31 patients with indolent lymphomas<sup>40</sup> whereas increased M-MDSC (CD14<sup>+</sup>HLA-DR<sup>low</sup>) numbers have been detected in another cohort of 22 patients with indolent lymphomas.<sup>42</sup> Increased PMN-MDSC and M-MDSC numbers and arginase-1 activity have been also identified in PB of HL patients.<sup>40,45</sup> As regards to T-cell lymphomas, even less is known about the potential role of MDSCs. Patients with mycosis fungoides and Sézary syndrome with stage IB and above have been shown to display increased production of ROS by MDSCs compared to patients with stage IA or healthy controls, despite the normal MDSC numbers and this abnormality was reversed following anti-CD25 denileukin difitox or IFN- $\alpha$ 2b treatment.<sup>50</sup> Collectively, all the above studies have pointed out the possibility of targeting MDSCs in future therapeutic trials with lymphoma patients by controlling their expansion and/or blocking their immunosuppressive functions.

## Multiple myeloma

Multiple myeloma (MM) is a B-cell malignancy characterized by expansion of monoclonal plasma cells preferentially in the BM and the accumulation of monoclonal immunoglobulins in the PB. It is now widely accepted that the BM microenvironment displays a prominent role in pathophysiology of the disease by providing a protective niche to the plasma cells that promotes the immune-escape, drug-resistance, and angiogenesis.<sup>51</sup> An interactive crosstalk between the malignant cells and the BM microenvironment is also responsible for many clinical characteristics of the disease such as the osteolytic lesions, anemia and immunosuppression.<sup>51</sup> According to recent studies, MDSCs are involved in the pathogenesis and progression of MM.<sup>52–54</sup> Experiments in 5T2 and 5T33MM mice models of MM have shown that the malignant plasma cells can induce the generation and survival of both PMN-MDSCs and M-MDSCs that accumulate in the BM in early stages and in PB at later stages, and display T-cell immunosuppressive activity, through production of NOS, arginase-1 and IL-10, which is highest among M-MDSCs.<sup>55,56</sup> In the DP42 MM mouse model, however, a more prominent role of the BM PMN-MDSC population was demonstrated for the induction of plasma cell growth and chemoresistance.<sup>57</sup> The malignant cells secrete factors such as IL-6, GM-CSF, VEGF, IL-1b and exosomes leading to the activation of STAT3 and STAT1 pathways, increase expression of Bcl-xL and Mcl-1 proteins and release of NO enhancing finally the BM angiogenesis as well as the survival and suppressive activity of MDSCs.<sup>55,58,59</sup> These

effects can be further potentiated by the MSCs in MM BM microenvironment.<sup>60,61</sup> Using the 5TGM1 mouse model it has also been shown that tumor-induced MDSCs, in addition to their immunosuppressive effect, can be differentiated into mature and functional osteoclasts contributing therefore to the bone destruction associated with the disease.<sup>62</sup> The importance of MDSCs in the development of MM was demonstrated in the S1009 knockout transgenic mice which display defective response to cancer and delay in the development of MM following inoculation of MM cells which is reversed following adoptive transfer of MDSCs.<sup>63</sup> In humans, early studies have shown that MDSCs isolated from the PB of patients with MM display a T-cell inhibitory effect which can be abrogated by drugs inhibiting arginase-1 and iNOS activity.<sup>64</sup> Similar to mice models, increased number of PMN-MDSCs with immunosuppressive properties has been reported in the BM and PB of patients with MM at diagnosis and relapse compared to healthy subjects.<sup>63,65,66</sup> The frequency of PMN-MDSCs has been shown to correlate with the disease activity and it is higher in MM patients compared to patients with monoclonal gammopathy of undetermined significance (MGUS) suggesting that these cells can be used as markers of disease activity and progression.<sup>65–67</sup> Contradictory results have been published so far regarding M-MDSC numbers and function in patients with MM. Increased numbers of M-MDSCs have been reported in BM and PB of newly diagnosed and relapsed MM patients compared to patients in remission or healthy donors suggesting their potential consideration as prognostic predictors of disease activity<sup>68</sup> whereas other studies have not identified such differences.<sup>63,65–67</sup> Discrepancies may be due to different flow-cytometric strategies and different quantitative and qualitative characteristics of patient cohorts.

In summary, all available evidence suggests that MDSCs are increased in the patients with MM and participate in the pathophysiology of the disease by inducing the survival and proliferation of malignant plasma cells both directly and indirectly through their immunosuppressive effects. Therefore, MDSCs can become therapeutic targets for MM. It has been shown that the immunomodulatory drug lenalidomide and the proteasome inhibitor bortezomib are able to downregulate molecules produced by MDSCs in MM; however, they cannot abrogate the number or immunosuppressive function of MDSCs.<sup>66</sup> These observations emphasize the importance of developing novel agents to overcome the immunosuppressive effects of MDSCs in MM patients.

