Current Cancer Research

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Advances in Tumor Immunology and Immunotherapy

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Introduction

The first decades of immunotherapy applied to cancer yielded modest and sporadic successes, largely confined to the treatment of a handful of solid tumors such as melanoma, renal cell carcinoma, and bladder cancer, either through the installation of local adjuvants such as BCG or through systemic administration of cytokines such as interferon-alpha and interleukin-2 in pharmacologic doses. Despite a dearth of mechanistic underpinning and immunologic insight, these successes demonstrated the potential power inherent in harnessing the immune system to combat malignant disease, as well as the durability of the responses in the handful of patients in which such responses were observed. Early therapeutic success derived from serendipitous application of newly discovered immune-effector molecules such as high-dose interleukin-2, and insight into underlying mechanisms was lacking. Recent advances have allowed for the application of our evolving understanding of immunologic principles and provided new avenues by which both innate and adaptive immune responses can be harnessed to augment antitumor therapies. There is growing appreciation that the cytolytic CD8+ T-cell, while necessary is but one actor in a complex environment in which tolerance and effector function may coexist and may facilitate or alternatively inhibit tumor growth. Immune response and/or tolerance is shaped from the inception of tumorigenesis by complex interaction between the tumor, its microenvironment, the innate and adaptive immune systems, and immune editing. Antigen processing and presentation, chemokines, cytokines, costimulatory ligands and their receptors, including members of the TNF receptor family, toll-like receptors and their ligands, NK-cells and activating and repressive signals, and a variety of cells with immune regulatory function act in coordinate fashion to shape the ultimate outcome of the encounter between the immune system and tumor. Such immune regulatory function has been ascribed to plasmacytoid dendritic cells, tumor-associated macrophages, myeloid suppressor cells, and T- and B-regulatory cells as well as the tumor cells themselves which may usurp normal cellular mechanisms conferring immune tolerance such as elaboration of TGF-β, and interleukin 10 (IL-10), expression of tolerogenic costimulatory ligands such as PDL-1 and ICOS-ligand, and soluble forms of NKG2D ligand which may serve to tolerize the host.

Despite manifest complexity, recent therapeutic successes leading to the approval of anti CTLA-4 antibody (ipilimumab), and successful targeting of the PD1 pathway in lung cancer, demonstrate the potential inherent in selective manipulation of even a single important pathway in altering the balance between immune tolerance and rejection. While a host of autoimmune phenomena have been encountered as a result of such manipulations, ultimately the increasingly frequently observed therapeutic successes offer real promise that manipulation of such key pathways is feasible and may be used to augment response in a variety of solid and hematologic malignancies.

In this volume leaders in the immune therapy field as well as clinically engaged investigators have summarized selected advances in our understanding of immune suppression and anti-tumor immunity and highlighted promising new approaches which may foretell the next generation of immune interventions. The volume is not meant to be all encompassing; this would not have been possible within the context of a volume of this size. Rather, it seeks to highlight new and evolving approaches and insights which may shape a new generation of immune therapies. The editors have elected to survey territory somewhat less well explored in an effort to take a fresh look at new trends in this rapidly evolving field. These include for example, the role of myeloid suppressor cells in human malignancies and the evolving body of knowledge relevant to the potential role of B-regulatory cells in addition to the better appreciated T-regulatory cell. While most human data regarding B reg function has been amassed in the setting of autoimmune disease, extensive murine studies point to a likely role for B cells in shaping of the human anti-tumor response, an area of emerging study surveyed by Zhang and Rosenblatt in this volume. Novel approaches harnessing potent innate pathways such as those involving biology of heat shock proteins which have already advanced into the clinic are reviewed by Schreiber and Podack who have pioneered the use of gp96 in secreted form now being tested in Phase I/II trials alone, and which will shortly be tested in combination with therapeutic manipulation of adenosinergic tolerizing pathways. Biology and manipulation of natural killer cells is summarized by George Weiner, who has pioneered the manipulation of NK cell biology in relation to therapeutic antibody administration. Drs. Paul Sondel and Lou Weiner extensively review developments in antibody engineering, and early experiences and challenges using bifunctional molecules incorporating both antibody targeting sequences as well as immune effector molecules such as cytokines. These approaches while still in their infancy have been unusually successful in murine models, yet have proven quite difficult to apply in the human setting. Nevertheless, they offer considerable promise and versatility and perspective is provided by leading researchers in the field.

