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REVIEW ARTICLE

Myeloid-derived Suppressor Cells in Cancer Patients: A Clinical Perspective

Alberto J. Montero, Claudia Marcela Diaz-Montero,* Christos E. Kyriakopoulos,* Vincenzo Bronte,† and Susanna Mandruzzato‡*

Summary: Myeloid-derived suppressor cells (MDSCs) represent a heterogenous collection of immature myeloid cells endowed with suppressive function on the immune response. Their presence has been extensively investigated in preclinical models, especially in the context of cancer. One of the major obstacles in their accurate identification has been the definition of an unambiguous phenotype, shared between mice and humans, and clearly correlating with their suppressive function. In this paper, we review the literature concerning the phenotype in mouse and in humans, showing that at least 2 subsets of MDSCs are present under different situations. We also address the role of MDSCs in tumor progression, evaluate the prognostic significance of MDSC in cancer patients, and their possible role as marker of clinical outcome and response to therapy. Finally, we examine the strategies designed to modulate MDSCs in cancer patients, which might represent an innovative approach to enhance the effectiveness of immune-based therapies.

Key Words: myeloid-derived suppressor cells, suppression

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Immune evasion was recently included in the list of hallmarks of cancer,[1] a sort of recognition of the last 2 decade efforts in understanding the immune response to tumor antigens. This research activity translated into new therapies and a proliferation of clinical trials targeting the immune system. One of the greatest challenges in exploiting the immune system clinically, is the presence of multiple control pathways, some redundant and distinct others, with intricate feedback loops. Long before regulatory T cells (Treg) were recognized, one of the earliest machineries of immune evasion in cancer was the presence of tumor-infiltrating macrophages as powerful negative regulators of intratumoral immunity.[2] However, immunosuppression is not limited to the tumor microenvironment, and circulating myeloid cells able to create dysfunctional immune responses have been repeatedly described. From the initial observation in the 90s, increasing evidence accumulated on a population of $CD11b^+/Gr\text{-}1^+$ myeloid cells expanding in tumor-bearing mice. Most recently, to account for their functional ability to suppress T cells, these cells were named myeloid-derived suppressor cells (MDSCs).[3]

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There is a large body of literature showing that MDSCs expand in a wide array of transplantable and autochthonous tumor models, suppress NK and T cells through direct cell contact, cytokines, and byproducts of metabolic pathways, can control expansion and activation of Tregs, and support neoangiogenesis and metastatic spread (extensively reviewed elsewhere[4–7]). MDSC accumulation is likely an early event in tumor progression, due presumably to the recruitment of cells from the bone marrow through secretion of tumor-derived factors and preclinical data have clearly indicated their progressive accumulation in blood, spleen, marrow, and tumor site. As previously discussed, MDSC levels appear to correlate proportionally with tumor burden, and thus directly contribute to tumor progression. The study of MDSCs in cancer patients, however, has lagged behind in part due to lack of cognate marker Gr-1 (Ly6G/C) in humans. This in turn has led to great heterogeneity in phenotypical definition of MDSCs, with the utilization of rather different cell surface markers. The primary aims of this article are to systematically review the published clinical literature on MDSCs in cancer patients, and discuss gaps in our knowledge and how/why these should be answered.

MOUSE MDSC PHENOTYPE

MDSCs have been extensively investigated in mouse models and it is now widely accepted that these cells comprise a heterogeneous immature population with at least 2 main subsets resembling either polymorphonuclear (PMN) or monocytic cells, which have been termed granulocytic and monocytic MDSCs, respectively. This distinction can be highlighted already with the sole use of the markers CD11b and Gr-1. In fact, thanks to the different expression intensity of the Gr-1 marker, at least 2 cellular fractions can be recognized, a $Gr\text{-}1^{high}$ subset mainly composed of immature and mature granulocytes, and a $Gr\text{-}1^{int}$ cell subset encompassing monocytes and other immature myeloid cells (ImCs).[8] These 2 MDSC subsets can be found in different proportions in vivo under different experimental conditions, including mice with cancer, sepsis, traumatic lesions, autoimmune disease and chronic infections.[4–7] It has been advanced that MDSC composition depends on tumor-derived soluble factors released from the cancer microenvironment, which can vary according to tumor histology and anatomical localization. Given the immaturity and the plasticity of MDSCs, it is not surprising that a different milieu can drive partial maturation of these cells toward different myeloid lineages.