## Immune-mediated cytopenias

Immune thrombocytopenia (ITP) is a disease entity characterized by low platelet count due to antiplatelet autoantibodies, abnormal effector T-cell activation and inappropriate platelet production in the BM.<sup>69</sup> Recent evidence suggests that MDSCs have a role in the pathophysiology of the disease but contradictory results have published thus far on the frequency and function of MDSCs at diagnosis probably due to different flow-cytometry strategies, that is, whole blood or PBMCs.<sup>70–72</sup> Circulating MDSCs in ITP patients increase following immunosuppressive treatment with high dose dexamethasone (DXM) and MDSC numbers correlate with platelet recovery suggesting that PB MDSCs could be used as markers of response to therapy.<sup>70–72</sup> It has also been shown that PB and splenic MDSCs in ITP patients display impaired immunosuppressive function contributing possibly to the pathogenesis of the disease and that DXM

treatment improves the immunomodulatory properties of MDSCs including the production of suppressive cytokines and their T-cell suppressive effects.<sup>70</sup> The effect of DXM on MDSCs was found to correlate with the transcription factor Ets1 both in ITP patients and a murine model of ITP generated following transfer of splenocytes from CD61 knockout mice immunized with CD61<sup>+</sup> platelets into severe combined immunodeficient mouse recipients.<sup>70</sup> Interestingly, adoptive cell transfer with MDSCs alleviated thrombocytopenia and resulted in higher survival rate in the ITP murine model.<sup>70</sup> Treatment with intravenous immunoglobulin (IVIG) has been reported to increase the number of MDSCs in spleen cell cultures from ITP patients indicating that, in addition to blocking the macrophage Fc receptors, IVIG may ameliorate ITP by increasing MDSC populations similar to DXM.<sup>73</sup> The findings provide novel insights linking MDSCs with the pathogenesis, disease activity and management of ITP that need further investigation in the clinic.

Chronic idiopathic neutropenia (CIN) is another immune-mediated disorder characterized by prolonged, unexplained reduction in the number of PMN associated in the majority of patients with the presence of activated T-lymphocytes with myelosuppressive properties that induce the apoptotic death of the granulocytic progenitor cells.<sup>74</sup> Preliminary data on the role of MDSCs have shown low frequency of PB PMN-MDSCs and M-MDSCs in CIN patients and this decrease might contribute to the aberrant T-cell activation and sustained chronic inflammation in CIN, a hypothesis that is currently under investigation.<sup>75</sup>

Aplastic anemia is the prototype of T-cell mediated BM failure syndrome and the potential implication of MDSCs in the abnormal T-cell responses associated with the disease remains an open field for research. In a mouse model of acquired aplastic anemia following deletion of the TGF $\beta$ -activated kinase-1 gene in hemopoietic cells, the BM failure was significantly progressed following inactivation of TNF $\alpha$  signaling and was associated with increased capacity of macrophages to prime T-helper type I cell development and reduced ability of MDSCs to suppress T-cell proliferation.<sup>76</sup>

## Graft versus host disease

Allo-HSCT represents the only curative treatment for a number of hematologic malignancies. The beneficial effect of the treatment is regularly implicated by the immunological attack of the recipient tissues, an effect known as graft-versus-host disease (GvHD) which is associated with significant morbidity and mortality.<sup>77</sup> Based on their immunosuppressive properties, there is an increasing interest in exploring the possible implication of MDSCs in the development of GvHD and their potential effect on the treatment and patients' clinical outcome.<sup>78,79</sup> Studies have shown that PMN-MDSCs and M-MDSCs are increased in the PB during G-CSF stem-cell mobilization in human donors<sup>80</sup> and that the MDSC content of the graft correlates inversely with the risk of acute GVHD risk in patients receiving allogeneic, G-CSF mobilized, PB stem cells.<sup>81,82</sup> Results from a recent study also showed that accumulation of MDSCs in the graft and in PB during engraftment results in successful control of severe acute GVHD and long-term survival without any influence on the risk of disease recurrence after allo-HSCT.<sup>83</sup> In accordance with the clinical data are results from murine models demonstrating that the adoptive transfer of donor MDSCs in fully MHC-mismatched allo-HSCT recipients can result in successful control of GvHD without compromising the graft versus tumor effects.<sup>84,85</sup>

Although there are no currently clinical trials using MDSC infusions in GvHD patients, the experimental data from animal models and observations from patients undergoing allo-HSCT indicate that MDSCs represent a promising therapeutic tool for the prevention and therapy of GvHD in the clinic.<sup>86</sup> Certainly, studies evaluating large patient cohorts and long observational periods are required to clarify the beneficial effects of MDSCs in patients receiving allo-HSCT versus potential risks from infections or immunosuppression.

## Discussion

In recent years, there has been an increasing interest in the investigation of the contribution of MDSCs in the pathogenesis/pathophysiology of hematologic diseases. Although different protocols and strategies have been used for MDSC investigation and characterization in hematologic diseases, there is conclusive evidence suggesting that similar to their role in cancer induction and progression, MDSCs have a decisive role in hematologic malignancies by suppressing the immune reactions against the malignant cells through previously described mechanisms<sup>10,11</sup> (Table 1). But also, decreased number and defective function of MDSCs may have a contributing role in the hematologic diseases other than malignancies such as immune-mediated cytopenias including ITP or CIN by augmenting the T-cell mediated platelet or neutrophil destruction.<sup>70,75</sup> Apparently, MDSCs represent regulatory components of the immune system with critical role in malignant and immune disorders of hemopoiesis.

