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Review

The Role of Myeloid Derived Suppressor Cells in Urothelial Carcinoma Immunotherapy

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Abstract. Myeloid derived suppressor cells (MDSC) are immune cells that dampen immune responses. In patients with cancer, MDSC are associated with adverse oncologic outcomes and therapeutic resistance. Pre-clinical evidence suggests that MDSC suppress anti-tumor immune responses. In this report, the biologic functions of MDSC are defined and evidence linking MDSC with the response to cancer immunotherapies in solid tumors are reviewed. Associations of MDSC in clinical bladder cancer cohorts are outlined in addition to evaluation of the suggested roles of MDSC in pre-clinical bladder cancer models. Human clinical trials that investigate possible MDSC modulators are highlighted, and therapeutic strategies to leverage MDSC biology in bladder cancer immunotherapy are outlined.

INTRODUCTION

Developing effective systemic therapy for bladder cancer continues to present a challenge to oncology physicians and researchers. Cisplatin-based chemotherapy has been the best option for decades, but only 50% of patients benefit in the form of objective responses, and just 13–25% experience a complete response [1]. For those who are ineligible for cisplatin-based regimens or experience progression of disease, in 2016–2017 the United States Food and Drug Administration approved five monoclonal antibodies that achieve immune checkpoint blockade by targeting the programmed cell death protein-1/programmed death-ligand 1 (PD-1/PD-L1) pathway. Immune checkpoint blockade can lead to durable complete responses for some patients, but overall objective response rates are only 15–31% [2–4].

An immune cell in the tumor microenvironment that may be important for inhibiting the immune response against bladder cancer is the myeloid derived suppressor cell (MDSC). This review summarizes what is currently known about MDSC function, known roles of MDSC in cancer, and

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how MDSC have been implicated in bladder cancer prognosis and in the context of different bladder cancer therapies. Completed and ongoing clinical trials that have evaluated potential MDSC-modifying therapeutics are highlighted. Finally, knowledge gaps and areas for advancement in the study of MDSC to enhance bladder cancer immunotherapy are presented.

OVERVIEW OF MDSC FUNCTION

Myeloid derived suppressor cells (MDSC) dampen immune responses. From a physiological standpoint, MDSC can be thought of as effectors of a homeostatic mechanism that regulate T cell-mediated inflammatory responses to pathogens [5]. In mice, MDSC can be identified by species-specific cell surface markers ($CD11b^+Gr\text{-}1^+$) and may be further classified as monocytic (M-MDSC, $Ly\text{-}6C^{hi}$) or granulocytic (G-MDSC, $Ly\text{-}6G^+$) based on additional cell surface markers [6]. MDSC are derived from monocyte, macrophage and dendritic cell progenitors (M-MDSC); or neutrophil, eosinophil, basophil and mast cell progenitors (G-MDSC) [5, 7]. G-MDSC are also referred to as polymorphonuclear (PMN)-MDSC, which differ from conventional neutrophils via expression of lectin-type oxidized LDL receptor 1 (LOX-1), which inhibits T cell proliferation [7, 8]. Intratumoral M-MDSC appear to differentiate into immune suppressive tumor associated macrophages (TAM) in response to tumor-hypoxia mediated STAT3 signaling [9]. In humans, MDSC can be identified with different cell surface markers: $CD33^+$ or $CD11b^+$, HLA^-DR^{low} and $(LIN)^-$. (LIN^- refers to cells negative for lineage markers CD3, CD19, CD56 and CD13) [6]. Additionally, human M-MDSC are $CD14^+CD15^-$ with stronger CD33 positivity than PMN-MDSC; PMN-MDSC are also $CD66b^+/CD14^{-/dim}CD15^+$ with dim CD33 expression [10].

MDSC are triggered by chronic inflammatory stimuli such as chronic infection or malignancy, [11] which is why circulating MDSC are often found to be elevated in patients with a variety of cancer types. MDSC act in multiple ways to suppress T cell function. They are capable of producing reactive oxygen species (ROS), arginase-1, nitric oxide (NO), prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO), inhibitory cytokines such as IL-10 and TGFβ, [5, 12] and inhibiting the ability of T cells to respond normally to IFNα- and IFNγ–mediated stimulation [13].