Dr. Eli Gilboa, highlights an unusual new approach to altering the inherent immunogenicity of tumors through manipulation of nonsense RNA editing functions within the cell, an innovative approach which has garnered significant recent attention. The creation of “space” for homeostatic T cell expansion and its utility is summarized by Bernie Fox who has pioneered understanding of this mechanism in relation to clinical immunotherapy. Perhaps nowhere is the complex interplay between tolerance, NK, B, and T cell repopulation more routinely and effectively manipulated than in the setting of allogeneic stem cell transplantation and lessons

learned from decades of preclinical and clinical investigation are summarized in a comprehensive chapter by Lazaros Lekakis and Krishna Komanduri.

The recent successes in the genetic manipulation of T-cell specificities as well as intracellular signaling within T-cells following encounter with tumor cells are highlighted in the two chapters by Zelig Esshar and Aaron Rapoport, pioneers in the development of T cell engineering and redirection of T cell specificity, and their application to hematologic malignancies, respectively. The striking results recently reported to great acclaim by Carl June and colleagues observed in a small number of patients with ALL and CLL following introduction of the CAR-T technology, highlight the considerable promise of the approach.

Renal cell carcinoma and melanoma continue to serve as principal examples of success of immunotherapeutic approaches. The current status of tumor immunotherapeutic approaches in renal cell carcinoma is reviewed by Jaime Merchan, providing perspective in an area in which immune and non-immune approaches are rapidly coalescing to alter prognosis.

Finally, the recent successes using anti CTLA4 antibody and other targets in the TNF receptor family in solid tumors are reviewed from a clinical vantage point as the underlying immunology has been extensively addressed elsewhere. These and other new approaches in the clinic have increased our need for improved means of assessing immune response, and correlation of such response with clinical outcomes are comprehensively reviewed by Theresa Whiteside, a leading authority in clinical immune assessment.

The strong association between the human papilloma virus infection and a subset of human head and neck squamous cell carcinoma suggests that head and neck cancer may be particularly susceptible to immune intervention, and also may afford unique accessibility of tumor for correlative study. Rationale and opportunities for manipulation of the immune response in head and neck cancer are carefully reviewed by Dr. Paolo Serafini and Donald Weed.

This volume could not have been expansive but rather is meant to highlight evolving new areas and critical recent advances in the field. The authors, recognized as leaders in an exciting field have been given free reign of thought and have been encouraged to raise critical questions for future investigation. The editors certainly hope that this volume will be of substantial interest to clinicians as well as basic and translational scientists working in the rapidly moving and exciting field of antitumor immunity.

We owe a special debt of gratitude to my coeditors and our accomplished colleagues who have contributed to this volume. Special thanks to Fiona Sarne, Cancer Research Editor at Springer for her tireless and enthusiastic encouragement and persistence in seeing this volume to completion and to my editorial assistants Zulema Rivero and Angie Monnar for their dedicated efforts. We truly hope you enjoy the volume.

Part I
Immune Activation, Suppression and Manipulation of the Immune Antitumor Response