A particular interest for the implication of MDSCs in hematologic diseases originates from the fact that these cells derive from the hemopoietic stem cells which are primarily affected in a number of myeloid malignancies such as MDS, MPN, and acute leukemias. Although MDSCs may be part of the malignant clone, this is not the case in all circumstances<sup>28</sup>; definitely, however, they exert systemic immune suppressive effects and may also contribute to a permissive BM microenvironment where the malignant cells can survive and proliferate by evading host immune-surveillance and antitumor therapies.<sup>11</sup> In parallel, the malignant cells secrete cytokines, chemokines and growth factors that may sustain and expand MDSCs in a vicious cycle that favors the malignant cell growth and expansion. In lymphoid malignancies, the malignant cells originate from progenitor/precursor or mature cells of the lymphoid system; therefore, any implication of MDSCs in disease pathophysiology, progression and outcome is mediated through their immune-regulatory effects rather than the participation in the malignant population.<sup>39-48</sup>

Apart from the current interest in exploring the reserves, distribution and function of MDSC in hematologic diseases and homogenization of the protocols and strategies for their study, there is also an emerging interest in the potential of developing novel therapeutic strategies targeting MDSCs. Interestingly, a number of therapies currently used for hematologic malignancies have been reported to impact on the number and function of MDSCs and have been extensively reviewed elsewhere.<sup>10,11</sup> For example, all-trans retinoic acid (ATRA) has been shown to induce the differentiation of MDSCs in mice models and patients with solid tumors resulting in reduction of the number and the immune-suppressive effects of MDSCs<sup>87-90</sup>; its potential effect on MDSCs and consequences in patients with acute promyelocytic leukemia remains to be studied. The pyrimidine nucleoside analog Gemcitabine has been reported to reduce the number of MDSCs inducing therefore an antitumor immunity in mice

Table 1

## Summary of Representative Studies Investigating the of MDSCs and Their Subpopulations in Different Hematologic Diseases and Their Effects on Disease Outcome