ROLE OF MDSC IN BENIGN CONDITIONS

Immunosuppression mediated by MDSC has been found to be important to physiological processes and benign conditions. For example, MDSC have been suggested to play an important role during human pregnancy. PMN-MDSC capable of suppressing T cell proliferation are elevated in the peripheral blood of pregnant women, suggesting a role in maternal-fetal tolerance [14]. In addition, MDSC derived from human placenta have also been shown to be capable of polarizing CD4+ T cells toward a Th2 cytokine response, which is thought to promote maternal tolerance [15]. MDSC may also be elevated near the end of life [16]. Verschoor et al. found significantly higher levels of circulating $CD11b^+CD15^+$ PMN-MDSC in in a cohort of frail elderly individuals, as compared to younger adults [17].

Obesity has been characterized as a pro-inflammatory state, [18] and pre-clinical evidence implicates obesity as a stimulus for MDSC generation. Clements et al. using the BALB/c and C57BL/6 murine models, discovered that mice fed a high fat diet had substantial elevations in $Gr\text{-}1^+CD11b^+$ MDSC, and that fatty diet induced MDSC were also required for somatic fat accumulation [19].

MDSC are also likely to play important functions in the biology of autoimmune disease, organ transplant tolerance and immunodeficiencies. For example, Crook et al. studying a murine model of rheumatoid arthritis, found that adoptive transfer of MDSC from subjects with moderate arthritis could improve the condition of mice prone to severe arthritis [20]. Meng et al. analyzed a cohort of patients with T cell mediated renal transplant rejection, and found that higher levels of circulating $CD33^+HLA\text{-}DR^-$ MDSC were strongly associated with increased graft function, which is consistent with the function of MDSC to suppress effector T cell function [21]. Murine studies have suggested that the ability of MDSC to delay allograft rejection depends on deficient Smad3 signaling (which is part of the TGFβ pathway) [22]. Patients with primary or secondary inflammatory disorders such as common variable immunodeficiency [23] and early Alzheimer's disease [24] have also been found to have elevated circulating MDSC.

The study of MDSC has several practical challenges [25]. Immunohistochemistry markers to identify human MDSC in formalin fixed paraffin embedded tissues are lacking. Though their surface ligand-based classification is well-defined, the gating

of HLA-DR$^{low/neg}$ populations during flow cytometry analysis can be subjective. Since PMN-MDSC may not withstand freezing and thawing, analysis of this population may only be valid on freshly collected sampled. Therefore MDSC-based biomarker discovery efforts necessitate strict adherence to a clearly stated protocol that should be reproducible.

ROLE OF MDSC IN SOLID TUMORS

MDSC are pro-tumorigenic, and cancers appear to promote the differentiation of myeloid progenitors into MDSC. Both PMN-MDSC and M-MDSC, through the mechanisms detailed above, suppress anti-tumor immune activity. Specifically, MDSC inhibit cytotoxic T cells and natural killer cells and promote the expansion of regulatory T cells [26] Additionally, Ortiz et al. found that MDSC promote melanoma carcinogenesis in a murine carcinogen model via specific recruitment of IL-17 producing CD4$^+$ T cells [27]. In turn, the mechanisms by which MDSC are induced by cancers include tumor-derived growth factors (such as GM-CSF), tumor stroma-produced cytokines (such as IL-6) and hypoxia [9, 28].

As a quantitative biomarker, MDSC are an adverse prognostic factor in cancer patients. Markowitz et al. studying a cohort of patients with pancreatic adenocarcinoma, found that patients with progressive disease had higher levels of circulating CD33$^+$HLA-DR^{neg} MDSC [29]. A 2016 meta-analysis performed by Zhang et al. combined 442 patients with different types of solid tumors (including hepatocellular carcinoma, melanoma and colorectal cancer) and evaluated the data regarding circulating MDSC levels and overall survival. They demonstrated that MDSC quantity is an adverse prognostic factor, as patients with elevated MDSC levels exhibited a significantly increased hazard of death from any cause [30]. A report analyzing a large cohort of patients with breast cancer also associated higher circulating MDSC levels with worse overall survival [31]. Li et al. studied a clinical cohort of ovarian cancer patients and reported that users of the anti-diabetic biguanide drug metformin had greater overall survival compared to non-users; performing *in vitro* studies of MDSC isolated from ovarian cancer patients, they demonstrated that metformin inhibited MDSC signaling via the AMKα/HIF1α pathway [32].