Myeloid-Derived Suppressor Cells in Cancer

Christos E. Kyriakopoulos, Alberto J. Montero, and Claudia Marcela Diaz-Montero

Abstract Immune evasion is an emerging hallmark of cancer. Many cancers evade the immune system through the overproduction of a wide array of immunosuppressive cells and cytokines, which not only inhibit the host's antitumor immune response, but also hinder the clinical efficacy of immune-based therapies. Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous collection of immature myeloid cells that play an important role in cancer immune evasion. Their presence has been extensively investigated in preclinical models. MDSCs arise from myeloid progenitor cells that have failed to terminally differentiate into mature granulocytes and macrophages and are recruited from the marrow to the tumor microenvironment through production of various cytokines. One of the major obstacles in developing clinical strategies targeting MDSCs in cancer patients has been their heterogeneity in humans, which thus far has prevented determination of an unambiguous phenotype, shared between mice and humans, that has clinical relevance and correlates with their suppressive function. In this chapter we review the current clinical literature on MDSCs in cancer patients, showing that there appear to be two major subsets of MDSCs which are present under different situations. We also discuss the potential use of MDSC as prognostic and predictive markers in cancer patients. Finally, we examine current strategies designed to modulate MDSCs in cancer patients, which represents an innovative and promising approach to enhance the effectiveness of immune-based therapies.

Keywords Myeloid derived suppressor cells • Cancer • Tumor immunology • Cancer immune evasion

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1 Introduction

Myeloid-derived suppressor cells (MDSCs), first described over 30 years ago in patients with cancer, are a heterogeneous population of myeloid cells with the ability to suppress the immune system. The biology of MDSCs in malignant disease has now been more thoroughly characterized as a result of work in preclinical models as well as a more refined understanding of the varied mechanisms by which tumor cells utilize them to evade the immune system. However, advances in clinical research have been hindered by their heterogeneous phenotype in humans, and thus far there is no uniform consensus regarding which is the most clinically relevant phenotype to study. In this chapter we provide an overview of what has been learned about the biology of MDSCs in the setting of cancer from preclinical models, review what has been learned from clinical studies, and discuss pharmacologic strategies to directly modulate MDSCs, as a novel therapeutic approach in oncology.

2 Preclinical Data

2.1 Phenotype

MDSCs constitute a diverse population of cells derived from bone marrow progenitor cells that are at varying stages of differentiation from early myeloid to more granulocytic or monocytic in phenotype. In murine tumor models, MDSCs have been isolated from peripheral blood, spleen, lymph nodes, and tumor sites and are known to have the ability to block both innate and adaptive immunity. MDSC recruitment to the tumor microenvironment is currently thought to be one of the central mechanisms by which tumor cells evade the immune system [1]. Our current understanding from the published literature is that there are two main subtypes of MDSCs with either polymorphonuclear or monocytic characteristics, termed granulocytic and monocytic MDSCs, respectively, each of which employs slightly different mechanisms to suppress antitumor immunity (Fig. 1).

The distinction between the two different phenotypes was initially based on the expression of Ly6G and Ly6C. Granulocytic MDSCs were described as $Ly6G^{+}Ly6C^{low}$, whereas the monocytic subpopulation was described as $Ly6G^{-}Ly6C^{high}$. In terms of their function, the granulocytic MDSCs are known to express high levels of arginase, but not inducible nitric oxide synthetase (iNOS), and have been shown to produce higher levels of reactive oxygen species (ROS). Monocytic MDSCs are known to express both arginase and iNOS but do not produce high levels of ROS [2]. The production of ROS is believed to be important, as this is one mechanism by which granulocytic MDSCs are able to suppress T-cells that are in close proximity through production of high levels of ROS, such as hydrogen peroxide and peroxynitrite, that can induce T-cell apoptosis. The production of ROS could also lead to nitration of tyrosine residues in the T-cell receptor (TCR)