| Disease                                | Patients' Origin                                    | Number of Patients/Controls (if Available)                                                                                                                                                             | Definition of MDSCs <sup>†</sup>                                                                                                                                                                                                                                                                                                     | Source | Observation/Outcome                                                                                                                                                                                                                                                                                                                                                                   | Refs. |
|----------------------------------------|-----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| CML                                    | Sweden                                              | 36 patients/18 controls                                                                                                                                                                                | CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup>                                                                                                                                                                                                                                                                               | PB     | PB of both high- and low-risk (Sokal score) patients showed higher % of CD34 <sup>+</sup> cells in the subpopulation of CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> and higher expression of PD-L1 in CD11b <sup>+</sup> cells than healthy controls. PB of high-risk patients contains higher numbers of CD34 <sup>+</sup> PD-L1 <sup>+</sup> cells than healthy controls | 14    |
| CML                                    | Italy                                               | 19 patients at diagnosis and 18 patients during therapy (13 of whom were also collected at diagnosis)/20 controls                                                                                      | MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup> ; G-MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup> ; M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                             | PB     | Patients at diagnosis have higher proportions of G- and M-MDSCs than healthy donors and patients after imatinib treatment: G-MDSCs of patients at diagnosis produce higher levels of arginase-1 and have a higher suppressive effect on T-cell proliferation in vitro than G-MDSCs from donors and patients after therapy                                                             | 15    |
| CML                                    | Italy                                               | 59 patients; available clinical characteristics in 42; 13 treated with dasatinib                                                                                                                       | G-MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup> ; M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                 | PB     | CML patients have higher levels of both G-MDSCs and M-MDSCs than healthy controls; patients with higher numbers of M-MDSC do not exhibit MMR after treatment with dasatinib                                                                                                                                                                                                           | 16    |
| CML                                    | Australia                                           | 78 patients; different stages of disease and treatment                                                                                                                                                 | MDSCs: HLA-DR <sup>-</sup> Lin <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> ; G-MDSCs: HLA-DR <sup>-</sup> Lin <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> CD66b <sup>+</sup> CD15 <sup>+</sup> ; M-MDSCs: HLA-DR <sup>-</sup> Lin <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> CD66b <sup>+</sup> CD14 <sup>+</sup> | PB     | G-MDSCs are increased in CML patients at diagnosis in comparison with pre-MMR, MMR, MR and TR; M-MDSCs are also increased at diagnosis in comparison with healthy donors and patients in MR and TR                                                                                                                                                                                    | 17    |
| CML                                    | Italy                                               | 30 patients. 20 controls; BM CML = 10, controls = 8                                                                                                                                                    | G-MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> CD15 <sup>+</sup> HLA-DR <sup>-</sup> CD66b <sup>+</sup>                                                                                                                                                                                                                               | PB, BM | Patients have higher levels of G-MDSCs than healthy controls: G-MDSCs from CML patients have higher immunosuppressive activity (inhibition of T-cell proliferation) than G-MDSCs from healthy donors; higher expression of immunomodulatory mRNAs by CML-MDSCs and -MDCs                                                                                                              | 18    |
| MPN                                    | USA                                                 | 55 patients, different subtypes of MPN/23 controls                                                                                                                                                     | CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup>                                                                                                                                                                                                                                                                               | PB     | MPN patients have higher level of MDSCs than healthy controls; MDSCs from MPN patients exhibit higher suppression of T-cell proliferation and express higher level of arginase-1 mRNA than MDSCs from healthy controls                                                                                                                                                                | 20    |
| AML                                    | China                                               | 27 patients/12 controls (anemia or hyperplastic patients)                                                                                                                                              | CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>low/-</sup>                                                                                                                                                                                                                                                                         | BM     | Increased level of MDSCs in AML patients in comparison with controls; MDSC levels correlate with plasma D-dimers; frequency of MDSCs decreases after AML treatment, but is higher in patients with MRD                                                                                                                                                                                | 21    |
| AML                                    | Israel, USA—data not given in the main body article | Numbers not given                                                                                                                                                                                      | M-MDSCs: CD11b <sup>+</sup> HLA-DR <sup>-</sup> CD14 <sup>+</sup> CD33 <sup>+</sup> CD15 <sup>-</sup> ; G-MDSCs: CD11b <sup>+</sup> HLA-DR <sup>-</sup> CD14 <sup>+</sup> CD33 <sup>+</sup> CD15 <sup>+</sup>                                                                                                                        | PB     | Higher levels of both M-MDSCs and G-MDSCs in AML patients than in healthy controls; in vitro experiments with AML cell lines MOLN-14 and THP-1 that release tumor-derived extracellular vesicles (EV); treatment of PBMCs with EV increases MDSCs                                                                                                                                     | 22    |
| B-ALL                                  | China                                               | 43 patients before treatment (after treatment 35 in remission, 8 not)/21 controls                                                                                                                      | G-MDSCs: CD45 <sup>+</sup> CD19 <sup>-</sup> HLA-DR <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> CD15 <sup>+</sup> ; M-MDSCs: CD45 <sup>+</sup> CD19 <sup>-</sup> HLA-DR <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>+</sup>                                                                                    | PB, BM | G-MDSCs higher both in PB and BM in patients before therapy than in healthy controls and in patients in remission; no significant difference in patients without remission; no differences observed in M-MDSCs                                                                                                                                                                        | 23    |
| B-ALL                                  | Egypt                                               | 43 patients                                                                                                                                                                                            | MDSCs: Lin <sup>-</sup> HLA-DR <sup>-</sup> CD33 <sup>+</sup> CD11b <sup>+</sup>                                                                                                                                                                                                                                                     | PB     | Patients at diagnosis have higher levels of MDSCs than healthy donors; during chemotherapy MDSCs numbers increased                                                                                                                                                                                                                                                                    | 24    |
| MDS                                    | Netherlands                                         | 12 MDS patients/8 non-MDS cancer patients (4 breast cancer and 4 lymphoma)/8 controls                                                                                                                  | Lin <sup>-</sup> HLA-DR <sup>-</sup> CD33 <sup>+</sup>                                                                                                                                                                                                                                                                               | BM     | MDS patients have higher % of MDSCs than both non-MDS patients and controls; in vitro: MDSCs have a direct suppressive effect on erythroid and myeloid progenitor cell development; proliferation of MDSCs is driven by the S100A9/CD33 pathway                                                                                                                                       | 28    |
| MDS                                    | Norway, UK                                          | 42 pretreatment patients, 23 from them BM sample/12 controls PB, 3 of them BM                                                                                                                          | MDSCs: Lin <sup>-</sup> CD33 <sup>+</sup> CD11b <sup>+</sup> ; G-MDSCs: Lin <sup>-</sup> CD33 <sup>+</sup> CD11b <sup>+</sup> CD15 <sup>+</sup> ; M-MDSCs: Lin <sup>-</sup> CD33 <sup>+</sup> CD11b <sup>+</sup> CD14 <sup>+</sup>                                                                                                   | PB, BM | MDSCs are increased in PB of patients than in healthy donors; MDSCs/G-MDSCs are more in patients with intermediate, high and very high risk than in patients with very low and low risk; M-MDSCs expression of CX3CR1 is higher in PB from intermediate and high-risk patients compared to BM                                                                                         | 31    |
| MDS                                    | USA                                                 | 40 patients/controls from blood bank                                                                                                                                                                   | Not available                                                                                                                                                                                                                                                                                                                        | BM     | Ex vivo: BM cells cocultured with Fc-engineered CD33 antibody BI 836858 resulted in blockage CD33-mediated IL-10 expression both in MDS patients and in healthy donors                                                                                                                                                                                                                | 36    |
| CLL                                    | USA                                                 | 29 patients/15 controls                                                                                                                                                                                | CD14 <sup>+</sup> HLA-DR <sup>low/-</sup>                                                                                                                                                                                                                                                                                            | PB     | Patients had higher number of MDSCs than healthy controls; numbers of MDSCs decreased with remission                                                                                                                                                                                                                                                                                  | 43    |
| CLL                                    | Germany                                             | 79 untreated patients/61 Binet stage A, 6 stage B, 12 newly diagnosed stage C/44 patients/40 healthy donors; analysis of frequency of MDSCs; in 4 patients suppressive activities of MDSCs were tested | CD14 <sup>+</sup> HLA-DR <sup>low/-</sup>                                                                                                                                                                                                                                                                                            | PB     | Patients have higher numbers of MDSCs than healthy donors, ex vivo and in vitro; IDO <sup>low</sup> /MDSCs from patients suppress T-cells and promote Treg induction; CLL cells induce conversion of monocytes into MDSCs                                                                                                                                                             | 44    |
| B-cell NHL (DLBCL and other lymphomas) | USA                                                 | 40 patients (4 newly diagnosed, 36 relapsed)/27 controls                                                                                                                                               | CD14 <sup>+</sup> HLA-DR <sup>low/-</sup>                                                                                                                                                                                                                                                                                            | PB     | PBMCs of patients showed lower recall response to influenza than controls—partly mediated by monocytes; patients have higher numbers of CD14 <sup>+</sup> HLA-DR <sup>low/-</sup> subpopulation than controls; the number of CD14 <sup>+</sup> HLA-DR <sup>low/-</sup> is associated with more aggressive disease                                                                     | 42    |

(continued)



Table 1  
(continued).

