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Review

Myeloid-derived suppressor cells and T regulatory cells in tumors: unraveling the dark side of the force

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ABSTRACT

Important conceptual advances in tumor immunology over the last years have shifted the paradigm from focusing on the malignant cell to the importance of host immune components in the design of successful immunotherapies. The immune system, through sophisticated innate and adaptive immune surveillance mechanisms, inhibits the growth and establishment of tumors. However, despite immune surveillance, tumors still escape and grow, mainly as a result of endowed tumor-induced immunosuppressive circuits. Regulatory T cells (T_{regs}) and myeloid-derived suppressor cells (MDSCs) are the major components of these regulatory networks that facilitate tumor immune escape and significantly compromise the efficacy of current immunotherapies. A better understanding of the induction, function, and expansion of these powerful regulatory compartments represents a major challenge on the clinical benefit of current treatments and may foster the design of novel cancer immunotherapies.
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Introduction

Cancer remains one of the leading causes of death globally, affecting both sexes equally. Although the immune system has the ability to recognize and destroy developing tumors, maintaining immune homeostasis, malignant cells often avoid immune recognition through use of multiple immunosuppressive mechanisms. The evolving, highly immunosuppressive TME comprises cells and molecules that facilitate tumor progression and metastasis and impedes immunotherapy [1]. Current, but also emerging, immune-based interventions in cancer aim in the induction of durable anti-tumor immune responses through reprogramming of TME. Although this still remains an unmet need, major efforts have been placed toward the in-depth delineation of the mechanisms and molecules that are used by malignant and host cells to form the TME. Specialized stromal cells [2], as well as recruitment of potent suppressive immune cells, such as MDSCs and T_{regs}, are considered as cardinal features of TME and have been shown to inhibit the function of TILs [3–5]. This review focuses on the role of T_{regs} and MDSCs in impeding antitumor immunity and discusses how the current therapeutic modalities could influence their suppressive programs. Finally, we propose a possible MDSC–T_{reg} interplay that might promote tumor progression.

Abbreviations: 5FU = 5-fluorouracil, ARG-1 = arginase 1, ATRA = all-trans retinoic acid, BM = bone marrow, COX-2 = cyclooxygenase 2, DC = dendritic cell, FAO = fatty acid oxidation, FLT3-L = FLT3 ligand, FoxP3 = forkhead box P3, G-MDSC = granulocytic myeloid-derived suppressor cell, GITR = glucocorticoid-induced TNFR-related protein, HIF-1α = hypoxia-inducible factor 1α, HMGB-1 = high-mobility group box 1,

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T_{regs}

Over the last two decades, strong evidence has emerged for a dominant role of T_{regs} in the regulation of tolerance and maintenance of immune homeostasis in both human and mice [6]. T_{regs} are either nT_{regs} that constitute 5–10% of the total peripheral $CD4^+$ T cells [7] or can be generated in the periphery upon exposure of naïve T cells to several tolerogenic stimuli (iT_{regs}) [8, 9]. A breakthrough in the field came with the identification of the transcription factor FoxP3 as a specific marker for T_{regs} [10–12], also crucial for T_{reg} development and function. Most importantly, the absence of T_{regs}, as a result of FoxP3 gene mutations, leads to the development of severe autoimmune disorders, known as scurfy phenotype in mice and immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome in men [13], highlighting their crucial role in the maintenance of immune homeostasis and the regulation of peripheral tolerance. Accumulating evidence supports the existence of multiple mechanisms and diverse molecules to be involved in T_{reg}-mediated suppression, including cell-to-cell contact, secretion of suppressive mediators, and deprivation of essential factors for cell growth (reviewed in Shevach [14]). However, the relative contributions of the aforementioned mechanisms in the T_{reg}-mediated suppression in vivo, the

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specific molecular and cellular targets, as well as the existence of disease-specific shaping of T_{reg} function remain to be determined.

T_{regs} IN CANCER PATIENTS AND CORRELATION WITH DISEASE STAGE AND PROGNOSIS

T_{regs} have a prominent role in antitumor immunity, as they support tumor immune evasion [15]. In humans, as a result of the lack of a T_{reg}-specific marker, their frequencies in malignant samples have been assessed by the expression of CD25 (IL-2Ra) [16–18] on $CD4^+$ T cells or of the transcription factor FoxP3 [19]. In all cases, percentages of T_{regs} in the peripheral blood of patients were elevated significantly compared with healthy individuals in nearly every type of malignacy, such as gastric, colorectal, pancreatic, liver, gynecologic, and breast cancers, as well as squamous cell carcinoma of the head and neck [16–21]. In accordance, several reports demonstrated increased frequencies of T_{regs} among TILs [17, 22–24]. For example, in ovarian epithelial cancer, tumor-infiltrating $CD4^+CD25^+$ T cells represented 23 ± 11% of the total $CD4^+$ TILs, whereas they were undetectable in normal ovarian tissues [25].

Whether increased numbers of T_{regs} in the periphery or in tumor site correlate with disease stage is still debatable. Various reports demonstrate that T_{reg} numbers increased in advanced disease stages of cancer patients with nonsmall cell lung cancer [26], breast cancer [20, 26], gastric cancer [24], and ovarian epithelial cancer [25]. Nevertheless, studies on multiple myeloma [27], epithelial malignancies [28], and gastric carcinoma [16] failed to show correlation of T_{reg} numbers with disease stage. In a similar way, the impact of the elevated T_{reg} frequencies in cancer patients regarding tumor outcome remains controversial. Although increased T_{reg} numbers infiltrating the tumor have been correlated with poor prognosis and worse disease outcome in diverse tumor settings [19, 25, 27, 29–31], in other studies, increased T_{reg} frequencies correlated with good prognosis [25, 30, 32–34]. Several explanations could be offered for these discrepancies. First, as mentioned before, CD25 and FoxP3 do not represent specific markers for human T_{regs}, as both of them are up-regulated by activated, nonsuppressive T cells [35, 36], and also, FoxP3 is expressed by several tumor cells [37]. Moreover, tumor milieu could de-stabilize T_{regs}, inducing ex-T_{regs} that have lost their suppressive activity, although they retain FoxP3 expression [38].

Overall, T_{regs} are increased in patients with solid tumors and hematologic malignancies, and a unified nomenclature system for their analysis is required for T_{regs} to be used as a prognostic marker in cancer.