MONOCYTIC MDSCs IN MURINE MODELS

In a mouse model of colon carcinoma engineered to produce high levels of granulocyte macrophage-colony

stimulating factor (GM-CSF), the 2 main subsets of MDSCs were induced and could be sorted on the basis of the expression of the alpha chain of the IL-4 receptor (IL-4Rα). Cells positive for this marker homogenously displayed a predominant monocytic morphology endowed with suppressive activity, while IL-4Rα–negative cells had the appearance of granulocytes at different stages of differentiation but lacked a strong suppressive activity.[9] Although IL-4Rα was later found to be upregulated in granulocytic MDSCs in other tumor models as well, the idea that monocytic compartment might contain the cells with main immunoregulatory activity was substantiated by other observations. By using anti–Gr-1 mAb it is possible to distinguish at least 3 subsets with different Gr-1 intensity: Gr-1^{high}, Gr-1^{int}, and Gr-1^{low} cells, endowed with different suppressive abilities.[8] In fact, in 3 different transplantable tumor models the Gr-1^{int} subset, mainly comprising monocytes and myeloid precursors, showed a constant suppressive activity, whereas Gr-1^{high}, mainly comprising granulocytes, exerted an only limited suppressive activity, which was tumor dependent.[8] Moreover, adoptively transferred MDSCs possessed dissimilar tolerogenic ability, with Gr-1^{high} cells increasing rather than decreasing the immune response, whereas only the transfer of Gr-1^{int} subset produced a statistically significant tolerance in vivo. It is interesting to note that knocking down GM-CSF in a mammary carcinoma model demonstrated that this cytokine was cardinal in driving Gr-$1^{int/low}$ suppressive MDSCs, whereas GM-CSF preferentially induced Gr-1^{high} cells with poor immunosuppressive activity.[8] GM-CSF administered exogenously was shown to influence myelopoiesis as it acted on GM progenitors in the bone marrow inducing local expansion of $CD11b^+$/Gr-1^{low} cells[8] suggesting that it can also influence the expansion of promyelocytes, as later shown for human MDSCs.[10]

These results were mirrored in an inflammatory setting. MDSCs with suppressive potential could be expanded in vivo by the injection of lipopolysaccharide plus interferon-γ (IFN-γ).[11] As previously reported, the PMN-like fraction expressing a Gr-1^{high} phenotype lacked suppressive activity, but the $CD11b^{int}$Gr-1^{high} cells with ring-shaped nuclei and the $CD11b^{int}$Gr-$1^{low}SSC^{low}$ monocytes were endowed with immunosuppressive activity.[11]

In agreement with these results, myeloid suppressive cells were identified on the basis of the markers CD115 (M-CSF receptor) and F4/80, in addition to Gr-1, and cells from bone marrow of tumor-bearing mice were sorted on the basis of these markers. Results from an in vitro suppression assay indicated that Gr-$1^+F4/80^+$ and Gr-1^+CD115^+ monocytic MDSCs had a strong suppressive activity, whereas Gr-$1^+F4/80^-$ or Gr-1^+CD115^- cells did not.[12] Moreover, this study provided evidence that Gr-1^+CD115^+ MDSCs can induce the development of Treg in vitro and in tumor-bearing mice, which was dependent on IFN-γ and IL-10.[12]

GRANULOCYTIC MDSCs IN MURINE MODELS

To study the phenotype of MDSCs induced by different tumor models, 10 transplantable tumor models were investigated in 3 different strains of mice. A significant increase in the proportion of Gr-1^+CD11b^+ MDSCs in the spleens was documented in all tumor models. In this work, granulocytic MDSCs were defined as $Ly6G^+Ly6C^{low}$ cells and monocytic MDSCs as $Ly6G^-Ly6C^{high}$ cells. Granulocytic MDSCs were consistently increased in all tumor models, whereas the frequency of monocytic MSDCs was significantly increased in only 3 models, although their overall suppressive activity among $CD11b^+$Gr-1^+ cells was comparable.[13] Recently the same group studied the relationship between granulocytic MDSCs and normal PMN cells, demonstrating that, although they share the morphology, they differ in terms of markers and functional properties and that granulocytic MDSCs are less mature cells than PMN and might represent a pathological activated precursors of PMNs whose transition has been halted.[14]

The presence of monocytic and granulocytic MDSCs was also investigated in 2 T-cell lymphoma models in which $CD11b^+$Gr-1^+ MDSCs purified from the spleen consisted of 2 main fractions characterized by a differential Ly6G expression. $Ly6G^+$ cells showed a PMN profile and a high side scatter characteristic (SSC) profile, corresponding to granulocytic MDSCs, whereas $Ly6G^-$ cells were mononuclear cells with a lower SSC, corresponding to monocytic MDSCs. Both subsets were able to suppress antigen-specific T-cell responses, but through distinct mechanisms, with granulocytic MDSC requiring IFN-γ acting through a STAT-1–independent pathway.[15] These data suggest that, even though the immunosuppressive power is lower on a cell per cell basis, granulocytic MDSCs might be still immunosuppressive in vivo because of their superior numbers over monocytic MDSCs.