| Disease        | Patients' Origin | Number of Patients/Controls (if Available)                                        | Definition of MDSCs <sup>‡</sup>                                                                                                                                                                                                                                                                        | Source     | Observation/Outcome                                                                                                                                                                                                                                                                                                                                          | Refs. |
|----------------|------------------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| HL, B-cell NHL | Italy            | 124 patients (117 analyzed for MDSCs and outcome)/48 controls                     | MDSCs: CD45 <sup>+</sup> CD66b <sup>+</sup> CD33 <sup>dim</sup> HLA-DR <sup>-</sup> ; LDNs (mature low-density neutrophils) subpopulation within MDSCs expressing CD11b <sup>+</sup> CD16 <sup>+</sup>                                                                                                  | PB         | Higher proportion of MDSCs in lymphoma patients than in healthy donors; patients with higher median numbers of MDSCs than patient have worse FFDP (freedom from disease progression)                                                                                                                                                                         | 40    |
| HL             | Italy            | 60 newly diagnosed HL patients/25 controls                                        | immature MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> CD34 <sup>+</sup> HLA-DR <sup>-</sup> ; G-MDSC: CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup> Lin <sup>-</sup> ; M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>low</sup>                                   | PB         | Patients have higher numbers of all subtypes of MDSCs than healthy controls; less CD34 <sup>+</sup> MDSCs associated with better outcome of chemotherapy                                                                                                                                                                                                     | 45    |
| DLBCL          | Israel           | 109 patients; PB from 23 patients, 15 controls                                    | M-MDSCs: CD45 <sup>+</sup> CD14 <sup>+</sup> HLA-DR <sup>low</sup>                                                                                                                                                                                                                                      | PB         | Newly diagnosed patients have higher numbers of M-MDSCs than patients with complete remission after treatment and healthy controls                                                                                                                                                                                                                           | 48    |
| DLBCL          | France           | Cohort 1: 76 patients/87 controls, cohort 2: 66 patients/45 MDSCs controls        | M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>low</sup> ; G-MDSCs: Lin <sup>-</sup> CD123 <sup>low</sup> HLA-DR <sup>-</sup> CD33 <sup>+</sup> CD11b <sup>-</sup>                                                                                                                                              | PB         | Both M-MDSCs and G-MDSCs were higher in proportion in patients than in healthy donors; M-MDSCs were increased in high risk than in low-risk patients; M-MDSCs suppressive activity on T-cell proliferation is independent of arginase-1 and IDO and is linked with IL-10, PDL-1 and S100A12                                                                  | 41    |
| DLBCL          | China            | 144 patients                                                                      | M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>low</sup> , monocytes: markers not given                                                                                                                                                                                                                         | PB         | M-MDSCs decrease after chemotherapy irrespective of the outcome; M-MDSCs > 8% before treatment are associated with better prognosis                                                                                                                                                                                                                          | 49    |
| MF and SZS     | USA              | 77 patients, 26 SZS and 51 MF without PB involvement/7 controls                   | CD33 <sup>+</sup> CD11b <sup>+</sup> CD86 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                            | PB         | MDSCs proportion is lower in MF patients in stage ≥IB than in healthy controls; MDSCs of MF patients in stage ≥ IB produce more ROS than MDSCs of other patients and healthy controls; treatment with IFN-α2b (7 patients) resulted in no change of MDSCs frequencies, responders (4 patients) had decreased serum arginase-1 and intracellular ROS in MDSCs | 50    |
| MM             | China            | Numbers not given                                                                 | CD11b <sup>+</sup> CD142 <sup>+</sup> CD33 <sup>+</sup>                                                                                                                                                                                                                                                 | BM         | In vitro: MSCs from patients promote MDSCs proliferation more than MSCs from healthy donors; MSCs stimulate production of arginase-1 and NOS2 by MDSCs                                                                                                                                                                                                       | 61    |
| MM             | USA              | BM: 15 patients/6 controls; PB: 11 patients/10 controls                           | MDSCs: CD11b <sup>+</sup> CD14 <sup>-</sup> CD33 <sup>+</sup> ; PMN-MDSCs: CD11b <sup>+</sup> CD14 <sup>-</sup> CD33 <sup>+</sup> CD15 <sup>+</sup> ; M-MDSCs: CD11b <sup>+</sup> CD14 <sup>-</sup> CD33 <sup>+</sup> CD15 <sup>-</sup> or CD11b <sup>+</sup> CD14 <sup>+</sup> HLA-DR <sup>-</sup> low | BM, PB     | Patients display higher % of MDSCs and PMN-MDSCs in BM and PB than healthy donors; same % of M-MDSCs was observed in PB and BM, but M-MDSCs from BM of patients exhibit higher suppressive activity of T-cells                                                                                                                                               | 63    |
| MM, MGUS       | Italy            | 45 MM patients (30 newly diagnosed, 15 relapsed), 30 MGUS/30 controls             | G-MDSCs: CD11b <sup>+</sup> CD15 <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                                     | PB         | Patients with MM have higher % of G-MDSCs than MGUS patients and healthy donors; in vitro: only MM G-MDSCs can suppress autologous T-cell proliferation; MM-MSC-educated G-MDSCs can digest bone matrix                                                                                                                                                      | 65    |
| MM             | USA              | 17 patients (4 newly diagnosed, 4 relapsed, 9 relapsed/refractory)/5 controls     | MDSCs: CD11b <sup>+</sup> CD14 <sup>-</sup> CD33 <sup>+</sup> HLA-DR <sup>-</sup> low and/or CD11b <sup>+</sup> CD14 <sup>-</sup> HLA-DR <sup>-</sup> low/CD15 <sup>+</sup>                                                                                                                             | PB, BM     | Patients have increased MDSCs both in PB and BM compared to healthy donors; frequency of MDSCs increases in relapsed/refractory patients; MDSCs from PB and BM patients suppress T-cell proliferation and promote tumor growth                                                                                                                               | 66    |
| MM             | Australia        | 35 patients (10 progressive disease, 25 stable disease)/11 controls               | G-MDSCs: CD33 <sup>+</sup> CD11b <sup>+</sup> HLA-DR <sup>-</sup> low/CD14 <sup>-</sup> CD15 <sup>+</sup> ; M-MDSCs: CD33 <sup>+</sup> CD11b <sup>+</sup> HLA-DR <sup>-</sup> low/CD14 <sup>-</sup> CD15 <sup>-</sup>                                                                                   | PB, BM     | Patients have higher % and absolute numbers of G-MDSCs than healthy donors; patients with progressive disease have higher numbers of G-MDSCs than patients with stable disease; G-MDSCs both from patients and healthy donors did not differ in T cell inhibition                                                                                            | 67    |
| MM             | China            | 93 patients (41 newly diagnosed, 12 relapsed, 40 remission)/30 controls (PB only) | M-MDSCs: CD14 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                                                                          | PB, BM     | Patients have higher % of M-MDSCs than healthy donors; higher % of M-MDSCs in newly diagnosed and relapsed patients than in patients in remission both in PB and BM                                                                                                                                                                                          | 68    |
| ITP            | China            | PB: 21 patients/18 controls; spleen 5 patients, 5 controls                        | MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>low</sup>                                                                                                                                                                                                                                       | PB, spleen | PB: patients display lower MDSCs, lower arginase 1, higher iNOS than healthy donors; treatment with high dose DXM results in expansion of MDSCs                                                                                                                                                                                                              | 70    |
| ITP            | China            | 25 patients/10 controls                                                           | MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                                                         | PB         | Patients have lower MDSCs, platelets and Tregs than healthy donors; treatment with DXM leads to increase of MDSCs and platelets, but not Tregs                                                                                                                                                                                                               | 71    |
| ITP            | China            | 33 patients/18 controls                                                           | MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                                                         | PB         | Patients have higher numbers of MDSCs than healthy donors; treatment with high dose DXM results in increase of MDSCs and M-2 like macrophages (CD68 <sup>+</sup> CD163 <sup>+</sup> ) in patients with complete response and decrease of MDSCs in patients with partial and no response                                                                      | 72    |
| ITP            | USA              | 7 patients/7 controls—trauma patients                                             | MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>-</sup>                                                                                                                                                                                                                                         | Spleen     | In vitro: treatment of spleen cells with intravenous immunoglobulin led to higher increase of MDSCs in ITP patients than in trauma controls                                                                                                                                                                                                                  | 73    |
| CIN            | Greece           | 34 patients/23 controls                                                           | PMN-MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>low</sup> /CD15 <sup>+</sup> ; M-MDSCs: CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>low</sup> /CD14 <sup>+</sup>                                                                                                                       | PB         | Patients have significantly decreased proportion of M-MDSCs but not PMN-MDSCs than healthy donors                                                                                                                                                                                                                                                            | 75    |
| allo-HSCT      | Belgium          | 6 G-CSF-treated PBSC donors/3 controls                                            | MDSCs: Lin <sup>-</sup> HLA-DR <sup>-</sup> CD11b <sup>+</sup> ; M-MDSCs: Lin <sup>-</sup> HLA-DR <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>high</sup> CD14 <sup>low</sup> CD15 <sup>high</sup>                                                                                                         | PB         | Increase of MDSCs in G-CSF-treated donors in comparison with controls; in vitro: both M-MDSCs and G-MDSCs from donors suppress T-cell proliferation, effect of M-MDSCs more pronounced                                                                                                                                                                       | 80    |
| aGVHD          | Italy            | 60 allo-HSCT patients/60 donors/60 controls                                       | M-MDSCs: Lin <sup>low</sup> HLA-DR <sup>-</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> CD14 <sup>+</sup>                                                                                                                                                                                                  | PB         | G-CSF treated donors have higher % of M-MDSCs than controls; in vitro: M-MDSCs from donors suppress T-cell proliferation; aGVHD in patients inversely correlated with % of M-MDSCs in the PBSC graft                                                                                                                                                         | 81    |