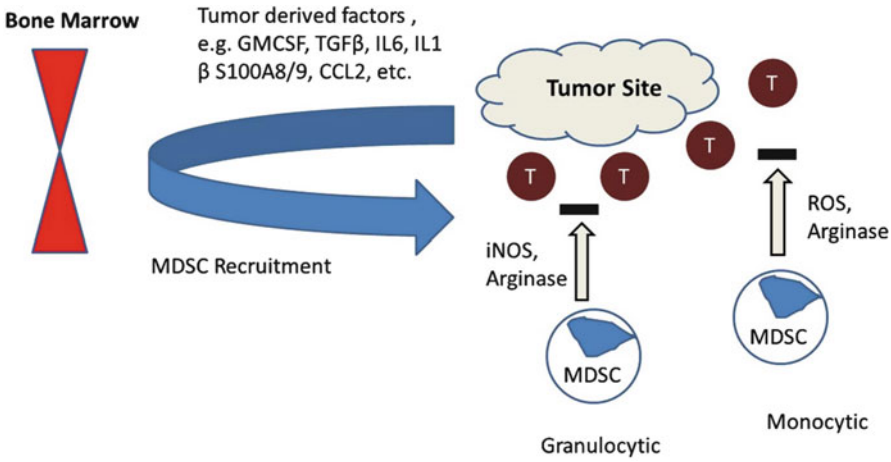

Fig. 1 Schematic of tumor-induced mobilization of MDSCs

during direct cell–cell contact which renders it unable to bind to antigen, thus blocking their activation [3].

Further classification of those cells in mice was based on the intensity of Gr-1 expression [4] which is associated with specific functional traits [5]. Monocytic MDSCs have been described as $CD11b^{+}/Gr\text{-}1^{int/low}$ and are capable of constantly suppressing the $CD8^{+}$ T-cell activation in tumor-bearing mice [6]. These cells show high expression of IL-4Rα when compared to granulocytic MDSCs, and their activity appears to be driven by tumor-secreted GM-CSF [6] and by IFN-γ released from T lymphocytes [7]. Granulocytic MDSCs have been described phenotypically as $CD11b^{+}/Gr\text{-}1^{high}$ and exert limited immune suppression in some tumor models and only when present in high numbers [6]. Although they require GM-CSF secretion in order to expand, they do not appear to respond when GM-CSF is given externally [6] since GM-CSF is a required but not a sufficient factor for their maturation [8].

2.2 Expansion and Activation of MDSCs in Tumor Models

In tumor-bearing mice, expansion and activation of MDSCs are controlled by several factors released by tumor cells, the surrounding stroma, and/or the immune system. Factors released from the tumors mostly induce MDSC proliferation through the stimulation of myelopoiesis and inhibition of their differentiation, whereas factors released from the tumor stroma or the immune system directly impact on their activation.

The majority of these tumor-derived factors are growth factors, cytokines, or chemokines and trigger different signaling pathways on MDSCs that are mainly mediated through the signal transducer and activator of transcription (STAT) family

of transcriptional factors [9]. The activation of STAT3 is known to lead to prolonged survival and increased proliferation of MDSCs through the induction and upregulation of genes that control proliferation and apoptosis, such as MYC, BCL-XL, and cyclin D1 [9]. Also, it is primarily through both STAT 3 and NADPH that ROS are overproduced in granulocytic MDSCs as well.

There are also more complex and interrelated chemokine and cytokine networks between tumor cells, stroma, and immune cells that ultimately lead to MDSC recruitment and activation, a process that is required before the MDSCs can exert their immunosuppressive activity. Those factors include IFNγ, ligands for Toll-like receptors, IL-4, IL-3, and TGFβ, among others [9].

2.3 Mechanisms of Immunosuppression of MDSCs in Cancer

MDSCs mediate immune suppression through various metabolic pathways and direct cell-to-cell contact. Even though most of the functional studies have been conducted in the preclinical setting, there is an increasing body of evidence supporting the notion that similar mechanisms are also involved in humans.

2.3.1 Metabolism of L-Arginine

While both granulocytic and monocytic MDSCs utilize a variety of mechanisms to suppress tumor immunity, both are known to utilize a strategy that involves depletion of an amino acid in the tumor microenvironment that is important for proper T-cell function. MDSCs produce high intracellular levels of arginase, the enzyme that catabolizes L-arginine. L-arginine is a semi-essential amino acid, and is fundamental for proper T-cell function. L-arginine serves as a substrate for two different enzymes implicated in MDSC-induced immunosuppression, arginase 1 and iNOS. Like most cells, both MDSCs and T-cells need L-arginine for protein synthesis, but as a direct consequence of MDSCs having high intracellular arginase levels, they need to import excess arginine through their CAT-2B transporter. This results in L-arginine depletion from the microenvironment which leads T-cells to cell cycle arrest [10].