The presence of G-MDSC and M-MDSC was also documented in the tumor microenvironment. In 2 different tumor models the presence of tumor-infiltrating $CD11b^+$ myelomonocytoid cells was characterized and more than 90% of these cells were Gr-$1^{low}F4/80^+$IL-$4R\alpha^+$ monocytes with suppressive activity.[16] It is interesting to note that suppression of $CD8^+$ T-cell–mediated antitumor response was shown to be dependent by the presence of Gr-1^{high} MDSC recruited at the tumor site by the generation of C5a complement fraction and regulating MDSC ability.[17] Moreover, presented data suggested that C5a was involved in the processes of MDSC migration and accumulation to peripheral lymphoid organs.

MDSCs AS INDICATORS OF TUMOR PROGRESSION IN MICE

Although considered a hallmark of tumor development, only a limited number of studies have addressed kinetically the correlation between MDSCs and tumor burden. In a transgenic mouse model in which the rat protooncogene c-erb-B2 is under the control of the mouse mammary tumor promoter and mice spontaneously develop metastatic mammary carcinoma, the development of these tumors was accompanied by the gradual expansion of MDSCs. Of note, the number of MDSCs in the spleen was directly associated with G-CSF transcript levels, while within the tumor it was directly correlated with splenic GM-CSF transcript levels, tumor volume, and tumor cell numbers.[18] In a similar oncogene-driven tumor, but in the BALB strain (BALB-neuT), a linear correlation between tumor progression and the numbers of immature Gr-$1^+CD11b^+CD131^+$ cells endowed with suppressive activity was also established. Moreover, expansion of myeloid immunosuppressive cells in the peripheral blood and in the spleen of tumor-bearing BALB-neuT mice directly correlated with tumor multiplicity, thus highlighting the role of MDSCs in tumor progression.[19]

CLINICAL DATA OF MDSCs IN SOLID TUMOR PATIENTS

Since the initial identification of MDSCs, several subsequent publications reported increased circulating levels of MDSCs in patients with a variety of human solid tumors (Table 1). One of the greatest challenges however, has been the lack of consensus over the definition and phenotype of MDSCs, and considerable heterogeneity in how they are defined clinically.

To the best of our knowledge, the first account of a population of cells of myeloid origin with T-cell suppressive properties was described in patients (n = 18) with cancers of the head and neck, mostly squamous cell carcinoma (HNSCC).[20] A significant direct correlation ($r^2 = 0.65$) was observed between the amount of secreted GM-CSF in tumor fragments and the levels of intratumoral $CD34^+$ myeloid cells. It is interesting to note that in the 4 tumors from patients with a diagnosis other than HNSCC, neither GM-CSF production nor $CD34^+$ cells were found. Moreover, depletion of $CD34^+$ cells was associated with a reversal of T-cell suppression, evidenced by increased IL-2 production from intratumoral lymphocytes. Subsequent studies[21,48] analyzed peripheral blood samples from patients with HNSCC, non–small-cell lung cancer, and breast cancer of unknown clinical stages (n = 44), identifying a population of circulating cells that was termed immature myeloid cells (ImC) within the dendritic cell (DC) fraction. A more comprehensive phenotyping of these ImCs revealed that approximately two thirds of the cells were IMCs at early stages of differentiation described as lineage negative (Lin^-), defined here as CD3, CD14, CD19, and CD57. Further phenotyping characterized them as $CD33^+$ and $CD11b^+$. When ImCs were cocultured with T cells, they were able to directly suppress T cells through a fully reversible process.

