



Modulation of Immunosuppression by Oligonucleotide-Based Molecules and Small Molecules Targeting Myeloid-Derived Suppressor Cells

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Abstract

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that exert suppressive function on the immune response. MDSCs expand in tumor-bearing hosts or in the tumor microenvironment and suppress T-cell responses via various mechanisms, whereas a reduction in their activities has been observed in autoimmune diseases or infections. It has been reported that the symptoms of various diseases, including malignant tumors, can be alleviated by targeting MDSCs. Moreover, MDSCs can contribute to patient resistance to therapy using immune checkpoint inhibitors. In line with these therapeutic approaches, diverse oligonucleotide-based molecules and small molecules have been evaluated for their therapeutic efficacy in several disease models via the modulation of MDSC activity. In the current review, MDSC-targeting oligonucleotides and small molecules are briefly summarized, and we highlight the immunomodulatory effects on MDSCs in a variety of disease models and the application of MDSC-targeting molecules for immuno-oncologic therapy.

Key Words: Myeloid-derived suppressor cells (MDSCs), Oligonucleotide-based molecules, Small molecules, Tumor microenvironment, Immune suppression, MDSC-targeting agents

INTRODUCTION

Myeloid-derived suppressor cells (MDSCs) are heterogeneous populations of immature myeloid cells and have been known to possess suppressive activity against immune responses through the reduction of T cell function and proliferation. MDSCs include the following two major subsets: monocytic (MO-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs, also known as granulocytic MDSCs (G-MDSCs)), and both MDSC subsets play crucial and diverse roles in immune-related pathological conditions (Gabrilovich and Nagaraj, 2009; Nam *et al.*, 2019). Evidence has shown that the induction of MDSCs in the tumor microenvironment or the reduction of MDSC activity in autoimmune diseases decreases the therapeutic efficacy of antitumor immunotherapy or immunosuppressive agents. Because of their strong immune suppressive function in various immune responses, a number of oligonucleotides and small molecules have been evaluated to modulate the function, proliferation and accumulation of

MDSCs. Furthermore, over the last decade, therapies using MDSC-targeting molecules alone or with other therapeutics, including immune checkpoint inhibitors and antitumor drugs, have been extensively studied in several disease models, such as autoimmune diseases, infections and cancers. In fact, in recent years, there has been convincing and accumulating evidence that MDSC modulation in combination with immunotherapy is able to provide a beneficial effect on a patient's prognosis, leading to an extended lifespan. In the current review, we will examine, in detail, the MDSC modulation in which small molecules are used to interfere with or to promote the expansion and function of MDSCs and the mechanisms by which they interrupt or facilitate the immune suppressive activity of MDSCs.

Open Access <https://doi.org/10.4062/biomolther.2019.069>

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Received Apr 25, 2019 Revised Jul 2, 2019 Accepted Jul 4, 2019

Published Online Aug 21, 2019

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MODULATION OF MDSC ACTIVITY BY OLIGONUCLEOTIDE-BASED MOLECULES

MDSC expansion and activation by microRNAs

MicroRNAs (miRNAs) are small noncoding RNAs that consist of ~22 nucleotides in length and have crucial and diverse roles in various biological processes and diseases, including the function and differentiation of immune cells, cancer, immunopathology as well as MDSC activity (El Gazzar, 2014; Chen *et al.*, 2015a). Recently, the correlation between MDSCs and miRNAs has been extensively studied. A set of miRNAs (miR-99b, miR-100, miR-125a, miR-125b, miR-146a, miR-146b, miR-155 and let-7e) from the extracellular vesicles (EVs) of melanoma patients has been found to be correlated with MDSCs by regulating the differentiation of monocytes into CD14⁺HLA-DR^{neg} MDSC cells, and they cause a reduction in the therapeutic efficacy of immune checkpoint inhibitors in melanoma patients (Huber *et al.*, 2018). miR-21 directly inhibits phosphatase and tensin homolog (PTEN) and activates signal transducer and activator of transcription 3 (STAT3) via PTEN inactivation, leading to the promotion of the activity and expansion of the CD11b⁺Gr1⁺ MDSCs. Moreover, it has been shown to have a synergistic effect with miR-155, which also induces STAT3 activation via targeting SHIP-1; in fact, these miRs increase in tumor-bearing mice (Li *et al.*, 2014). In a similar fashion, inoculated tumors or the multiplicity of colonic neoplasms by azoxymethane (AOM) and dextran sodium sulfate (DSS) occurred less frequently in miR-155-deficient mice than the occurrence in syngeneic WT mice. These mice show delayed tumor growth, and miR-155 depletion reduces the differentiation and suppressive activity of CD11b⁺Gr1⁺ MDSCs by inducing suppressor of cytokine signaling 1 (SOCS1) expression (Chen *et al.*, 2015b). It has been reported that hypoxia-induced human or mouse glioma cells or human primary glioblastoma-derived exosomes, which contain miR-10a, miR-21, miR-29a and miR-92a, promote the expansion and activity of MDSCs via targeting RAR-related orphan receptor alpha (RORA), PTEN, high-mobility group box transcription factor 1 (*Hbp1*) and protein kinase cAMP-dependent type I regulatory subunit alpha (*prkar1a*) (Guo *et al.*, 2018a, 2018b). In addition, miR10a induces MDSC expansion and activation through another target. The induced expression of miR-10a in MDSCs by PGE₂ from doxorubicin-resistant cancer cells activates AMP-activated protein kinase (AMPK) signaling and leads to the increased expansion and activation of CD11b⁺Gr1⁺ MDSCs (Rong *et al.*, 2016). Since miR-21 induces MDSCs, the inhibition of miR-21 and miR-181b by antagomir, a chemically modified miRNA inhibitor, decreases Gr1⁺CD11b⁺ myeloid progenitors and late-sepsis mortality in an *in vivo* mouse sepsis model, and this is related to the reduction of NF- κ B, which is a transcription factor that plays a role in myeloid cell differentiation (McClure *et al.*, 2014). In addition, miR-34a, which is known to show distinct expression levels in diverse immune cells, induces the expansion of MDSCs. It reduces apoptosis but not proliferation by directly binding to the 3' untranslated region (3'UTR) of N-myc, leading to suppressed expression (Kuchen *et al.*, 2010; Huang *et al.*, 2014; Chen *et al.*, 2016). Hypoxia-inducible factor (HIF1 α) plays a role in tumor-associated macrophages (TAMs) to induce suppressive activity on T cells and tumor progression in a mouse breast cancer model *in vivo* (Doedens *et al.*, 2010). HIF1 α -induced miR-210 under hypoxic conditions has been found in tumor-infiltrated MDSCs and can increase MDSC-mediated T cell suppression via inducing ar-