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HSC = hematopoietic stem cell, iT_{reg} = inducible regulatory T cell, M-MDSC = monocytic myeloid-derived suppressor cell, MDSC = myeloid-derived suppressor cell, MHC II = MHC class II, miR = micro RNA, nT_{reg} = thymic-derived (natural) regulatory T cell, Opn = osteopontin, PD-L1 = programmed death ligand 1, PD1 = programmed death 1, PMN = polymorphonuclear, PNT = peroxynitrile, RMAS = Rauscher murine leukemia virus-induced T-cell lymphoma cell line, ROS = reactive oxygen species, SCF = stem cell factor, tdLN = tumor draining lymph node, TIL = tumor-infiltrating lymphocyte, TIM-3 = T cell Ig and mucin-domain containing 3, TME = tumor microenvironment, T_{reg} = regulatory T cell, VEGF = vascular endothelial growth factor

T_{regs} IMPEDE THE SUCCESS OF CANCER THERAPIES

The success of cancer immunotherapies has been hampered by T_{regs}. In clinical studies of head and neck squamous carcinoma, cervical cancer [39], and glioblastoma [40, 41], a combination of chemo-radiotherapy depleted $CD4^+$ and $CD8^+$ T effector cells in the peripheral blood and tdLNs of patients, whereas highly suppressive $FoxP3^+$ T_{regs} were unaffected or expanded. The efficacy of peptide vaccines in cancer mouse models [42] and human cervical cancer [43] has been limited as a result of the induction of tumor-specific T_{regs}. Moreover, histone deacetylase inhibitors have been shown to augment T_{reg}-suppressive function [44]. Transplantation of allogeneic HSCs to leukemic patients resulted in the killing of leukemic blasts [45]. However, an increased number of T_{regs} helped leukemic cells evade immune surveillance [46].

Several modalities have been designed to target T_{regs} specifically in cancer, mainly focusing on their phenotype, but their therapeutic efficacy is still controversial. In experimental models, targeting of T_{regs} through their CD25 expression has provided encouraging results. For instance, in a murine prostate tumor model, the administration of anti-CD25 antibody significantly improved the efficacy of radiation, resulting in delayed tumor growth and transient tumor regression by eliminating T_{regs} [47]. Moreover, in the B16 mouse melanoma model, tumors in mice receiving radiation therapy plus T_{reg}-depleting antibodies were significantly smaller than tumors in mice treated only with radiation [48]. The translation of these results into the clinic has, so far, yielded controversial results. Specifically, clinical trials with daclizumab, a mAb against CD25, have reported beneficial effects in breast cancer patients [49] but very low efficacy in metastatic melanoma patients and only upon administration of DC vaccines [50]. In addition, infusions of patients with Hodgkin's lymphoma, with radiolabeled daclizumab (^{90}Y-daclizumab), showed some promising results in almost 50% of patients [51]. Thus, the urgent need to understand in more detail the mechanism of recruitment, expansion, and function of T_{regs} to design more effective therapeutic modalities is evident.

T_{regs} IN TME

T_{reg} recruitment

Chemokines produced by tumor or host cells play a pivotal role in the recruitment of T_{regs} in tumor sites. CCL22, CCL5, CCL28, CCL2, and CXCL12 have been closely associated with the intratumoral presence of T_{regs}. Specifically, CCL22, CCL5, and CCL28 were shown to be secreted by pancreatic [52] and colon tumor cells [53] in both humans and experimental models, as well as by human ovarian and hepatocellular carcinoma cells [54]. In addition, CCL22 was produced by DCs, macrophages [25], tumor cells [55], and $CD8^+$ T cells in diverse tumors [56], whereas tumor stroma cells, such as cancer-associated fibroblasts, have been characterized as CCL5 producers in a mouse model of mammary carcinoma [57]. Furthermore, T_{regs} of cancer patients preferentially express the respective receptors CCR4, CCR1, CCR5, CCR10, and CXCR4, and the blockade of the chemokine-chemokine receptor interaction resulted in inhibition of the in vitro migration of human T_{regs} [55] or

in impaired recruitment of T_{regs} in solid tumors models [25, 52, 56–59].

T_{reg} proliferation and de novo conversion

Although characterized as anergic in vitro, T_{regs} have been reported to proliferate efficiently in the peripheral blood and particularly, within the tumor tissues of colorectal and metastatic prostate cancer patients. Indeed, tumor-infiltrating T_{regs} in liver metastases of colorectal cancer were positive for the proliferation marker Ki67 [60]. However, the mechanism responsible for T_{reg} proliferation in a tumor site remains poorly understood. TNF-α has been implicated in the intratumoral expansion of T_{regs} in a mouse model of colon carcinoma. TNF-α/TNFR2 interaction promoted T_{reg} proliferation and enhanced their suppressive activity against $CD8^+$ T cell-mediated anti-tumor immune responses, both in vitro and in vivo [61].

A large body of literature supports that TME enhances the peripheral conversion of $CD4^+CD25^-$ T cells into T_{regs} (iT_{regs}) that possess all of the features of nT_{regs} [62]. Intratumoral conversion of $FoxP3^-$ T cells to $FoxP3^+$ iT_{regs} has been mainly attributed to factors produced by cells of the TME. For instance, lymphoma B cells [63] and monocyte-derived DCs from breast cancer patients [64] significantly contributed to the in vitro FoxP3 expression in $CD4^+CD25^-$ T cells. Several molecules have been implicated in this process, such as COX-2 and PGE2, produced by head and neck squamous cell carcinomas [65], and TGF-β, produced by renal carcinoma (RENCA) or transgenic adenocarcinoma of the mouse prostate C2 (TRAMP-C2) cell lines [66] and ovarian carcinoma cells [67]. The importance of TGF-β in the de novo formation of T_{regs} in tumors has also been demonstrated in vivo in an animal model of hepatocellular carcinoma. SM-16, which is a specific inhibitor for TGF-βR reduced the percentage of T_{regs} in liver tissue and tumor progression [68]. Hypoxia and nonsoluble factors, such as IDO, which are enriched in TME, have also been linked to the de novo conversion of T_{regs} in the tumor. Indeed, in mice, intrasplenic injection of IDO^+ leukemia/lymphoma A20 cells promoted the conversion of $CD4^+CD25^-$ T cells to T_{regs} [69], and the exposure of Jurkat cells to hypoxic conditions resulted in increased levels of the HIF-1α, which in turn, led to FoxP3 induction [70]. In another study, hypoxic conditions were shown to potentiate glioblastoma multiforme through induction of an immunosuppressive environment rich in $Foxp3^+$ T_{regs} with STAT3 and HIF-1α to play a crucial role [71]. Increased frequencies of $Foxp3^+$ T_{regs} were also demonstrated in cancer patients upon administration of low doses IL-2 upon HSC transplantation [72]. In this study, it was shown that IL-2, through STAT3/STAT5 signaling, induces Foxp3 expression to T cells, rendering them T_{regs}. In support, it was more recently documented that high-dose IL-2 therapy in melanoma patients resulted in a robust increase of $ICOS^+Foxp3^+$ T_{regs} and correlated with a worse clinical outcome [73]. Finally, MDSCs have been proposed to influence the induction and/or expansion of T_{regs} (discussed in more details below).