The next major clinical study of MDSCs in human cancer patients described the presence of a granulocytic population of cells capable of suppressing T cells, in

AQ5 **TABLE 1.** Phenotype of MDSCs in Human Malignancies

Phenotype	Cancer Type	References
$CD34^+$	HNSCC	Pak et al[20]
Lin^-/HLA-DR$^-$*	Breast carcinoma	Almand et al[21]
	HNSCC	
	NSCLC	
$CD15^+$ granulocytes	Breast carcinoma	Schmielau and Finn[22]
	Colon carcinoma	
	Pancreatic cancer	
$CD11b^+/CD14^-/CD15^+$	Renal cell carcinoma	Zea et al[23]
$CD14^+$/Arginase$^+$	HNSCC	Serafini et al[24]
	MM	
$CD14^+$/HLA-DR$^{-/low}$	Melanoma	Fillipazzi et al[25]
$CD11b^+/CD33^+$	NSCLC	Srivastava et al[26]
$Lin^{-/low}$/HLA-DR$^-/CD33^+/CD11b^+$†	Multiple solid tumors (Breast cancer, esophageal, gastric, colorectal and other solid malignancies)	~~Solito and colleagues~~[10,27,28]
Lin^-/HLA-DR$^-/CD33^+$†	Melanoma	Daud et al[29]
$CD11b^+/CD14^-/CD33^+/CD15^+$	NSCLC	~~Wang and colleagues~~[30,31]
$CD14^+$/IL-4Ra$^+$	Colon cancer	Mandruzzato et al[32]
	Melanoma	
$CD14^+$/HLA-DR$^{-/low}$/B7-H$^+$	Melanoma	Wilcox et al[33]
$CD11b^+/CD14^-/CD33^+$	HNSCC	Corzo et al[34]
$CD11b^+/CD13^+/CD34^+/CD14^-/CD45^+$	Hodgkin lymphoma	Parrinello et al[35]
$CD14^+$/HLA-DR$^{-/low}$	Melanoma	Poschke et al[36]
DC-Sign$^+/CD80^+/CD83^+$		
$CD11b^+/CD13^+/CD14^-/CD34^+/CD45^+$	MM	Parrinello et al[37]
	MGUS	
Lin^-/HLA-DR–/$CD33^+$‡	MDS	Wei et al[38]
$CD11b^+/CD16^{low}/CD62L^{low}/CD66b^+$/VEGFR1$^+$	Renal cell carcinoma	Rodriguez et al[39]
$CD14^+/CD15^+/CD33^+$/HLA-DR$^-$	Bladder cancer	Shepard et al[40]
$CD14^+$/HLA-DR$^{-/low}$	MM	Brimnes et al[41]
	NHL	Lin et al[42]
	HCC	Hoechst et al,[43,44]
SSC^{high}/$CD66b^+/CD125^-/CD33^+$/HLA-DR$^-$	Bladder cancer	Brandau et al[45]
	HNSCC	
	NSCLC	
$CD34^+/CD45^+/CD116^+/CD13^+/CD14^-$	NHL	Pitini et al[46]
$CD11b^+$/CD15^{high}/CD33^{low}	Bladder cancer	Eruslanov et al[47]

*−CD3, −CD14, −CD19, and −CD57.
†−CD3, −CD14, −CD19, and −CD56.
‡Lin not defined in the paper.
AQ6 §−CD3, −CD14, −CD16, −CD19, −CD20, and −CD56.
HCC indicates hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; MDS, myelodysplastic syndrome; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NSCLC, non–small-cell lung cancer.

apparent contrast to the previously cited studies, which defined a more immature myeloid population.[23] In this study, peripheral blood levels of granulocytic cells in patients with metastatic RCC without previous treatment (n = 123) were compared with normal controls (n = 33), and a statistically significant ($P = 0.037$) increase in the subset of cells with immunosuppressive properties was found. This PMN population of cells was described as $CD11b^+/CD14^-/CD15^+$. Further phenotyping determined that this population was negative for the expression of CD11a, CD80, CD83, CD86, and HLA-DR, and had increased arginase activity. Arginase, which metabolizes L-arginine to L-ornithine, plays an important role in T-cell suppression through depletion of arginine, which is requisite for T-cell proliferation and cytokine production.[49] This study was also unique in that it was the largest clinical study at that point (n = 123) with a homogenous population of cancer patients, that is patients with metastatic RCC. In a subsequent study of patients with RCC (n = 27), increased levels of granulocytic MDSCs with a similar phenotype, that is $CD11b^+/CD15^+/CD14^-$ were also detected.[39] These granulocytic MDSCs also had measurable vascular endothelial growth factor receptor (VEGFR1) expression, but low CD62L and CD16 expression. VEGF has been found to correlate with high numbers of immature DCs in patients with cancer,[50] and it was therefore hypothesized that blockade of VEGFR1 with bevacizumab would decrease the number of MDSCs in the peripheral blood. However, even though VEGFR1 overexpression in MDSCs was confirmed, the addition of bevacizumab to IL-2 did not reduce neither their numbers nor the level of arginase 1 in the peripheral blood of the patients.