MDSC may also be predictive of response to therapy. Kitano et al. reported a novel method of quantifying MDSC via a computational algorithm that reproducibly classified CD11b$^+$CD14$^+$ MDSC as HLA-DR$^{low/neg}$, and found that low MDSC levels were associated with longer overall survival among a pooled clinical trial-based cohort of melanoma patients treated with the anti-CTLA-4 monoclonal antibody ipilimumab [33]. Weber et al. found that high circulating MDSC levels (defined as >12.6% of CD14$^+$CD11b$^+$HLA-DR^{low} cells among viable peripheral blood mononuclear cells) was associated with substantially increased overall survival in ipilimumab-refractory melanoma patients treated with nivolumab [34].

Just as MDSC are related to prognosis among patients with many different tumor types, MDSC themselves may be modulated/inhibited by tumor-directed therapies. Elements that drive MDSC development include endoplasmic reticulum stress and the transcription factors STAT3, IRF8 and C/EBPβ [7, 8]. Cancer therapies that are thought to modulate MDSC include tyrosine kinase inhibitors (TKI) such as sunitinib [35–39] and sorafenib; [40–44] vascular endothelial growth factor (VEGF) inhibitors such as bevacizumab; [45] mammalian target of rapamycin (mTOR) inhibitors; [46–50] deacetylase (HDAC) inhibitors; [51, 52] fibroblast growth factor receptor (FGFR) inhibitors; [48, 53] chemotherapeutic agents such as gemcitabine, [54] 5-fluorouracil (5-FU), [55–58] and cisplatin; [59] and radiation therapy [39, 60]. Clinical trials in bladder cancer that have investigated these agents are presented in Table S1.

EVIDENCE SUGGESTING TARGETING MDSC CAN ENHANCE CANCER IMMUNOTHERAPY

In addition to playing roles in carcinogenesis and conferring adverse oncologic outcomes, MDSC are also thought to underlie resistance to different types of cancer therapies. Several investigators have shown that the therapeutic efficacy of anti-PD-(L)1 or anti-CTLA-4 immune checkpoint blockade can be meaningfully increased in pre-clinical models that employ an MDSC-inhibiting strategy. MDSC targeting approaches in these studies have included histamine [61] TGFβ inhibition, [62] phenformin (an anti-diabetic biguanide) [63] CXCR2 inhibition, [64] sorafenib, [41] all-trans-retinoic-acid (ATRA), [65] ibrutinib (an inhibitor of the Bruton's tyrosine kinase pathway in MDSC), [66] inhibition of

CSF-1R, [67] PI3K inhibition, [68, 69] entinostat (a HDAC inhibitor), [52] bromodomain inhibition, [70, 71] CCRK inhibition, [72] and activation of Liver-X receptors (LXR) [73]. These agents, along with the disease settings and/or models in which they were investigated, as well as applicable immunotherapies with which they were evaluated, are summarized in Table 1.

CORRELATIVE AND FUNCTIONAL CHARACTERIZATION OF MDSC IN BLADDER CANCER

Early pre-clinical evidence implicating a role for MDSC in the progression of bladder cancer was demonstrated by Eruslanov et al. who showed the SW780 bladder cancer xenografts in nu/nu mice were infiltrated with $CD11b^{+}Ly6C^{+}$ MDSC [74]. The authors also identified prostaglandin E2 (PGE2) produced from cancer cells as a factor that promoted the differentiation of myeloid progenitors to MDSC, which had been previously observed in a different (4T1) tumor model by Sinha et al. [75]. Prima et al. further defined the role of PGE2 in bladder cancer-associated MDSC. Studying murine MBT2 bladder cancer cells co-cultured with bone marrow cells, the authors found that tumor cells induced PD-L1 expression specifically on MDSC and tumor-associated macrophages, and that this PD-L1 expression was dependent on COX2 and PGE2 signaling [76].