Arginase 1 secretion by murine MDSCs is modulated by several cytokines such as IL-4, IL-13, TGF-β, and GM-CSF [11]. Arginase 1 metabolizes L-arginine to L-ornithine and urea, thus depleting L-arginine from the tumor microenvironment. The exact mechanism of inhibition of T-cell proliferation through L-arginine depletion is still unclear; however different potential mechanisms have been postulated. One possible mechanism that has also been observed in humans is that depletion of L-arginine may lead to decreased expression of CD3 ζ-chain of the T-cell receptor, thereby interfering with their function [12]. Furthermore L-arginine depletion prevents T-cell upregulation by cyclin D3- and cyclin-dependent kinase 4 [13]. In addition, increased expression of arginase 1 by MDSCs in a lymphoma mouse model has been shown to induce antigen-specific tolerance through recruitment and expansion of regulatory T-cells (T_{reg}) [14].

2.3.2 ROS and Peroxynitrite

ROS are another important mechanism by which MDSCs can directly suppress T-cells. High levels of ROS, mainly H_2O_2, have been found at sites heavily infiltrated by MDSCS in both cancer patients and animal models [4, 15–18]. ROS production is mainly regulated by NADPH oxidase (NOX2) whose expression is regulated by STAT3 [15]. The exact mechanism of immunosuppression triggered by ROS is not fully elucidated; however, it has been shown that high levels of ROS correlate with either impaired dendritic cell maturation [19] or decreased CD3ζ-chain expression of the T-cell receptor and thus diminished T-cell proliferation and cytokine production [20]. These immunosuppressive properties have only been observed in granulocytic MDSCs [4, 15], and they were abrogated by eliminating ROS [15, 17].

In addition to ROS, peroxynitrite, which in vivo has been ascribed to the reaction of the free radical superoxide with the free radical nitric oxide (NO), is a powerful prooxidant that has emerged as a crucial mediator of MDSC-related suppression of T-cell function. In both cancer patients and tumor models increased levels of peroxynitrite accumulate in areas of tumor progression [21–25]. Even though the immunosuppressive properties of peroxynitrite are not fully understood, it has been shown that it promotes apoptosis of T-cells [26] and alteration of their function [3]. In the latter, nitration of tyrosine residues in the T-cell receptor–CD8 complex by MDSCs, through ROS and peroxynitrite production, resulted in marked decrease in the binding of specific peptide-major histocompatibility complex (pMHC) to the $CD8^+$ T-cells and thus resulted in T-cell tolerance.

3 Clinical Data

Since the initial identification and description of MDSCs, in the preclinical literature, there have been many studies in cancer patients with solid and hematologic malignancies that have evaluated the presence and clinical significance of MDSCs (Table 1). One of the main challenges has been the absence of a universally accepted clinical definition of MDSCs. This is due to their highly heterogeneous nature and also in part due to the absence of the cognate Gr-1 molecule in humans [1].

One of the first published clinical studies that evaluated the presence of MDSCs in cancer patients was in the tumor of patients with head and neck cancer, mostly squamous histology ($n=18$) [51]. This study reported the presence of intra-tumoral $CD34^+$ myeloid cells that were significantly correlated ($r^2=0.65$) with levels of secreted GM-CSF in tumor fragments. Moreover, depletion of $CD34^+$ cells by immunomagnetic separation was associated with a reversal of T-cell suppression, evidenced by increased IL-2 production from intra-tumoral lymphocytes. A subsequent study [27] analyzed peripheral blood samples from patients with HNSCC, NSCLC, and breast cancer of unknown clinical stages ($n=44$) that identified a population of immature myeloid cells (ImC). These cells were described as lineage negative (Lin^-), defined here as $CD3^-$, $CD14^-$, $CD19^-$, and $CD57^-$. The immunosuppressive properties of