The role of arginase as a mechanism of T-cell suppression may be tumor dependent, as evidenced by the work by Filipazzi et al[51] in patients with metastatic melanoma (n = 16) who were treated with a GM-CSF–based antitumor vaccine and interferon alpha. In this study, the circulating MDSCs population was described as $CD14^+/HLA\text{-}DR^{low/-}$. These cells were shown to have suppressive activity on T cells, mediated through a transforming growth factor β (TGF-β)–dependent mechanism and not arginase.

One possible explanation for the significant heterogeneity of MDSCs in the literature in terms of overall levels, mechanisms of suppression, and phenotype is that MDSCs may not be universally present in human cancers due to differences in tumor-derived factors. To begin to address this issue, a subsequent study prospectively evaluated MDSCs in patients (n = 123) with newly diagnosed solid tumors, clinical stages I to IV.[27] Approximately 50% of patients in this study had breast cancer, 30% had gastrointestinal cancers, and the remainder 20% comprised patients with melanoma, sarcoma, prostate cancer, or other cancers. Enumeration of MDSCs was performed on freshly collected whole blood and MDSCs were defined by FACS as $Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$. In this study, Lin1 was defined by as CD3, CD14, CD16, CD19, CD20, and CD56. Overall circulating MDSCs levels were found to be significantly higher in cancer patients relative to a smaller cohort of matched healthy controls ($P < 0.0001$). Moreover, MDSCs were present to varying degrees in all solid tumor patients, and overall levels were found to be directly proportional to clinical cancer stage. Patients with advanced stage IV disease were found to have significantly higher levels ($P < 0.0001$) than patients with early-stage disease. Furthermore, MDSC levels in patients with widely metastatic disease were higher than in patients with more limited metastatic involvement. This same study also provided evidence for the induction of MDSCs as a result of cyclophosphamide treatment; a phenomenon widely described in preclinical models. Cyclophosphamide-induced MDSCs were also found to have T-cell suppressive capabilities. Looking for a similar phenotype, another study found that circulating levels, of $Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$ MDSCs, were aberrantly elevated in 131 cancer patients (46 pancreatic, 60 esophageal, and 25 gastric) relative to healthy controls.[28] Numbers of MDSCs correlated with levels of Tregs, and increased circulating MDSC levels were an independent adverse prognostic factor for overall survival.

Differences in CD14 expression exemplify the challenges thus far in studying MDSCs in cancer patients. Although the $Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$ MDSC phenotype has been shown by 2 independent groups to correlate well with cancer clinical stage and prognosis, CD14 is part of the Lin1 cocktail and therefore this MDSC population is expected to have no or very low expression of this myeloid marker. Likewise, the granulocytic MDSC population described in renal cancer patients was also $CD14^-$.[23] However, in at least 7 different clinical studies (Table 1), MDSCs have been described as cells expressing the CD14 marker. In a large study of patients with hepatocellular carcinoma (n = 111),[43] increased levels of circulating $CD14^+/HLA\text{-}DR^{-/low}$ MDSCs were described. This subpopulation had also increased arginase activity, and was capable of T-cell suppression. In another study, a population of MDSCs defined as $CD14^+/IL\text{-}4R\alpha^+$ was also detected in colon cancer (n = 15) and melanoma (n = 14) patients.[32] MDSCs with ether granulocytic or mononuclear features were expanded in the PMN and mononuclear fraction, respectively, and both cell subsets overexpressed the receptor for IL-4Rα but the presence of this marker correlated with an immunosuppressive phenotype only for the mononuclear cells.[32]

Another study also described a population of $CD14^+$ and $HLA\text{-}DR^{-/low}$ circulating MDSCs in melanoma patients (n = 34).[36] Subsequent phenotyping suggested that this population of MDSCs was more differentiated, as cells also expressed CD80, CD83, and DC-sign (CD209). It was also demonstrated that only the subpopulation of $CD14^+$ and $HLA\text{-}DR^{-/low}$ myeloid cells that expressed IL-4Rα was suppressive. Moreover, S100A9, a calcium-binding protein that is overexpressed in MDSCs in murine models, was not found to be uregulated.[52]