ginase activity and nitric oxide (NO) production. Accordingly, anti-miR-210 reduces the immunosuppressive activity of MDSCs and tumor progression in a tumor-bearing mouse model (Noman *et al.*, 2015). miR-30 increases in both PMN-MDSCs and MO-MDSCs derived from a B cell lymphoma (non-Hodgkin lymphoma; NHL) mouse model and promotes the differentiation and suppressive activity of MDSCs through SOCS3 inhibition. Treatment with miR-30a agomir in a B-cell lymphoma mouse model leads to an increase in the MDSC population and a reduction in the CD3⁺CD4⁺ and CD3⁺CD8⁺ T cell populations and tumor progression in the spleen, and the observation is reversed by treatment with antagomir (Xu *et al.*, 2017). Similarly, miR-494 promotes the accumulation and function of tumor-infiltrated MDSCs via the downregulation of PTEN and increases tumor invasion and metastasis in an *in vivo* mouse model (Liu *et al.*, 2012). It has been reported that miR-690 is induced by Δ^9 -tetrahydrocannabinol (THC) and promotes functional MDSCs from bone marrow (BM) via the inhibition of transcription factor CCAAT/enhancer-binding protein α (C/EBP α) expression. However, it did not increase as much in tumor-bearing mice as it did in the control mice (Hegde *et al.*, 2013).

Inhibition of MDSCs by microRNAs

Although the vast majority of studies have reported a positive correlation of miRs with MDSCs, studies have shown a negative correlation to exist. It has been documented that miR-223 is downregulated in tumor-associated MDSCs. The transfection of miR-223 into bone marrow cells shows a reduced differentiation of tumor-induced CD11b⁺Gr1⁺ MDSCs by inhibiting myocyte enhancer factor 2C (MEF2C) and miR-223 impairs the tumor infiltration and suppressive activity of MDSCs on CD8⁺ T cells and tumor growth in tumor-bearing mice (Liu *et al.*, 2011). Furthermore, it has been reported that MDSCs play a crucial role in multiple sclerosis (MS) and that the miR-223 knockout (miR-223^{-/-}) mouse MS model, which is an experimental autoimmune encephalomyelitis (EAE) model, shows an enhanced MDSC population and less severe EAE by targeting STAT3 (Cantoni *et al.*, 2017). Likewise, miR-17-5p and miR-20 have suppressive roles in MDSCs, and they are found in tumor-associated MDSCs at a concentration less than in disease-free mice. These miRs directly inhibit STAT3 expression, leading to a reduction in the expression of reactive oxygen species (ROS) and H₂O₂, which are activity markers of MDSCs. In addition, miR-17-5p and miR-20 reduce the suppressive function of MDSCs against CD4⁺ and CD8⁺ T cells and tumor growth *in vivo*. Interestingly, although a reduction in miRs has been found in both PMN-MDSCs and MO-MDSCs, they inhibit the activity of PMN-MDSCs only (Zhang *et al.*, 2011). Collectively, these reports suggest that miRNAs are related with modulation of MDSC function through several mechanisms and could be utilized in diverse immune-related diseases models.