Table 1 Heterogeneity of MDSC phenotypes utilized in clinical studies

Phenotype	Cancer type	References
Lin^{-} [a]/$HLA\text{-}DR^{-}$	Breast	[27]
	HNSCC	
	NSCLC	
$CD15^{+}$ granulocytes	Breast	[20]
	Colon	
	Pancreatic	
$CD11b^{+}/CD14^{-}/CD15^{+}$	Renal cell	[28]
$CD14^{+}$/arginase^{+}	HNSCC	[29]
	MM	
$CD14^{+}/HLA\text{-}DR^{-/low}$	Melanoma	[30]
$CD11b^{+}/CD33^{+}$	NSCLC	[31]
$Lin1^{-/low}$ [b]$/HLA\text{-}DR^{-}/CD33^{+}/CD11b^{+}$	Multiple solid tumors (breast, esophageal, gastric, colorectal, and other solid malignancies)	[32–34]
Lin^{-}[c]$/HLA\text{-}DR^{-}/CD33^{+}$	Melanoma	[35]
$CD11b^{+}/CD14^{-}/CD33^{+}/CD15^{+}$	NSCLC	[36, 37]
$CD14^{+}/IL\text{-}4Ra^{+}$	Colon	[38]
	Melanoma	
$CD11b^{+}/CD13^{+}/CD34^{+}/CD14^{-}/CD45^{+}$	Hodgkin lymphoma	[39]
$CD14^{+}/HLA\text{-}DR^{-/low}$	Melanoma	[40]
DC-Sign^{+}/ $CD80^{+}/CD83^{+}$		
$CD14^{+}/CD15^{+}$/ $CD33^{+}/HLA\text{-}DR^{-}$	Bladder	[41]
$CD14^{+}/HLA\text{-}DR^{-/low}$	MM	[42]
	MGUS	[43]
	NHL	[44, 45]
	HCC	
$SSC^{high}/CD66b^{+}/CD125^{-}/CD33^{+}/HLA\text{-}DR^{-}$	Urothelial tract	[46]
	HNSCC	
	NSCLC	
$CD34^{+}/CD45^{+}/CD116^{+}/CD13^{+}/CD14^{-}$	NHL	[47]
$CD11b^{+}/CD15^{high}/CD33^{low}$	Bladder	[48]
Lin^{-}[b]$/HLA\text{-}DR^{-}/CD33^{+}$	Multiple solid tumors	[49]
$CD14^{+}/HLA\text{-}DR^{low/-}$	Prostate	[50]

[a]Lineage defined as -CD3, -CD14, -CD19, and -CD57

[b]Lineage-1 defined as-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56; HNSCC: head and neck squamous cell carcinoma; NSCLC: non-small-cell lung cancer; MDS: myelodysplastic syndrome; MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; NHL: non-Hodgkin lymphoma

[c]Lineage defined as -CD3, -CD14, -CD19, and -CD56

those cells were confirmed by restoration of the ability of the dendritic cells to stimulate allogeneic T-cells in vitro when the ImC were depleted.

The next major study of MDSCs in cancer patients described a more mature granulocytic population of circulating cells with T-cell immunosuppressive properties in metastatic renal cell carcinoma (RCC) patients [28]. In this study, peripheral blood levels of granulocytic cells ($CD11b^{+}$, $CD14^{+}$, and $CD15^{+}$) in patients without

previous treatment ($n=123$) were found to be significantly higher ($p=0.037$) than in healthy controls ($n=33$). Additional phenotypic characterization of this population revealed negative expression of CD11a, CD80, CD83, CD86, and HLA-DR and increased arginase activity. A subsequent study in patients with metastatic RCC ($n=27$) confirmed the presence of a granulocytic population of MDSCs that were $CD11b^+/CD15^+/CD66b^+$ and $CD14^-/CD16^{low}/CD62L^{low}$ [52].