T_{reg}-MEDIATED SUPPRESSION OF ANTITUMOR IMMUNE RESPONSES

T_{regs} have been shown to exert their suppressive function through multiple mechanisms, affecting almost all cells of the immune system, such as effector $CD4^+$ and $CD8^+$ T cells, NK cells, and DCs (**Fig. 1**).

$CD4^+$ and $CD8^+$ T cells

Extensive literature demonstrates the important role of T_{regs} in the suppression of proliferation and function of anti-tumor T cell-mediated immune responses. For instance, $CD4^+CD25^+$ T cells isolated from malignant ascites of ovarian carcinoma inhibited the polyclonal in vitro proliferation of autologous $CD3^+CD25^-$ T cells [25]. Moreover, T_{regs} infiltrating pancreatic tumors suppressed the antigen-specific proliferation of TILs [74]. In the experimental models of RMAS lymphoma and B16 melanoma T_{regs} were able to kill $CD8^+$ T cells through granzyme B and perforin [75]. In line with this, peripheral T_{regs} from lung cancer patients or epithelial ovarian carcinoma potently suppressed IFN-γ production by $CD8^+$ T cells in a CTLA-4- or TIM-3-dependent mechanism [76, 77], whereas in the murine model of colon cancer (CT26), T_{regs} suppressed the in vivo cytotoxicity of $CD8^+$ T cells with a mechanism involving TGF-β [5].

T_{regs} could also impair $CD4^+$ T cell responses. Thus, $FoxP3^{hi}$ T_{regs} infiltrating gastric cancer suppressed the proliferation of autologous $CD4^+CD25^-$ T cells, and this effect was reversed by COX inhibitors and PGE2 receptor-specific antagonist [78]. Moreover, treatment with soluble GITR ligand induced a decrease in the suppression mediated by the activated hepatocellular carcinoma-infiltrating T_{regs} and restored the proliferative capacity and cytokine production of $CD4^+CD25^-$ T cells [60].

NK cells

NK cells belong to the innate arm of the immune system and play an important role in anti-tumor immune responses. Limited studies in murine tumor models have demonstrated the suppressive function of T_{regs} on NK cell cytotoxicity. For example, transfer of T_{regs} into athymic mice depressed the cytotoxic activity of the endogenous NK cells against the tumor cell line YAC-1 [79]. As for the mechanism, it was shown that circulating T_{regs}, isolated from the gastrointestinal tumor, suppressed NK cells via membrane-bound TGF-β and down-regulation of the activating NK cell receptor (NKG2D) [79]. Similar findings were reported in cervical carcinomas, where T_{regs} potently suppressed NK cell activity in vitro [80]. Furthermore, in mice bearing RMAS lymphoma and B16 melanoma, $CD4^+FoxP3^+$ T_{regs} induced NK cell death in a granzyme B- and perforin-dependent fashion. Adoptive transfer of wild type T_{regs}, but not granzyme B- or perforin-deficient T_{regs}, into granzyme B-deficient mice partially restored susceptibility to tumor growth [75].

DCs

APCs are required for the development of a potent anti-tumor immune response. It is well known that T_{regs} modulate DC maturation and function through secretion of immunomodulatory cytokines or cell-to-cell contact. Specifically, $CD4^+CD25^+FoxP3^+$ T_{regs} from leukemia-bearing mice positive for breakpoint cluster region-abelson murine leukemia viral oncogene homolog 1 fusion gene (BCR-ABL) were shown to impede DC function by down-regulating the expression of the costimulatory molecules CD80, CD86, and CD40 and the production of TNF-α, IL-12, and CCL5/RANTES. This suppression mechanism required TGF-β and IL-10 [81]. Moreover, $FoxP3^+$ T cells induced DC death in