Additional clinical evidence demonstrating the presence of MDSC within bladder cancers was shown by Brandau et al. in 2011 [77]. Among a cohort of patient with different tumor types, 16 patients with urothelial cancers were found to have elevated circulating quantities of $CD33^{+}$HLD-DR^{-} MDSC, which were found to also have the capacity to inhibit T cell proliferation and IFNγ production from patient-derived T cells [77]. Adding insight into the suppressive mechanisms of MDSC in bladder cancer, Yuan et al. showed that the ability of bladder cancer patient-derived $CD14^{+}$HLA-$DR^{-/low}$ MDSC to decrease peripheral blood mononuclear cell-mediated IFNγ production could be reversed by supplementation with L-arginine or anti-TGFβ. This finding demonstrated the potential importance of canonical MDSC mechanisms (arginase, TGF-β secretion) in bladder cancer [78].

Zamanian-Daryoush showed in an immune competent MB49 model of bladder cancer that subcutaneous allograft growth was significantly diminished in the setting of myeloid-specific conditional knockout of the ABCA1 cholesterol transporter, [79] suggesting lipoprotein metabolism as a determinant of MDSC tumor-promoting function. Zhang and Chin, studying a murine MB49 orthotopic model of bladder cancer, found that transgenic mice deficient for the kinase Rip2 had tumors that were much larger, highly infiltrated with MDSC and, compared with Rip2-competent subjects, had higher intratumoral levels of G-CSF. Thus Rip2 may be part of a signaling axis necessary for MDSC recruitment [80].

MDSC may also inhibit the adaptive arm of the tumor immune response in bladder cancer. Bennet et al. found in a 1978 report that bone-marrow-derived 'natural suppressor cells' from BCG-immunized mice could inhibit cell-mediated immunity against allogeneic tumor cells [81]. Smith et al. studied a murine MB49 orthtopic model of bladder cancer and found that one of the correlates of successful treatment and tumor immunity induced by an IL-12 based intravesical treatment was decreased MDSC in the bladder [82].

MDSC AND BLADDER CANCER STAGE

Several studies indicate that MDSC quantities are directly associated with increasing stage in bladder cancer patients. Initially, Eruslanov et al. made the qualitative observation that MDSC, paradoxically, were more highly infiltrative into non-muscle invasive bladder cancers than invasive bladder cancers [83]. However, the strength of these results was limited by small sample size. In a different study that analyzed 113 bladder cancer patients, Yang et al. found that $CD11b^{+}CD33^{low}$HLA-DR^{-} circulating MDSC quantities were higher in patients harboring high grade malignancies ($p = 0.009$) and in those with high stage disease (pT2-4, $p < 0.0001$) [84]. Among a contemporary cohort of 36 patients with invasive localized bladder cancer undergoing neoadjuvant chemotherapy (predominantly cisplatin-based) followed by radical cystectomy, the quantity of circulating $CD33^{+}$HLA-DR^{-} MDSC was significantly lower among patients who were found to be complete responders to neoadjuvant therapy (defined as stage pT0N0 at radical cystectomy) [85].

MDSC AND BLADDER CANCER PROGNOSIS

MDSC are directly associated with adverse oncologic outcomes in clinical bladder cancer cohorts.

Table 1
Pre-clinical development of MDSC-targeting agents that may enhance cancer immunotherapy

Agent	Target/Mechanism	Setting	Evaluated in combination with
Histamine [61]	Myeloid cell NADPH oxidase (NOX2)	EL-4 (lymphoma), 4T1 (breast), MC38 (colon) [murine]	Anti-PD-1
Anti-PD-L1			
TGF-βR Inhibitor [62]	TGF-β	EC109 esophageal squamous cell carcinoma [murine]	Anti-PD-1
Phenformin [63]	Biguanide (mitochondrial complex I)	BP01 melanoma [murine]	Anti-PD-1
Anti-CXCR2 mAb [64]	CXCR2	M3-9-M rhabdomyosarcoma [murine]	Anti-PD-1
Sorafenib [41]	Tyrosine kinase inhibitor (VEGFR, PDGFR, c-kit)	RENCA (renal cell carcinoma) [murine]	Anti-CTLA-4
All-trans-retinoic acid (ATRA) [65]	Decrease in circulating MDSC	Melanoma [human]	Anti-CTLA-4
Ibrutinib [66]	Bruton's tyrosine kinase, IL-2-inducible T cell kinase	EMT6 (breast), 4T1 (breast) [murine]	Anti-PD-L1
Anti-B7-H3 mAb [67]	Decrease in circulating and intra-tumoral MDSC	*Tgfbr1/Pten* 2cKO Head and neck squamous cell carcinoma [murine]	
IPI-145 [68]	PI3Kδ, PI3Kγ	MOC Head and neck squamous cell carcinoma [murine]	Anti-PD-L1
IPI-549 [69]	PI3Kγ (polarization of tumor associated myeloid cells from M2 to M1 phenotype)	4T1 (breast), B16 (melanoma), B16-GMCSF (melanoma) [murine]	Anti-PD-1 +/– Anti-CTLA-4
Eninostat [52]	Class I HDACs (inhibiting PMN-MDSC differentiation and function)	RENCA (renal cell carcinoma), LLC (lung carcinoma) [murine]	Anti-PD-1
JQ1 [70]	Bromodomain proteins (BRD2, BRD4, BRD9)	AB1 Malignant mesothelioma [murine]	
PLX51107 [71]	Bromodomain proteins, Myc	EMT6 (breast) [murine]	Anti-CTLA-4
CCRK KO [72]	Inhibition of CCRK-mediated IL-6 (MDSC promoting cytokine)	Hepa1-6 (hepatocellular carcinoma) [murine]	Anti-PD-L1
RGX-104 [73]	Liver-X receptor (LXR)/ApoE agonist	Multiple tumor types [murine]	Anti-PD-1, Adoptive T cell transfer, Gvax