Eruslanov et al[47] examined the presence of 2 distinct populations of MDSCs in bladder cancer patients (n = 32) with superficial noninvasive and invasive disease. In this study, both peripheral blood and fresh tumor samples were collected and analyzed by flow cytometry. Two different populations of myeloid cells were isolated from the peripheral blood: (i) $CD11b^+/CD15^{high}/CD33^{low}$ with coexpression of the neutrophil markers CD114 and CD117; and (ii) $CD11b^+/CD15^{low}/CD33^{high}$ with coexpression of the monocyte-macrophage markers CD14, CD115, CD116, and CCR2. When patient peripheral blood samples were compared with samples from healthy volunteers, only the $CD11b^+/CD15^{high}/CD33^{low}$ cells were found to be present in higher levels in bladder cancer patients, whereas the $CD11b^+/CD15^{low}/CD33^{high}$ cells were also found to be present in significant amounts in healthy volunteers as well. Both populations were found to secrete substantial

amounts of cytokines, but only the $CD11b^+/CD15^{high}/CD33^{low}$ population was noted to have immunosuppressive activity. In the tumor specimens, 2 distinct MDSC populations were found to infiltrate the tumors: 60% to 70% of those cells were described as $CD11b^+/HLA\text{-}DR^+$ with the remainder 30% to 40% described as $CD11b^+$ and $CD15^+$. The clinical significance of those cells though was not fully explored.

Based on a synthesis of the clinical data about MDSCs in cancer patients, which is also reflected in the preclinical literature, it appears that MDSCs mainly consist of: (i) a monocytic population characterized by the presence of CD14 and absence of CD15, which could also comprise a cell subset expressing CD15 at low levels, possibly representing a more immature stage of monocyte development, likely less differentiated than monocytic $CD15^-$ MDSCs; (ii) a more differentiated granulocytic population having the opposite pattern of expression, that is $CD15^+$ and ~~CD14~~.

MDSCs IN HEMATOLOGIC MALIGNANCIES

MDSCs in solid tumor patients have been studied extensively, whereas their presence in patients with hematologic malignancies is less well established. In patients with multiple myeloma (MM), MDSCs have been described as $CD14^+/arginase^+$[24] and $CD14^+/HLA\text{-}DR^{low/-}$.[41] It is interesting to note that in a separate study of patients with monoclonal gammopathy of undetermined significance (MGUS) and MM, whereas the number of circulating MDSCs in MGUS patients was similar to that measured in normal controls, overall MDSC levels were highest in MM patients.[37] MDSCs have also been described in both Hodgkin and non-Hodgkin lymphomas (NHL). The phenotype in Hodgkin lymphoma patients (n = 14) was described as $CD11b^+/CD13^+/CD34^+/CD14^-/CD45^+$ and overall MDSC levels correlated with cancer clinical stage, with the highest levels detected in patients with more advanced disease.[35] In NHL patients (n = 40), MDSCs isolated from the peripheral blood were described as $CD14^+/HLA\text{-}DR^{low/-}/CD120b^{low}$. The highest percentages of MDSCs were found in patients with advanced clinical stage ($P = 0.002$), more aggressive NHL histology ($P = 0.01$), and faster rates of disease progression ($P = 0.01$).[42] In follicular lymphoma also, a $CD14^+$ population of MDSCs have been described that were also positive for CD13, CD34, CD45, and CD116.[46]

The presence of MDSCs was confirmed in the bone marrow of 12 patients with low risk myelodysplastic syndrome.[38] The authors of this study compared the number of MDSCs in the bone marrow of 12 patients with low risk myelodysplastic syndrome, and 8 healthy individuals, showing increased numbers of MDSCs only in the first group. The same study also showed elevated levels of the cytokines (TGFβ, VEGF, IL-10), which may play a role in the immune-suppressive effects of MDSCs and in the maturation of stem cells in the bone marrow microenvironment.

PROGNOSTIC SIGNIFICANCE OF MDSCs IN CANCER PATIENTS: A WORK IN PROGRESS

Despite the fact that immune evasion is an emerging hallmark of cancer,[1] there is a clear paucity of biomarkers related to either innate or adaptive immunity and associated with prognosis and clinical outcome. In the setting of breast cancer, the most established and validated prognostic markers are all tumor related, for example HER-2/neu gene amplification, hormone receptor status, tumor histologic grade, etc.[53–57] However, more recent comprehensive microarray analyses underscored the importance of tumor host interactions with immune gene signatures having prognostic relevance in localized breast cancer and other solid tumors.[58] Another example is the presence of tumor-infiltrating lymphocytes, which have been shown to be of prognostic relevance in different solid tumors.[59,60] MDSCs are clearly an important mechanism of immune evasion by tumors, but thus far there is an overall paucity of studies that have explored in detail the overall prognostic or predictive significance of MDSCs in cancer patients. Even if we put aside the problems on how to best define MDSCs, very few studies addressed the clinical implications of circulating MDSCs.