To address the question of whether MDSCs aberrantly accumulate in cancer patients with a variety of different malignancies and whether levels in circulation were proportional to clinical stage, a subsequent study [33] by Diaz-Montero et al. prospectively evaluated MDSC levels in patients ($n=106$) with newly diagnosed solid tumors of various clinical stages. Approximately 50 % of patients had breast cancer, followed by 30 % of patients with gastrointestinal cancers and 20 % of various other types of cancer. In that study MDSCs were defined as a population of cells that were $Lin1^{-/low}/HLA\text{-}DR^-/CD33^+/CD11b^+$. Lineage-1 here was a cocktail of antibodies against CD3, CD14, CD16, CD19, CD20, and CD56. Overall circulating levels of MDSCs were significantly higher in patients with cancer ($P<0.0001$) compared to a cohort of matched healthy individuals ($n=21$). Furthermore, levels of circulating MDSCs were directly proportional to clinical stage of disease, with the highest overall numbers in patients with stage IV disease compared to patients with stage I/II disease ($P<0.0001$). Levels in patients with advanced metastatic disease also appeared to be highest among patients experiencing extensive metastatic burden.

Another study [48] examined the presence of two distinct populations of MDSCs in patients with superficial noninvasive and invasive bladder cancer. Both peripheral blood and fresh tumor samples were collected and analyzed by flow cytometry. Two different circulating MDSC populations were described: (1) $CD11b^+/CD15^{high}/CD33^{low}$ with co-expression of the neutrophil markers CD114 and CD117; and (2) $CD11b^+/CD15^{low}/CD33^{high}$ with co-expression of the monocyte–macrophage markers CD14, CD115, CD116, and CCR2. When circulating levels were compared, only the population of $CD11b^+/CD15^{high}/CD33^{low}$ cells were found to be present in higher levels in bladder cancer patients, whereas the $CD11b^+/CD15^{low}/CD33^{high}$ population was also found to be present in significant amounts in healthy individuals. Only the $CD11b^+/CD15^{high}/CD33^{low}$ population was noted to have immunosuppressive activity. Additionally, two distinct MDSC populations were found to infiltrate the tumors: 60–70 % of those cells were described as $CD11b^+/HLA\text{-}DR^+$ with the remaining 30–40 % described as $CD11b^+$ and $CD15^+$. The clinical significance of those cells though was not fully explored.

In summary, MDSCs in cancer patients consist of (1) 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, and (2) a more differentiated granulocytic population having the opposite pattern of expression, i.e., $CD15^+$ and $CD14^-$.

Despite the fact that immune evasion is an emerging hallmark of cancer, there is a clear paucity of validated immune related biomarkers that are known to correlate

with prognosis and clinical outcome. In the setting of breast cancer, the most established and validated prognostic markers are tumor related, for example HER-2/neu gene amplification, hormone receptor status, tumor histologic grade, and circulating tumor cells [53]. However, recent comprehensive microarray analyses have validated immune gene signatures as valuable prognostic indicators in localized breast cancer and other solid tumors [54, 55]. MDSCs are clearly an important mechanism of tumor-mediated immune evasion, but thus far there are few published studies that have explored in detail the overall prognostic or predictive significance of MDSCs in cancer patients. Even if we put aside the issue of how to best define MDSCs, very few studies have fully addressed the clinical implications of circulating MDSCs. To the best of our knowledge, only three published studies have shown that overall levels of a monocytic population of MDSCs ($Lin1^{-/low}$/$HLADR^{-}$/$CD33^{+}$/$CD11b^{+}$) in the peripheral blood correlate with clinical stage [32–34]. Another study reported MDSC levels in NHL patients correlated with clinical cancer stage and aggressiveness of disease; however a different phenotype was utilized ($CD14^{+}$/HLA-$DR^{-/low}$) [43]. Moreover, two studies [32, 34] have independently shown that in patients with advanced breast cancer and gastrointestinal malignancies, higher MDSC levels were associated with poorer overall survival times. In the study by Solito et al. 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 months [95 % confidence interval (CI), 0.5–11.3] and 19.32 months (95 % CI, 8.7–infinity), respectively ($P<0.048$) [32]. Similarly, in the study by Gabitass et al., 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 [34]. Patients with elevated MDSCs (>2 %) were found to have an overall poorer prognosis, with a median OS of only 4.6 months (95 % CI, 2.2–6.0), relative to a median OS of 9.3 months (95 % CI, 6.3–12.1) ($P<0.001$), in patients with circulating MDSCs <2 %. Although these studies were retrospective in nature and involved relatively small number of patients, they provided important initial data using a similar MDSC phenotype, i.e., $Lin1^{-/low}$/HLA-DR^{-}/$CD33^{+}$/$CD11b^{+}$, on the prognostic significance of MDSCs. It is presently unknown whether blood MDSC levels are an independent prognostic factor in different cancers; future appropriately powered prospective studies are needed to address this.