(continued)



Table 1  
(continued).

| Disease     | Patients' Origin | Number of Patients/Controls (if Available)                        | Definition of MDSCs <sup>†</sup>                                                                                                                                                                                                                                                                                                                                                               | Source | Observation/Outcome                                                                                                                                                                 | Refs.         |
|-------------|------------------|-------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| aGVHD/cGVHD | China            | 62 allo-HSCT patients (leukemia, MDS-refractory anemia)/20 donors | M-MDSCs: SSC <sup>low</sup> CD11b <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>-</sup> Lin <sup>-</sup> CD15 <sup>dim</sup> CD16 <sup>-</sup> CD14 <sup>+</sup> ; G-MDSCs: SSC <sup>high</sup> CD11b <sup>+</sup> CD33 <sup>dim</sup> CD15 <sup>+</sup> HLA-DR <sup>-</sup> Lin <sup>-</sup> ; Promyelocytic(P)-MDSCs: CD33 <sup>+</sup> CD11b <sup>dim</sup> CD16 <sup>-</sup> Lin <sup>-</sup> | BM, PB | Treatment of donors with G-CSF led to increase of M/GP-MDSCs in both BM and PB; early reconstitution of M-MDSCs in PB of patients was associated with a low risk of aGVHD and cGVHD | <sup>82</sup> |
| aGVHD       | China            | 8 ALL, 15 AML, 5 MDS and 2 CML patients undergoing allo-HSCT      | MDSCs: CD14 <sup>+</sup> CD11b <sup>+</sup> CD11c <sup>+</sup> CD13 <sup>+</sup> CD15 <sup>+</sup> CD33 <sup>+</sup> CD62L <sup>+</sup> HLA-DR <sup>-</sup> low (authors call them M-MDSC, but CD15 is also present)                                                                                                                                                                           | PB     | Patients that do not develop aGVHD have higher numbers of MDSCs and Tregs in the graft (relationship to body weight) than those that develop aGVHD and healthy controls             | <sup>83</sup> |