Controlling for clinical and pathologic variables in a multivariable Cox proportional hazards analyses, multiple groups have found that a high quantity of MDSC (either circulating or infiltrating the tumor) is significantly associated with a higher hazard of death [84, 86]. The previously referenced study by Ornstein et al. while strictly describing an association of MDSC quantity with pathologic stage after neoadjuvant chemotherapy [85] nevertheless provides an additional indicator that MDSC may be prognostic due to the fact that response to neoadjuvant chemotherapy is a well-validated predictor of favorable survival after radical cystectomy [87]. Recently, Tzeng et al. analyzed a cohort of 41 patients with metastatic urothelial carcinoma treated with systemic anti-PD-1 or anti-PD-L1 immune checkpoint blockade; their analysis did not show MDSC to be a prognostic biomarker. But these authors did find that patients undergoing anti-PD-1 treatment sustained a decrease in PD-1^+ MDSC after therapy, and patients undergoing anti-PD-L1 treatment, similarly, sustained a decreased in PD-$L1^+$ MDSC after therapy [88].

MDSC have even been found to be predictive of response among patients undergoing intravesical Bacille-Calmette Guerin (BCG) immunotherapy for high-risk non-muscle invasive bladder cancer. Chevalier et al. isolated $CD33^+CD11b^+HLA\text{-}DR^{low}$ MDSC, among other immune cell populations, from the urine of patients before and after BCG therapy. The authors discovered that patients with urinary MDSC:T cell ratios >1 experienced substantially lower recurrence-free and progression-free survival; that pre-treatment and post-treatment MDSC:T cell ratios did not appreciably change after BCG therapy; and that resistance to BCG may be mediated by type 2 innate lymphoid cells (ILC2), which promote the recruitment and immune suppressive functions of MDSC via IL-13 [89].

IMPACT OF BLADDER CANCER TREATMENTS ON MDSC

Emerging evidence suggests that multiple types of bladder cancer directed therapies in clinical use can modulate MDSC, which may in part explain their effectiveness. For patients with high-risk non-muscle invasive bladder cancers ('superficial' high grade stage cTis, cTa and cT1 bladder cancers that are associated with high recurrence rates), the gold standard treatment is immunotherapy with Bacille-Calmette Guerin (BCG). BCG is a live attenuated bacterium, *Mycobacterium bovis*, that induces infiltration of activated $CD4^+$ and $CD8^+$ T cells into the bladder with repeated intravesical instillations [90]