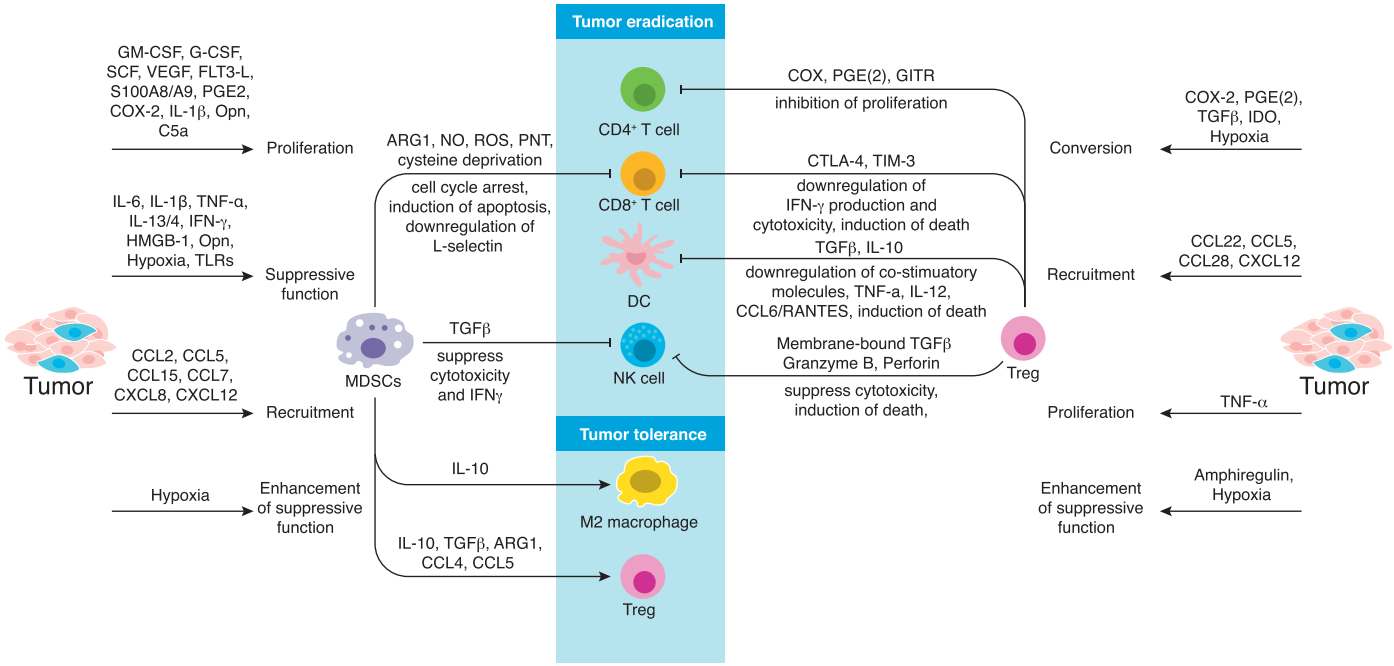

Figure 1. Tumors escape immune surveillance by promoting MDSCs and T_{reg} proliferation and suppressive function. Several factors produced by tumor and tumor stroma promote the proliferation, recruitment, and suppressive function of MDSCs and T_{regs}. MDSCs and T_{regs} suppress the immune cells that are responsible for tumor eradication, such as $CD4^+$ and $CD8^+$ T cells, DCs, and NK cells, whereas they induce other tumor-promoting subsets, such as M2 macrophages.

tdLNs of MCA101-bearing mice in a perforin-dependent fashion [82].

T_{regs} AND CANCER THERAPY

Effect of current therapies on T_{regs}

Various therapeutic regimens currently used for the treatment of cancer have been shown to affect T_{regs}: 1) low doses of cyclophosphamide eliminated T_{regs} in both mouse [79, 83] and human studies [84, 85] and also attenuated T_{reg} function by down-regulating FoxP3 and GITR [86]. 2) Fludarabine and gemcitabine, which inhibit DNA synthesis, have been shown to disrupt the proliferation, increase the apoptosis, and decrease the inhibitory functions of T_{regs} [85, 87–89]. 3) Paclitaxel, a mitotic inhibitor, has been reported to induce T_{reg} apoptosis selectively [90]. 4) Tyrosine-kinase inhibitors, such as imatinib, sorafenib, and sunitinib, attenuated T_{reg} numbers and decreased CD69, GITR, CTLA-4, and FoxP3 expression on T_{regs}, allied with a decrease in IL-10 and TGF-β secretion [91]. 5) The checkpoint inhibitor anti-CTLA-4 has been shown to suppress T_{reg} activity and reduce tumor-infiltrating T_{regs} [92–94], whereas other studies demonstrated that $FoxP3^+$ T_{regs} are stably maintained or even increased in peripheral bloods or TILs upon ipilimumab or tremelimumab treatment [95–98]. 6) Nivolumab (an anti-PD1-blocking antibody) was able to abrogate T_{reg}-suppressive function [99, 100]. Finally, the coadministration of anti-TIM-3 and anti-PD-L1 antibodies in mice bearing CT26 cell-derived colon carcinomas reduced the immunosuppressive functions of $TIM\text{-}3^+$ T_{regs} and altered their homing to and/or retention within neoplastic lesions [101].

Although checkpoint inhibitors and chemotherapy inhibited directly the in vitro proliferation and function of T_{regs} [99], at the same time, they could also influence malignant cell growth, which in turn, endows the T_{reg} potency. In line with this, it was recently demonstrated that tumor cells secret amphiregulin, which activated immunosuppressive function of T_{regs} to inhibit $CD8^+$ T cell responses [102, 103]. Therefore, it remains unknown whether the efficacy of cancer therapies directly affects T_{reg} frequency and function.

Finally, it has been demonstrated that phosphatase and tensin homolog-mediated control of PI3K is required for T_{reg} lineage homeostasis and function [104]. However, attempts to interfere with PI3K signaling in T_{regs} to diminish their function have yielded controversial results in both mice and men. Thus, α and δ isoform-specific PI3K inhibitors were shown to have minimal effects on T_{reg} proliferation [105], whereas with the use of different PI3K inhibitors, another study demonstrated significant suppression of T_{reg} proliferation both in vitro and in vivo [106]. In support of the latter, inactivation of the p110δ isoform of PI3K in T_{regs} in mice ($p110\delta^{D910A}$) induced tumor regression through promotion of $CD8^+$-mediated cytotoxic responses [107]. Collectively, whether PI3K inhibition in T_{reg} function could be exploited therapeutically in tumors is under intense investigation.

Emerging therapies targeting T_{regs}

Molecules highly expressed by T_{regs} have recently emerged as targets for immunotherapy. Specifically, an anti-OX-40 agonistic antibody reduced T_{reg} frequencies in tumor tissues, and this was accompanied by augmented anti-tumor immunity in experimental models of melanoma, colon cancer, glioma, breast cancer, sarcoma, renal cancer, and prostate cancer [108, 109]. A phase I trial of an OX-40 agonist demonstrated anti-tumor

activity in melanoma and renal cell cancer [110]. Clinical trials evaluating OX-40 agonists in head and neck, breast, and prostate cancer and in B cell lymphoma are now in progress. In addition, anti-GITR antibody specifically depleted T_{regs} in tumors and directly inhibited T_{reg} suppression in melanoma, colorectal carcinoma, malignant glioma, and fibrosarcoma mouse models, resulting in enhanced anti-tumor responses [89, 111–114]. Phase I clinical trials evaluating GITR agonists in solid tumors are currently tested.