aGVHD = acute graft versus host disease, ALL = acute lymphoblastic leukemia, Allo-HSCT = allogeneic hematopoietic stem cell transplantation, AML = acute myeloid leukemia, BM = bone marrow, cGVHD = chronic graft versus host disease, CIN = chronic idiopathic neutropenia, CLL = chronic lymphocytic leukemia, CML = chronic myeloid leukemia, DLBCL = diffuse large B-cell lymphoma, DMR = dexamethasone, G-CSF = granulocyte-colony stimulating factor, G-MDSCs = granulocytic myeloid-derived suppressor cells, HL = Hodgkin lymphoma, IDO = indoleamine 2,3-dioxygenase, IFN = interferon, IL = interleukin, iNHL = indolent B-cell NHL, INOS = inducible nitric oxide synthase, ITP = immune thrombocytopenia, Lin<sup>-</sup> = CD3-CD14-CD16-CD20-CD56-(HLA-DR<sup>-</sup>), MDSCs = myeloid-derived suppressor cells, MF = mycosis fungoides, MGUS = monoclonal gammopathy of undetermined significance, MM = multiple myeloma, M-MDSCs = monocytic myeloid-derived suppressor cells, MMR = major molecular response  $BCR-ABL/J \leq 0.1\%$ , MPN = myeloproliferative neoplasms (BCR-ABL) negative, MR = molecular response  $BCR-ABL/J \leq 0.0032\%$ , MRD = minimal residual disease, MSCs = mesenchymal stromal cells, NHL = non-Hodgkin lymphoma, PB = peripheral blood, PBMCs = peripheral blood mononuclear cells, PBSC = peripheral blood stem cell, PDL-1 = programmed death receptor ligand-1, PMN-MDSCs = polymorphonuclear myeloid-derived suppressor cells, pre-MMR = pre-major molecular response  $BCR-ABL/J > 0.1\%$ , ROS = reactive oxygen species, S/S = Sézary syndrome, TR = treatment free remission.

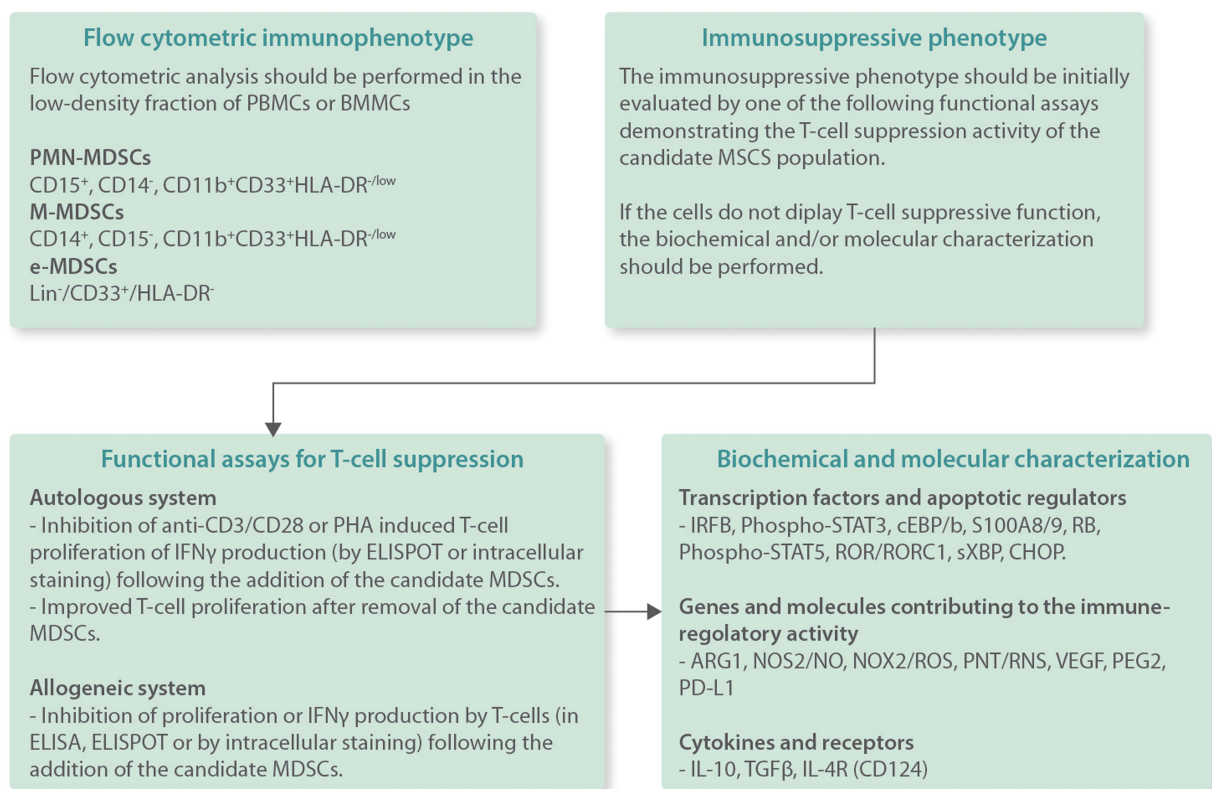
<sup>†</sup>Control samples were obtained from healthy blood donors if not stated otherwise (Ref.<sup>21</sup>).