Wang et al. studied the effect of intravesical BCG administered to Sprague-Dawley rats that had developed endogenous orthotopic bladder cancers after exposure to the carcinogen N-methyl-N-nitrosurea (MNU). They demonstrated that intravesical BCG and systemic anti-PD-L1 therapy independently and synergistically decreased the quantity of $CD11b^+Gr\text{-}1^+$ MDSC in tumor-bearing bladders [91]. A decrease in intratumoral MDSC was also demonstrated with anti-PD-L1 therapy in the murine subcutaneous MB49 model by Shao et al. [92]. Similarly, Huang et al. showed in C3H mice with orthotopically implanted MBT2 bladder cancers that the quantity of circulating $CD11b^+Gr\text{-}1^+$ MDSC was decreased in dose dependent fashion with intravesical BCG instillations [93]. On balance, others have shown that BCG may induce bladder cancer cells to secrete MDSC-attracting chemokines. Muthuswamy et al. studied an *in vitro* model system consisting of TS4 bladder cancer cells, fibroblasts and $CD14^+$ monocytes isolated from blood; in this model, BCG was associated with increased supernatant concentrations of CXCL8 and CCL22, which are MDSC chemoattractants [94]. As discussed in the prior section, systemic BCG has been reported to promote 'natural suppressor cells' that inhibit cell-mediated immunity as well [81]. Therefore there is conflicting data as to whether BCG promotes or antagonizes MDSC in the bladder cancer microenvironment.

Cisplatin is the foundation of frontline systemic chemotherapy regimens for patients with locally advanced or metastatic bladder cancers. Wu et al. studied the effect of *in vitro* cisplatin administration on peripheral blood mononuclear cells isolated from patients with bladder cancer and found a decrease in $CD33^+CD11b^+CD14^-CD15^+$ PMN-MDSC [95]. In this study it was also observed that cisplatin-pretreated PMN-MDSC had a diminished ability to suppress $CD8^+$ T cell proliferation, [95] suggesting that the therapeutic effect of cisplatin in bladder cancer may be due in part to its deleterious effects on PMN-MDSC number and function.

Gemcitabine chemotherapy is another fundamental component of cytotoxic chemotherapy regimens directed against bladder cancers (frequently in combination with cisplatin) in the neoadjuvant, adjuvant, and metastatic settings; and its use also extends to intravesical instillations in patients with localized, low-grade non-muscle invasive bladder cancers [96]. Gemcitabine has also been shown to inhibit MDSC. Studying several cancer types in immunocompetent murine models, Suzuki et al. showed that splenic $CD11b^+/Gr\text{-}1^+$ MDSC were substantially decreased after gemcitabine treatment of tumor-bearing subjects and enhanced the therapeutic efficacy of intratumoral IFN-β [97]. Gemcitabine may also decrease the prevalence of tumor-infiltrating $CD11b^+/Gr\text{-}1^+$ MDSC, as reported by Sawant et al. who were studying the combination of gemcitabine plus superoxide dismutase in the Lewis Lung murine lung carcinoma model [98]. Finally, as discussed previously, gemcitabine has been shown to decrease $CD11b^+/CD14^-/CD33^+/HLA\text{-}DR^-$ PMN-MDSC levels in the circulation of patients with pancreatic cancer [54].

5-fluorouracil (5-FU), when used with cisplatin or mitomycin, is a commonly used radio-sensitizing chemotherapy agent administered to patients with invasive bladder cancer who opt for a radiation therapy based bladder-sparing approach [99]. Liljenfeldt et al. explored the effect of intratumoral MDSC when C57BL/6 mice with orthotopic MB49 bladder cancers were administered 5-FU +/– CD40L expressing adenovirus (CD40L is an activator of antigen presenting cells) [55]. The authors noted that the combination treatment regimen (5-FU plus Ad-CD40L) led to a significant increase in the ratio of $Gr\text{-}1^{high}$:$Gr\text{-}1^{intermediate}$ MDSC. Because it had been previously demonstrated that $Gr\text{-}1^{int}$ MDSC were more suppressive to T cell proliferation than $Gr\text{-}1^{high}$ MDSC, [100] Liljenfeldt et al. associated the efficacy of 5-FU plus Ad-CD40L with that particular regimen's effect on suppressive MDSC. The ability of 5-FU to decrease MDSC number and function has also been observed in a murine colorectal carcinoma model [101].

Systemic treatments for patients with bladder cancer include monoclonal antibodies that target immune checkpoints such as PD-1, PD-L1, and CTLA-4. The PD-1 inhibitor nivolumab is an FDA-

approved agent for patients with bladder cancer whose disease has progressed after treatment with cisplatin-based chemotherapy. Updated results of the Checkmate 275 trial (platinum-resistant urothelial carcinoma) reported by Sharma et al. suggest that high baseline MDSC levels are associated with lower survival after nivolumab treatment [102] While baseline MDSC levels may be predictive, they may not necessarily change after systemic therapy. Galsky et al. demonstrated in a phase 2 cohort of patients with urothelial carcinoma treated with two cycles of gemcitabine/cisplatin followed by four cycles of gemcitabine/cisplatin in combination with the anti-CTLA-4 monoclonal antibody ipilimumab found that neither circulating PMN-MDSC nor M-MDSC levels changed after chemotherapy or chemo-immunotherapy [103].