MDSCs

MDSCs comprise a heterogeneous population of cells that arise from multipotent HSCs and serve diverse functions during innate and adaptive immune responses. MDSCs have been closely implicated in tumor pathogenesis, growth, and metastasis (reviewed in Gabrilovich et al. [115]) and today, have been largely involved in various disease settings, such as autoimmunity, infectious diseases, transplantation, etc. [116–119]. Comprehensive investigation of MDSCs has sometimes been hampered by the lack of a unified characterization system, mainly as a result of the phenotypic and morphologic heterogeneity of these cells. Recently, this issue has been addressed in a comprehensive review on recommendations for MDSC nomenclature [120]. To this end, in mice, MDSCs are identified by the coexpression of the myeloid-cell lineage differentiation antigen Gr-1 and CD11b and can be divided further based on their morphology, as M-MDSCs or G-MDSCs, which are defined as $CD11b^{+}Ly6G^{-}Ly6C^{hi}$ and $CD11b^{+}Ly6G^{+}Ly6C^{low}$, respectively. In humans, MDSCs have been characterized extensively in peripheral blood as $HLA\text{-}DR^{lo/-}CD14^{+}CD33^{+}CD15^{-}$ MDSCs with monocytic morphology or $HLA\text{-}DR^{lo/-}CD14^{-}CD15^{+}$ PMN-MDSCs. The lack of a harmonized phenotypic characterization of MDSC subsets has hampered, in many cases, the sole distinction of PMN-MDSCs from differentiated neutrophils. Cell surface markers that are used to characterize PMN-MDSCs in various studies are also expressed by classic neutrophils. Thus, today, only gradient centrifugation and nuclear morphology are used to separate the two populations, and a precise characterization of the two populations remains an unmet need in the field. Similar to T_{regs}, several mechanisms have been proposed for MDSC-mediated suppression of immune responses. These include production of ROS and nitrogen species; depletion of essential amino acids (i.e., arginine, cysteine); and production of regulatory cytokines, such as IL-10 and TGF-β. A better understanding of MDSC biology will result from the combination of an in-depth characterization of MDSC phenotype and MDSC-mediated suppression mechanisms.

MDSCs IN CANCER PATIENTS AND CORRELATION WITH DISEASE STAGE AND PROGNOSIS

MDSCs, although first identified and characterized as natural suppressor cells, 30 yr ago, their role in tumors was appreciated when increased numbers of $CD34^{+}$ myeloid cells were described in blood of patients with head and neck carcinoma [121]. Since then, increased accumulation of MDSCs has been monitored in both periphery and tumors of most cancer patients and in many cancer models, such as renal carcinoma [122], melanoma [123], prostate cancer [124], hepatocellular carcinoma [125], head and neck cancer [126], rectal cancer [127], colon and breast carcinoma [128], glioma [129], pancreatic [130], nonsmall cell lung cancer [131], and lung tumors [132]. In tumor-bearing mice, they accumulate in the BM, tumor, spleen, and blood and to a smaller degree, in tdLNs [133–135]. Importantly, MDSCs have been associated with poor prognosis [136], metastatic spread [137], and reduced effectiveness of immunotherapeutic approaches [138].

MDSC RECRUITMENT IN TME

Similar to T_{regs}, recruitment of MDSCs into tumors is facilitated mostly by chemokines. CCL2 and CCL5 are the main chemokines involved in MDSC attraction to tumor sites [139], as MDSCs isolated from patients with ovarian, breast, or gastric cancer exhibited ubiquitous expression of CCR2 (the specific receptor of CCL2), and CCL2 was shown to mediate their migration to tumor cells in vitro. Moreover, CCR2-deficient MDSCs failed to infiltrate tumors in vivo [140]. Finally, deletion of CCL2 in a mouse colorectal cancer model reduced the migration of MDSCs into tumor sites [141]. In the same line, the absence of the CCL5–CCR5 interaction led to aberrant generation of MDSCs in the BM, with impaired capacity to suppress cytotoxic $CD8^{+}$ T cells in orthotopic and spontaneous mammary tumors [142]. CCL7, CXCL8 (also known as IL-8), and CXCL12 have also been shown to mediate the mobilization of MDSCs. PGE2 has been reported to induce the production of CXCL8 and CXCL12 and induce MDSC accumulation in ovarian and gastric cancer [143, 144]. Finally, CCL15 secreted from SMA-and MDA (Mother Against Decapentaplegic)-related protein 4 (SMAD4)-deficient colorectal cancer cells was demonstrated to recruit $CCR1^{+}$ MDSCs and to result in aggressive tumor growth [145].

GENERATION AND ACTIVATION OF MDSCs

MDSCs are the progenitors of macrophages, granulocytes, or DCs. During cancer development, tumor and stroma cells produce factors adequate to expand MDSCs through promotion of myelopoiesis and inhibition of differentiation and also provide the necessary signals to endow their suppressive function.

Mediators of chronic inflammation, such as the growth factors GM-CSF [146], G-CSF [147], M-CSF [148], SCF [149], VEGF [150], and FLT3-L [151], are well-characterized mediators of MDSC expansion in diverse tumors. Indeed, the blocking of GM-CSF [152–154], M-CSF [155], VEGF [156], or G-CSF [147], using neutralizing antibodies, led to inhibition of intratumoral accumulation of MDSCs and subsequent reduction in tumor growth.

Furthermore, a family of proinflammatory proteins, termed S100A8/A9, has been shown to bind to cell surface glycoprotein receptors on MDSCs and promote MDSC accumulation in an autocrine-feedback loop [135]. Mice, genetically deficient for S100A9, were not able to develop colon carcinoma, and this was reversed upon adoptive transfer of wild-type MDSCs [157]. In

support, in human colon tumor tissues, MDSCs expressing S100A8 and S100A9 proteins infiltrated regions of dysplasia and adenoma, whereas administration of glycan-specific antibody blocked tumorogenesis in a mouse model of colitis [158], suggesting that the S100 proinflammatory cytokines promote MDSC expansion in cancer.

PGE2 and COX-2 have also been implicated in MDSC accumulation in tumors. They are produced by tumor cells and tumor-infiltrating macrophages [159, 160]. PGE2 was shown to skew the differentiation of BM stem cells toward $Gr1^+CD11b^+$ MDSCs, whereas receptor antagonists blocked it. Tumor-bearing mice deficient for EP-2 (a PGE2 receptor) displayed reduced tumor growth, and treatment of tumor-bearing mice with a COX-2 inhibitor reduced MDSC levels and delayed tumor progression [161]. Patients with renal cancer were shown to exhibit elevated expression of PGE2 in their MDSC compartment [162]. These finding support that PGE2 promotes tumor invasion by enhancing MDSC accumulation.