<sup>‡</sup>The term G-MDSCs has been currently substituted by the term PMN-MDSC. However, in this table we use the terminology of the authors.

models and patients with pancreatic cancer.<sup>91,92</sup> The N-bisphosphonate zoledronic acid has been reported to decrease MDSCs by downregulating MMP9, among other proteins, in patients with pancreatic cancer.<sup>93</sup> The drug has also been shown to inhibit the bone resorption in MM animal models by reducing the osteoclast formation by MDSCs.<sup>62</sup> It has also been indicated that the anti-CD38 monoclonal antibody Daratumumab currently used for the eradication of plasma cells in MM patients may additionally eliminate patient MDSCs which also express CD38 according to recently reported data.<sup>94</sup> Therefore, we may speculate that the well-recognized beneficial effect of all the above treatments in patients with hematologic malignancies might be partially related to their effect on the reserves and functional properties of MDSCs, a hypothesis that needs further investigation. On the other hand, a number of novel therapies currently used in hematologic malignancies target pathways in tumor cells that are also involved in the regulation of physiological processes in MDSCs.<sup>95</sup> Representative examples are the PI3K and Jak/Stat signaling pathways which are therapeutic targets in lymphomas and MPNs as well as key signaling pathways in MDSCs.<sup>95</sup> The possible effect of these target therapies on MDSCs and the possibility of an additive effect on the therapeutic outcome is an interesting field of research. It has also been shown that epigenetic modulation of genes such as retinoblastoma 1 (Rb1) may alter the reserves and function of MDSCs.<sup>96</sup> It is therefore interesting to investigate whether epigenetic regulators including the hypomethylating agents widely used in the treatment of MDS/AML, have also an impact on the MDSC component of the BM microenvironment. Even the FLT3 pathway, representing currently a target for patients with AML and advanced systemic mastocytosis,<sup>97</sup> has been associated with the expansion and function of MDSCs<sup>98</sup>; therefore the potential alterations of MDSCs in patients treated with the FLT3 inhibitor midostaurin remain to be investigated. Beyond the potential effect of existing therapies on the quantitative and qualitative characteristics of MDSCs, the development of novel therapies targeting MDSC-related signaling pathways or surface molecules, such as CD33, is challenging.

Despite the increasing knowledge on the biology of MDSCs, a number of issues related to the generation, expansion, and circulation of these cells and their precise role in the microenvironment of BM and lymphoid tissues in malignant and immune-mediated hematologic disorders undoubtedly need further investigation. The homogenization of the methods for MDSC assessment is also an important issue because there is considerable variability in how MDSCs have been measured and reported in different studies and the lack of uniform protocols can lead to inconsistencies, uncertainties and erroneous conclusions.<sup>99</sup> Even the standard method, that is, the immunophenotyping, currently used for the measurement of MDSCs in the low density fraction of PBMCs and BMMCs,<sup>5</sup> needs further improvement because it prevents the accurate quantification of absolute MDSC numbers whereas markers unique for MDSCs have not been identified so far. Therefore, the functional characterization, and specifically the T-cell suppression activity, remains a key assay for MDSC definition (Fig. 2). Recently, guidelines for the standardization and harmonization of the functional assays were published by a working group from EU COST Mye-Euniter consortium (<http://www.mye-euniter.eu>) aiming to diminish variations across laboratories for the study of MDSCs from different diseases and tissue sources.<sup>100</sup>

Overall, the better characterization of MDSCs and the elucidation of the molecular and signaling pathways implicated



**Figure 2. Algorithm for the characterization of MDSCs in human PB samples or BM aspirates.** For the characterization of a candidate cell population as MDSCs, both the specific immunophenotypic characteristics and the immunosuppressive potential of the cells should be determined. The immunosuppressive potential of the cells should preferentially be performed by a functional assay demonstrating their T-cell suppressive capacity. If the candidate MDSC population lacks a T-cell suppressive function, then this potential should indirectly be demonstrated by the biochemical and/or molecular characterization of the cells. The figure depicts representative functional assays and biochemical and molecular characteristics of MDSCs according to recent recommendations.<sup>5</sup> ARG1=arginase-1, BM=bone marrow, BMMCs=bone marrow mononuclear cells, C/EBP $\beta$ =CCAAT/enhancer binding protein  $\beta$ , CHOP=C/EBP homologous protein, ELISA=enzyme-linked immunosorbent assay, ELISPOT=enzyme-linked immunospot, e-MDSCs=early MDSCs, IFN $\gamma$ =interferon  $\gamma$ , IL=interleukin, IRF8=interferon regulatory factor 8, MDSC=myeloid-derived suppressor cells, M-MDSCs=monocytic MDSCs, NOS=nitric oxide synthases, NOX=NADPH=oxidase, PB=peripheral blood, PBMCs=peripheral blood mononuclear cells, PD-L1=programmed death-ligand 1, PGE2=prostaglandin E2, PHA=phytohemagglutinin, PMN-MDSCs=polymorphonuclear MDSCs, PNT=peroxynitrite, RB=retinoblastoma, RNS=reactive nitrogen species, ROR=RAR-related orphan receptors, ROS=reactive oxygen species, STAT=signal transducer and activator of transcription, sXBP=spliced X-box binding protein, TGF=transforming growth factor, VEGF=vascular endothelial growth factor.

in MDSC generation, expansion and function are anticipated to unravel novel pathogenetic mechanisms in normal and abnormal hemopoiesis and may offer novel therapeutic approaches for patients with malignant and immune-mediated hematologic disorders including patients undergoing allo-ASCT.

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