Nutritional interventional has been recently studied among patients with bladder cancer, along with effects on MDSC. Hamilton-Reeves et al. reported on a group of 29 men with bladder cancer undergoing radical cystectomy. Patients were randomized to peri-operative oral intake of a standard versus arginine-supplemented nutritional product. The arginine-supplemented product was found to be associated with a significantly lower quantity of circulating $CD11b^{+}CD33^{+}LIN^{-}CD14^{+}CD15^{-}$ M-MDSC two days after surgery and a slightly lower rate of post-operative infectious complications [104].

CLINICAL TRIALS INVESTIGATING AGENTS WITH POTENTIAL MDSC EFFECTS

MDSC signaling can be modulated by a wide range of pharmacologic agents, as reviewed by Wesolowski et al. [105] and as discussed in the preceding sections. Clinical trials investigating systemic therapies for bladder cancer in the metastatic and salvage settings (up to date as of December 2018), registered on ClinicalTrials.gov, that include agents known to modulate MDSC, are summarized in Table S1.

An example of a protocol that incorporates an MDSC-inhibiting chemotherapy (cisplatin) with a PD-1 inhibitor (pembrolizumab) is NCT02662062 – this phase 2 trial sponsored by Australian and New Zealand Urogenital and Prostate Trials Group also assesses radiation therapy as a bladder-sparing alternative to radical cystectomy. NCT02351739 is an example of a clinical trial that investigates the use of a tyrosine kinase inhibitor (acalabrutinib) that regulates Bruton's Tyrosine Kinase signaling (which is a key functional pathway in MDSC [106]) in combination with pembrolizumab – this combination is being evaluated in patients with metastatic urothelial carcinoma whose tumors progressed after first line cisplatin-based chemotherapy regimens. These notable trials are listed among the comprehensive list in Table S1. Any preliminary or final results regarding response and survival rates posted on ClinicalTrials.gov or published as papers or abstracts are included in the table summary.

MDSC IN BLADDER CANCER: FUTURE RESEARCH PRIORITIES

Taken together, MDSC represent a second immune checkpoint that may form the basis of intrinsic therapeutic resistance of most bladder cancers to anti-PD-(L)1 immunotherapy, BCG intravesical immunotherapy and cisplatin-based chemotherapies. Therefore targeting MDSC may be relevant for multiple states of bladder cancer. A notable unmet clinical need is for patients with non-muscle invasive bladder cancers that are unresponsive to intravesical BCG [107, 108]. It has not yet been established that MDSC underlie BCG resistance for this particular disease state. As reviewed above, MDSC infiltration may predict response to BCG, but BCG may either promote or antagonize MDSC. Therefore a combination strategy where BCG is given in sequence after an MDSC-depleting therapy (such as gemcitabine, a well-established intravesical bladder-cancer treatment) [96] may be appropriate for future pre-clinical and clinical study.

For patients with localized muscle-invasive bladder cancers (cT2-4 N0), cisplatin is the mainstay of combination chemotherapy regimens in the neoadjuvant setting. Cisplatin-based chemotherapy regimens are also the frontline treatments administered to patients with metastatic bladder cancer. Because cisplatin has been suggested to deplete circulating MDSC, current combination chemo-immunotherapy trials (cisplatin plus anti-PD-(L)1 therapy) (listed in Table 1) may offer promising response rates; non-responders in these clinical trials may reveal redundant MDSC signaling pathways that persist despite cisplatin.

Beyond general strategies to inhibit MDSC in the setting of bladder cancer, it is important to recognize that the subtypes of MDSC that promote bladder carcinogenesis, progression and therapeutic resistance

may be uniquely driven by bladder cancer-specific signaling derived from epithelial and stromal compartments of the tumor. For this reason, basic science efforts to delineate dominant signaling pathways in bladder cancer derived MDSC may generate unique insights to enhance systemic therapy for this disease.

SUPPLEMENTARY MATERIAL

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