Notably, proinflammatory cytokines, such as IL-6, IL-1β, TNF-α, IL-13/4, and IFN-γ, have been implicated in both MDSC accumulation and activation. Specifically, mice inoculated with tumor cells secreting IL-1β exhibited elevated numbers of MDSCs [163]. Neutralization of IL-6, a cytokine that inversely correlates with clinical outcome in many tumors [164], was shown to inhibit MDSC differentiation in a model of pancreatic cancer [165]. Cytokines, such as IL-4 and IL-13, bound to IL-4Rα to activate the immunosuppressive pathway of MDSCs by inducing the transcription of ARG-1 [166]. Furthermore, IFN-γ and IL-13, produced by MDSCs, acted in an autocrine loop to sustain IL-4Rα expression. In line with the above, knockdown of IL-4Rα attenuated the immune-suppressive phenotype of MDSCs in mouse models of colon and mammary carcinoma [167, 168]. Furthermore, TNF-α has been demonstrated to exhibit a dual function on MDSCs during cancer: blocking their differentiation to terminally differentiated subsets via the S100A8 and S100A9 inflammatory proteins and their receptor (receptor for advanced glycation endproducts) and enhancing their suppressive nature [169]. Finally, Opn, a pleiotropic cytokine produced both by tumor and myeloid cells, was found to promote myelopoiesis toward enhanced MDSC generation [170]. Trametinib, a known MEK inhibitor was shown to inhibit the expansion of M-MDSCs in a Kirsten ras oncogene homolog (KRas)-driven breast tumor model and to reduce the production of Opn by tumor cells, thus impairing the M-MDSC mobilization [171]. Studies using *Opn*-deficient mice, along with gene silencing of Opn in cancer cells, demonstrated that Opn produced by tumor cells supported their survival, whereas both tumor- and myeloid cell-derived Opn rendered the metastatic site more immunosuppressive [170].

Most of the aforementioned factors elicit JAK/STAT signaling pathways in MDSCs (mainly STAT3) [172, 173]. To this end, STAT3 mediated MDSC expansion through C/EBPβ [174] and IFN regulator factor 8 [175, 176], whereas phosphorylation of STAT3 led to MDSC differentiation and regulation of ARG-1 production [165, 170, 177]. Furthermore, activation of STAT3 in myeloid precursors by estrogen led to increased mobilization of MDSCs and enhanced their immunosuppressive program in vivo [178]. The transcriptional regulation of MDSCs has been reviewed recently [179].

Furthermore, increased levels of the alarmin HMGB-1 in the TME were shown to trigger NF-κB signaling in MDSCs and to control their suppressive fate by enhancing IL-10 production [180]. Recently, HIF-1α has been closely linked to the differentiation and function of MDSCs in tumors [4]. In support, it was also demonstrated that PD-L1 was a novel, direct target of HIF-1α, and its blockade during cancer development enhanced MDSC-mediated T cell activation [181]. In line with this, TLR family members, phospholipase Cγ2 and SHIP-1, two enzymes involved in physiologic hematopoiesis [182–184], and complement component C5a [185] have been implicated in the enhanced activation and function of MDSCs during cancer. Of interest, endoplasmic reticulum stress has recently gained attention in shaping the MDSC-suppressive function. Specifically, up-regulation of the cellular stress sensor C/EBP-homologous protein on MDSCs enhanced STAT3-mediated IL-6 production in Lewis lung carcinoma, a process that was dependent on ROS and NO species [186].

Finally, overexpression of miRs miR-155 and miR-21 enhanced, whereas depletion of miR-155 and miR-21 reduced, the frequencies of cytokine-induced MDSC and were both required for IL-6-mediated MDSC activation in a Lewis lung carcinoma model [187]. On the other hand, another miR (miR-142-3p) must be repressed to allow acquisition of immune-suppressive properties of MDSCs [188].

MDSC-MEDIATED SUPPRESSION

MDSCs use a plethora of mechanisms to promote tumor development, either directly by affecting angiogenesis, stroma deposition, epithelial-to-mesenchymal transition, and induction of metastatic spreading (reviewed in Marvel and Gabrilovich [189]) or indirectly through inhibition of anti-tumor immune responses (discussed in the following sections; Fig. 1).

The main mechanisms linked with the suppression of immune cells by MDSCs can be categorized in the following: deprivation of amino acids essential for the development and function of lymphocytes, secretion of ROS, NO resulting in oxidative stress, induction of lymphocyte apoptosis, blocking of lymphocyte trafficking, and finally, induction of T_{regs}.

One of the best-characterized mechanisms of MDSC-suppressive function is the production of the enzyme ARG-1, which uptakes L-arginine from the TME and enhances its catabolism. MDSC-mediated shortage of L-arginine resulted in inhibition of T cell proliferation by decreasing the expression of the CD3-ζ chains [190] and in induction of cell cycle arrest of T cell proliferation in the G_0–G_1 phase [191]. In a similar fashion, IFN-γ and TNF-α in the tumor triggered iNOS production in MDSCs, which has also been closely linked to MDSC-mediated suppression. Production of iNOS resulted in the generation of NO, which in turn, blocked activation of the IL-2R signaling cascade and led to suppression of T cell proliferation through induction of apoptosis [192–194] and inhibition of MHC II expression on macrophages [195]. In addition, MDSCs inhibited T cell activation by restraining availability of cysteine from the environment. Cysteine is an essential amino acid for T cell function that cannot be synthesized by T cells or MDSCs. An elegant study, using mammary

gland tumors, demonstrated that MDSCs competed with APCs for extracellular cystine, therefore reducing the availability of exported cysteine and thus affecting T cell function [196].

One of the main characteristics of activated MDSCs is the production of ROS and PNT. Increased ROS levels produced by MDSCs triggered the STAT3 signaling pathway through up-regulation of NADPH in both tumor-bearing mice and cancer patients [197]. Furthermore, ROS and PNT catalyzed the nitration of tyrosines in a TCR–CD8 complex, thus preventing T cell–peptide–MHC interactions [198]. Finally, MDSC-derived ROS exerts pleiotropic effects, such as promotion of DNA damage in immune cells and intratumoral MDSC recruitment (reviewed in Gabrilovich and Nagaraj [199]).