Thus far, only 3 studies have shown that overall levels of a monocytic population of MDSCs ($Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$) in the peripheral blood correlate with clinical stage.[10,27,28] The previously discussed study by Lin et al[42] also demonstrated that overall MDSC levels in NHL patients correlated with clinical cancer stage and aggressiveness of disease, however with a different phenotype ($CD14^+/HLA\text{-}DR^{-/low}$). Moreover, 2 of these studies[10,28] have independently shown that in patients with advanced breast cancer and gastrointestinal malignancies, higher MDSC levels were associated with shorter survival times. In the study by Solito et al,[10] patients with stage IV breast cancer (n = 25) with circulating MDSC levels > 3.17% (median) at baseline, had significantly shorter median OS times, than patients with circulating MDSCs less than the median at 5.5 [95% confidence intervals (CI), 0.5-11.3] and 19.32 months (95% CI, 8.7-infinity), respectively ($P < 0.048$). Similarly, in the study by Gabitass et al,[28] levels of circulating MDSCs > 2.0% were found to be an independent prognostic factor in patients with pancreatic, esophageal, and gastric cancers in a multivariate analysis. Patients with elevated MDSCs (> 2%) were found to have an inferior OS, with a median OS of only 4.6 months (95% CI, 2.2-6.0), and 12-month survival rate of 10.4% to a median OS of 9.3 months (95% CI, 6.3-12.1) and 12-month survival of 39% ($P < 0.001$), respectively, in cancer patients with MDSCs < 2%.

Although these studies were retrospective in nature and involved relatively small number of patients, they provide important initial data using similar MDSC phenotypes, that is $Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$, on the clinical relevance of MDSCs by correlating levels with overall survival and chemotherapy response. It is presently unknown whether blood MDSC levels are an independent prognostic factor in different cancers; future appropriately powered prospective studies will have to define this issue.

MDSCs AS PREDICTIVE MARKER FOR CANCER IMMUNOTHERAPY

To date, there is only 1 study that has explored whether MDSC levels are predictive of response to immunotherapy in cancer patients.[61] In this study, percentages of circulating MDSCs ($Lin^-HLA\text{-}DR^-CD33^+$) and mature DCs were evaluated in patients with advanced kidney cancer or melanoma (n = 36) who received high-dose IL-2. A high DC-to-MDSC ratio and low numbers of circulating MDSCs were able to discriminate the responder

subset within the cohort of patients treated with high-dose IL-2.

MDSCs AS A THERAPEUTIC TARGET IN CANCER PATIENTS

Finding novel ways to pharmacologically modulate or differentiate MDSCs represents a promising strategy in oncology, particularly if combined with immune-based therapies. Drugs tested in humans that may modulate MDSCs can be divided into 3 different categories: (i) agents that decrease MDSCs through promotion of MDSC differentiation; (ii) agents that alter the suppressive function of MDSCs, without altering their numbers; (iii) non-differentiating agents that decrease MDSC levels.

Two different agents (Table 2) have thus far been shown to promote the differentiation of MDSCs in humans: 25-hydroxyvitamin D_3 and all-trans-retinoic acid (ATRA). The effect of escalating doses of 25-hydroxyvitamin D_3 on circulating levels of $CD34^+$ MDSCs in patients with locally advanced or metastatic HNSCC (n = 18) was examined.[62] 25-hydroxyvitamin D_3 therapy, especially at the highest doses examined, was found to be associated with decreased numbers of $CD34^+$ MDSCs, and increased the number of HLA-DR^+ cells. Moreover, IL-12 and IFN-γ plasma levels were increased with vitamin D_3, and improved T-cell proliferation was also observed. However, this study was not designed to evaluate whether these changes correlated with improved clinical outcomes, and there was no clear clinical or antitumor response for the patients that received the drug. The modulatory effect of ATRA on MDSCs, was explored in a small cohort of patients (n = 18) with metastatic RCC.[63] In this study, different phenotypes of myeloid cells were examined, however MDSCs were ultimately defined as Lin^-/HLA-DR^-/$CD33^+$. ATRA was given in escalating doses of 50, 100, and 150 mg/m^2/d divided in 3 daily doses for 7 days, followed by subcutaneous IL-2. ATRA therapy was found to be associated with: decreased numbers of circulating MDSCs; improved myeloid/lymphoid DC ratios; and improved antigen-specific T-cell responses as measured by stimulation with tetanus toxoid. Of interest, the effect of ATRA was observed only in patients with high serum levels of ATRA (> 150 vs. < 135 ng/mL). Finally, in a separate study, ATRA therapy was shown to have the ability to reverse the immunosuppressive effect of MDSCs in patients with stages III to IV RCC, and improve T-cell function by direct differentiation of MDSCs into antigen-presenting cell precursors.[67] These findings were confirmed in vivo; however, whether these differences translated into improved clinical outcomes was not explored.