4 Pharmacologic Modulation of MDSCs

The myriad strategies utilized by MDSCs to promote evasion of the immune system represent major hurdles for the clinical success of any type of cancer immunotherapy. Moreover, recruitment of MDSCs to pre-metastatic niches appears to be an early event in the development of metastatic disease. Several drugs known to pharmacologically modulate MDSCs have been tested clinically and can be classified into at least three different categories: (1) drugs that decrease MDSCs through

Table 2 Drugs known to modulate MDSCs

Agent	Cancer type	References
25-Hydroxyvitamin D3	HNSCC	[56]
ATRA	Renal cell carcinoma	[57, 58]
	Breast cancer	[59]
	Sarcoma	
Nitroaspirin	Colon cancer	[60]
Sildenafil	HNSCC	[29]
	Multiple myeloma	
Sunitinib	Renal cell carcinoma	[61]
	Transitional cell bladder cancer	[41]
Taxane	Melanoma	[62]
Gemcitabine	Pancreatic and esophageal cancer	[63]
Fluropyrimidine		
Gemcitabine	Breast cancer	[64]
5-Fluorouracil	Thymoma	[65]
Triterpenoid	Multiple solid tumors (colon, lung, thymoma, renal cell, sarcoma)	[66]
Celecoxib	Mesothelioma	[67]

promotion of cell differentiation; (2) drugs that modulate one or more different immunosuppressive mechanisms of MDSCs, without affecting overall levels; and (3) non-differentiating agents that decrease MDSCs levels, through decreasing their recruitment or production in the bone marrow (Table 2).

Two agents that have been shown to promote the differentiation of MDSCs include 25-hydrooxyvitamin D3 and all-trans-retinoic acid (ATRA). Treatment of locally advanced or metastatic head and neck squamous cell carcinoma (HNSCC) patients with 25-hydrooxyvitamin D3 resulted in a decrease of $CD34^+$ suppressive cells and an increase in the frequency of $HLA\text{-}DR^+$ cells, increased plasma levels of IL-12 and IFN-γ, and improved T-cell proliferation [56]. However, the small nature of this study prevented the determination of any clinical correlates.

ATRA was initially found to promote the in vivo differentiation of $Gr\text{-}1^+CD11b^+$ MDSCs into mature dendritic cells, macrophages, and granulocytes, thereby improving T-cell-mediated immune response in fibrosarcoma and mammary adenocarcinoma mouse models [59]. Further vaccination of the pretreated animals with two different types of cancer vaccines resulted in a prolonged antitumor effect through immune-mediated mechanisms.

Subsequent testing of ATRA in metastatic renal cell carcinoma patients with subcutaneous IL-2 revealed decreased number of $Lin^-/HLA\text{-}DR^-/CD33^+$ MDSCs, improved myeloid/lymphoid dendritic cell ratios, and was associated with an improvement in antigen-specific T-cell responses as measured by stimulation with tetanus-toxoid [57]. Similar results were observed when ATRA was used in patients with stage III–IV renal cell carcinoma [58].

Several drugs have been shown to modulate the immunosuppressive properties of MDSCs both in vivo and in vitro without affecting their overall accumulation.