Apart from T cell activation and proliferation, MDSCs can also modulate T cell recruitment to a tumor site. It has been demonstrated that naive T cells cocultured with tumor-induced MDSCs have reduced L-selectin (CD62 ligand), an essential homing receptor on T cells. Moreover, reduced expression of L-selectin on T cells of cancer patients inversely correlated with MDSC levels in TILs [200]. MDSCs not only inhibited the infiltration of effector T cells but also actively promoted the generation of T_{regs} inside of the tumor. MDSCs isolated from various cancer models exhibited enhanced production of IL-10 and TGF-β and had the potency to induce the development of T_{regs} [201].

Finally, MDSCs may also affect the function of other lymphoid cells. More specifically, MDSCs, through production of TGF-β, weakened NK cell response by blocking their IFN-γ production and inhibiting their cytotoxicity [202]. Another report implicated MDSCs in skewing the phenotype of macrophages toward type 2 with decreased production of IL-12 [203].

Overall, the mechanisms of action of MDSCs in the periphery in response to cancer have been well characterized. However, whether MDSCs use the same mechanisms to exert their suppressive function inside of the tumors has only recently emerged and is discussed below.

Recent studies on intratumoral MDSCs have put forward a concept that TME enhances the immunosuppressive strength of MDSCs. Actually, studies using direct comparison of MDSCs isolated from spleens and tumors demonstrated that tumor MDSCs were more suppressive on a per-cell basis [4, 204–206]. More specifically, MDSCs infiltrating tumors increased their FAO, mitochondrial mass, and oxygen consumption rate. Inhibition of FAO modulated the immunosuppressive functions of MDSCs and enhanced the efficacy of cancer therapies [204]. MDSCs, isolated from tissues of different types of tumor, possessed immediate capacity to inhibit T cell function via up-regulation of ARG-1 and iNOS, whereas those isolated from peripheral tissues were not suppressive without previous activation by exposure to IFN-γ [204, 206].

Several recent studies propose that peripheral and tumor-infiltrating MDSCs exert their function via different mechanisms [4, 207]. In particular, MDSCs from peripheral lymphoid organs suppressed antigen-specific $CD8^+$ T cells in a ROS-dependent manner but failed to inhibit nonspecific T cell function. In contrast, HIF-1α, in the TME, dramatically altered the function of MDSCs, as tumor MDSCs were able to suppress both antigen-specific and nonspecific T cell activity, mainly via NO production and secretion of ARG-1 [4]. HIF-1α also drove a rapid differentiation of MDSCs to tumor-associated macrophages, mainly in the tumor site, whereas MDSCs lacking HIF-1α acquired a DC phenotype [4]. Furthermore, tumor-associated HIF-1α increased expression of PD-L1 on the surface of tumor-infiltrating MDSCs, resulting in more potent, suppressive activity of tumor MDSC than splenic MDSC [181].

Finally, studies using gene-expression analysis of tumor-infiltrating G- and M-MDSCs compared with MDSCs from peripheral blood, demonstrated that M-MDSCs not only produced high levels of NO and ARG-1 but also increased levels of the CCR5 ligands CCL3, CCL4, and CCL5. Moreover, CCL4 and CCL5 were able to attract T_{regs} in a CCR5-dependent manner [207].

Thus, it appears that MDSCs, upon exposure to TME, acquire a more suppressive phenotype than in peripheral lymphoid organs or blood. The outcome is the sculpture of a highly suppressive TME that facilitates immune escape. This could be attributed further to the differential ratio of MDSC subsets that infiltrate tumors [208] or the diverse suppressive mechanisms used by these subsets. Specifically, G-MDSCs exerted their function via ROS production in a mechanism that required cell-to-cell interaction with T cells [209] and have been reported as less immunosuppressive compared with M-MDSCs. In contrast, in some tumors, M-MDSCs produce molecules with a much longer half time (NO, ARG-1) that does not require close contact with T cells [210]. Finally, despite direct suppression of T cell responses, a bidirectional cross-talk between MDSCs and macrophages/DCs has been shown to generate a robust immunosuppressive environment. In brief, MDSCs have been shown to dampen the antigen-presenting machineries of professional APCs, for example, through secretion of, i.e., IL-10, failing to support T cell activation and proliferation. This concept is reviewed extensively elsewhere [211]. To conclude, the diverse mechanisms reported to operate in MDSC-mediated suppression in the tumor milieu, as well as the differential role of MDSC subsets in tumor evasion, could pave the way for the design of novel immunotherapies.

THERAPEUTIC TARGETING OF MDSCs

Immunotherapy targeting MDSCs is quite promising, because as described above, the restraining of MDSC frequencies and blocking of their immunosuppressive functions have been demonstrated to prolong survival both in tumor-bearing mice and in cancer patients. It has been proposed that therapeutic targeting of MDSCs can be accomplished by the following: 1) interfering with their generation through controlling myelopoiesis in the BM, 2) driving their differentiation toward mature myeloid subsets (such as DCs), 3) depleting MDSCs or arresting their trafficking to tumor sites, and 4) restraining their immunosuppressive function.

Regulation of myelopoiesis

Regulation of myelopoiesis can be achieved by means of blocking MDSC generation from precursor cells inside of the BM niche.

Many studies have implied SCF and c-kit (the receptor of SCF) as the major factors regulating MDSC generation [149, 212]. The knocking down of SCF using small interfering RNA during cancer development resulted in reduced MDSC expansion and restored proliferative responses of TILs. Importantly, administration of the antibody against the c-kit prevented T_{reg} development and tumor angiogenesis [149]. In support, blockade of the c-kit through sunitinib, pazopanib, and sorafenib resulted in a significant reduction in the frequency of circulating MDSCs in both tumor-bearing mice and cancer patients [212].

Promotion of MDSC differentiation

As mentioned above, MDSCs are progenitors of macrophages, DCs, and granulocytes. Therefore, an attractive therapeutic approach would be to direct the reprogramming of MDSCs toward fully mature myeloid cells. In line with this, administration of ATRA, along with IL-2, in renal cell carcinoma patients significantly reduced the numbers of circulating MDSCs and improved the myeloid/lymphoid DC ratio and the ability of patients' mononuclear cells to stimulate allogeneic T cells [213]. Furthermore, a combination of ATRA with vaccination not only decreased the number of MDSCs and suppressed their function but also generated significantly increased tumor antigen-specific T cells with elevated IFN-γ secretion and enhanced cytotoxic T cell activity [214].