Sildenafil is an example of a drug that has been shown to favorably modulate suppressive properties of MDSCs in humans.[24] Sildenafil is a phosphodiesterase-5 inhibitor that is used in the treatment of erectile dysfunction and pulmonary hypertension. Sildenafil has been shown to downregulate arginase 1 and nitric oxide synthase 2 in murine tumor models. The effect of Sildenafil in human PBMCs from patients with MM and HNSCC was observed only in vitro. Presently it is unknown whether a similar effect can be observed clinically in cancer patients in vivo.

Several drugs have been shown to decrease the overall number of MDSCs in humans and animal models. Sunitinib is a pan receptor tyrosine kinase inhibitor that is widely used in the treatment of RCC and other malignancies. The effects of sunitinib on circulating MDSCs in patients with metastatic RCC (n = 23) has been studied.[64] Sunitinib therapy was found to be associated with a decrease in the number of circulating MDSCs, whereas at the same time was associated with improved T-cell function, evidenced by increased IFN-γ production. These changes, though, did not correlate with radiographic responses or improved progression-free or overall survival.

Even though there is extensive literature on the effect of chemotherapy on MDSCs in animals, only 2 studies have shown any direct effect in humans so far. The first study[33] examined the effect of taxane-based chemotherapy on circulating MDSCs [$CD14^+$/HLA-DR^-] in stages I to IV melanoma patients (n = 77). In this study, MDSC levels were found to correlate with clinical cancer stage, and overall levels were found to decrease after taxane-based chemotherapy. The second study[66] included patients with pancreatic cancer (n = 16) treated with gemcitabine-based chemotherapy and patients with esophagogastric cancer (n = 23) treated with 5-FU–based chemotherapy. When posttreatment levels of MDSCs were compared with the pretreatment levels, there was a statistically significant decrease in percentages with chemotherapy ($P < 0.0001$); however, the decreases in MDSC number was apparently independent of response to treatment, and was also observed in patients with progressive disease.

TABLE 2. Drugs Known to Modulate MDSCs in Humans

Agent	Cancer	References
25-hydroxyvitamin D_3	HNSCC	Lathers et al[62]
ATRA	Renal cell carcinoma	Mirza et al[63]
Sildenafil	Multiple myeloma HNSCC	Serafini et al[24]
Sunitinib	Renal cell carcinoma	Ko and colleagues[64,65]
	Transitional cell bladder cancer	Shepard et al[40]
Taxane	Melanoma	Wilcox et al[33]
Gemcitabine Fluropyrimidine	Pancreatic, esophageal cancer	Gabitass et al[66]

ATRA indicates all-trans-retinoic acid; HNSCC, head and neck squamous cell carcinoma; MDSCs, myeloid-derived suppressor cells.

PROBLEMS AND PERSPECTIVES

The literature provides substantial evidence that MDSCs are present in patients with solid and hematologic malignancies. However, one of the major obstacles in the clinical study of MDSCs in cancer patients is the diversity of the cell populations analyzed. Despite this heterogeneity, from a clinical perspective, the most extensive clinical data demonstrating an inverse correlation between MDSC levels and prognosis and cancer clinical stage has involved an early and immature myeloid population ($Lin1^{-/low}$ HLA-DR^- $CD33^+$ $CD11b^+$). Early data showing that patients with high circulating MDSCs were less likely to respond to immunotherapy with high-dose IL-2 also suggest that this may be a useful predictive marker for immune-based cancer therapy. Although these initial studies are interesting, and suggest that MDSCs could be a potential marker correlating clinical outcome and response to therapy, they need larger prospective trials to be validated.

Another important aspect related to MDSC expansion is the comprehension of the essential factors produced by human tumors that control both recruitment of MDSCs from the bone marrow to the tumor site and MDSC activation, which remain largely unexplored. Finally, as MDSC are an attractive target, especially for a combined therapy of cancer, it is undeniable that a greater understanding of the biology of these cells will help to accelerate clinical development of strategies aimed at modulating MDSCs function to enhance the effectiveness of immune-based therapies.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

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