Elimination of MDSCs

Depletion of MDSCs, combined with known anti-cancer therapies, could provide a good tool for the break of immune tolerance and reduction of tumor progression. Administration of the chemotherapeutic drug gemcitabine was reported to reduce specifically the number of MDSCs in the spleen without any toxic effects on other leukocytes [215]. In support, another study demonstrated that 5FU was selectively cytotoxic on MDSCs. Treatment of tumor-bearing mice with 5FU showed a stronger efficacy over gemcitabine in depleting MDSCs from spleens and tumors of animals [216]. In line with this, inhibition of TRAIL receptors resulted in MDSC elimination in mice and humans with lung carcinoma [217]. Finally, it was elegantly demonstrated that administration of MDSC-specific peptibodies (peptides of S100A family proteins fused to Fc proteins) in multiple tumor models efficiently depleted MDSCs and were associated with reduced tumor development [218].

Blockade of MDSC function

As described previously, MDSCs use NO and ARG-1 production to mediate their immunosuppressive function. Inhibition of phosphodiesterase 5 using agents, such as sildenafil, tadalafil, and vardenafil, induced a potent anti-tumor immune response by down-regulating ARG-1 and NO production, thus limiting the suppressive function of MDSCs [219]. In support, use of nitroaspirin, which is also known to block ARG-1 and NO production, resulted in significant T cell activation and reduced tumor progression [220].

Although current therapies for targeting MDSCs in cancer have proven to be partially effective, there are still major drawbacks that need to be considered. Specifically, MDSC targeting for cancer therapy has been hampered by various impediments, such as the lack of specific markers. Compounds used for eliminating MDSCs are not specific, as they can result in elimination of other immune cell subsets as well. In addition, administration of compounds targeting MDSC function, generation, or accumulation does not target only the tumor site and thus, could result in an unwanted, generalized immunosuppression. Overall, further studies are required to achieve the goal of specific targeting of MDSCs for cancer therapy.

CROSS-TALK OF T_{regs} AND MDSCs

Strong evidence has been provided for a predominant role of T_{regs} in the suppression of anti-tumor immunity and promotion of cancer development. However, several questions regarding T_{reg} generation, accumulation, and function in TME remain unanswered. Specifically, the cell subsets, as well as the mechanisms involved in T_{reg} induction/proliferation during tumor development, are unknown. In addition, how the TME influences antigen uptake and presentation by APCs and whether such conditions favor T_{reg} development remain elusive. Although MDSCs have drawn attention as a possible candidate for T_{reg} induction and expansion in the tumor milieu, the molecular and cellular mechanisms involved in MDSC-mediated T_{reg} induction are poorly understood.

To this end, a positive correlation of MDSCs and T_{reg} frequencies with tumor progression in multiple myeloma patients has been demonstrated [221], whereas in experimental tumor models, expansion of MDSCs was not always correlated with the presence of T_{regs} [222], making the picture more complicated. Nevertheless, early reports described that expression of the CD80 costimulatory molecule by MDSCs in a mouse model of ovarian carcinoma was required for the MDSC-mediated suppression that was dependent on $CD4^+CD25^+$ T_{regs} and CD152 [223]. In addition, in colon carcinoma-bearing mice, $Gr\text{-}1^+CD115^+$ MDSCs induced the development of highly suppressive and anergic $Foxp3^+$ T_{regs} in vivo in an IL-10/TGF-β-dependent but NO-independent mechanism [201]. On the other hand, in a B-cell lymphoma mouse model. MDSCs mediated tumor-induced tolerance through development and proliferation of T_{regs} [224]. In this study, MDSCs were expressing low levels of MHC II and served as tolerogenic APCs that engulfed and presented tumor antigens to induce tumor-specific T_{regs} in a mechanism dependent on ARG-1 but not on TGF-β. Expression of the CD40 molecule by MDSCs has also been implicated in MDSC-mediated T_{reg} induction. Specifically, administration of agonistic anti-CD40 antibodies or adoptive transfer of CD40-deficient MDSCs failed to induce tumor-associated expansion of T_{regs} [225]. Finally, in a clinical setting, $CD14^+HLA\text{-}DR^{-/low}$ MDSCs, isolated from patients with hepatocellular carcinoma, induced $CD4^+CD25^+$ $Foxp3^+$ T_{regs} in vitro upon culture with autologous T cells [125].

By taking into account the increased heterogeneity of both regulatory populations, as well as their increased plasticity, it would be important to delineate better the pathways and molecules that are involved in the MDSC–T_{reg} cross-talk during cancer development. For example, what is the MDSC genetic and proteomic/metabolomic signature that influences the induction of iT_{regs} or the expansion of nT_{regs}? How do current immunotherapies alter the phenotype and function of MDSCs and T_{regs} to shift the balance from tolerance to immunity? What are the mechanism(s) implicated in tumor antigen uptake, and how are tumor antigens processed by MDSCs to be presented in a tolerogenic manner that

will influence the induction/expansion of T_{regs} in TME? Finally, which molecules secreted by MDSCs could drive the intratumoral accumulation of T_{regs}, and could these molecules serve as potential therapeutic targets? Various molecules and signatures have been described to distinguish better the diverse T_{reg} subsets and should be considered in the future analysis of tumor-associated MDSC–T_{reg} cross-talk. This knowledge is of clinical importance and should be considered in the design of more effective immunotherapies.

CONCLUDING REMARKS

Recent insights into immune-based mechanisms that govern tumor development and progression have generated a number of new therapeutic opportunities and designs of clinical trials aiming to shift the immune system in favor of potent anti-tumor responses. However, the efficacy of anti-tumor immunotherapy is still limited, and the presence of immunosuppressive MDSCs and T_{regs} in TME further impedes its success. Importantly, an interplay between MDSCs and T_{regs} is becoming apparent in the establishment of tumor escape from immune recognition. The picture becomes more complicated, as the relative contribution of MDSCs and T_{regs} in the development of cancer remains unknown. Furthermore, whether MDSCs and T_{regs} act in concert or whether they have distinct roles in inducing immunosuppression has not been elucidated. Finally, the way that different tumors in distinct anatomic sites shape the function of these regulatory populations is unknown. Therefore, the deciphering of mechanisms and molecules used by suppressive networks that lead to tumor development and progression is of paramount importance for the design of successful cancer immunotherapies.

AUTHORSHIP

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DISCLOSURES

The authors declare no conflicts of interest.

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KEY WORDS:
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