Inhibition of MDSCs by RNAi

RNA interference (RNAi) was first identified in plants and fungi and has a role in regulating gene expression through posttranscriptional silencing. Since the identification of the function of RNAi, it has been studied for diverse disease models to regulate target genes, especially the genes encoding undruggable proteins (Kanasty *et al.*, 2013; Setten *et al.*, 2019). C/EBP β is known to play a role in MDSC suppressive function (Marigo *et al.*, 2010). It has been reported that both C/EBP β

silencing and miR-142-3p overexpression using poly (amidoamine) (PAMAM) dendrimer carriers show therapeutic effects in tumor-bearing mice, and this result suggests the possibility of RNAi-based therapies to target myeloid cells (Zilio *et al.*, 2017). RNAi, such as shC/EBP β or miR142-3p with encapsulated polyarginine nanocapsules (NCs), facilitates the uptake by MDSCs and reduces the C/EBP β -mediated immunosuppressive activity of MDSCs as well as the generation of TAMs *in vitro* and in tumor bearing mice *in vivo* (Ledo *et al.*, 2018). These findings suggest the possibility to develop the strategy for blocking immune suppressive function through RNAi by targeting crucial factors in MDSCs.

Ambivalent effects of CpG on MDSC modulation

The CpG motif that is included in synthetic oligodeoxynucleotides (ODNs) reveals the ability to stimulate immune responses similar to bacterial DNA in the innate immune system. CpG ODN, which triggers Toll-like receptor (TLR) 9-expressing cells, plays a role in immunomodulation and has been used for immunotherapy of several cancers (Klinman, 2004). CpG oligonucleotides (CpG) increase the proportion of Gr1⁺CD11b⁺ MDSC-like cells and do not affect the suppressive activity of MDSCs on T cells, but the Ly6G⁺ MDSC-like cells among the Gr1⁺CD11b⁺ MDSC-like cells are reduced in the spleen and BM of tumor-free mice. Interestingly, CpG treatment inhibits tumor progression and MDSC-mediated T cell suppression in the CT-26 tumor-bearing mouse model, in which CpG inhibits Ly6G⁺ MDSCs similar to that seen in tumor-free mice. The suppressive activity of MDSCs on T cells is also reduced by CpG treatment in the spontaneously developed gastric tumor model. Furthermore, CpG increases the expression of myeloid cell markers, such as F4/80, MHC II, CD11c and CD80, and induces MDSC maturation and differentiation *in vivo*. In addition, these effects are dependent on TLR9-expressing DCs secreting IFN- α , which elicits a reduction in MDSC function *in vitro* and *in vivo* (Zoglmeier *et al.*, 2011). Similarly, it has been reported that the intratumoral injection of CpG decreases tumor growth, leading to a reduction in the MO-MDSC population in CT26 tumor-bearing mice, and CpG treatment down-regulates the suppressive activity of MO-MDSCs on CD8⁺ T cells through the inhibition of Arg1 expression. Moreover, CpG induces the expression of the macrophage marker F4/80 in MO-MDSCs obtained from the spleens of tumor-bearing mice, which reflects MDSC differentiation into macrophages (Shirota *et al.*, 2012). In contrast, although DCs are differentiated from BM by treatment with GM-CSF and IL-4, the additional long-term treatment with CpG ODN induces cells to exhibit different phenotypes with a distinct cytokine profile, which is identified as MDSCs. CpG ODN-induced MDSCs also have suppressive activity on CD4⁺ T cell proliferation. In addition, a *Listeria monocytogenes* infection, which has been known to exhibit a cytokine profile similar to that of CpG ODN, reduces the DC proportion and can increase the MDSC proportion in mice (Chen *et al.*, 2013).

AMBIVALENT ROLES OF KINASE INHIBITORS IN THE MODULATION OF MDSC ACTIVITY

Tyrosine kinase inhibitor (TKI)

The receptor tyrosine kinases (RTKs) are composed of 20 subfamilies with 58 types of receptors including epidermal

growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR) and have been found to be dysregulated in a variety of tumors. For that reason, various receptor tyrosine kinase inhibitors (TKIs), such as lapatinib, sunitinib and erlotinib, have been evaluated for their cancer therapeutic potential (Hojjat-Farsangi, 2014; Yamaoka *et al.*, 2018). Recently, many studies have verified the role of TKIs not only in cancer but also in MDSCs. Sunitinib (Sutent, Pfizer, New York, NY, USA) has been known to target several RTKs, including VEGFR, PDGFR, stem cell factor receptor (c-Kit) and colony-stimulating factor (CSF)-1 receptor, and has been used therapeutically for metastatic renal cell carcinoma (mRCC) as a first-line therapy (Motzer *et al.*, 2006; Roskoski, 2007). Intriguingly, it has been reported that sunitinib inhibits the accumulation and suppressive activity of MDSC and T-regulatory cells (Tregs; CD4⁺CD25^{hi}/Foxp3⁺) in RCC tumor-bearing mice as well as in other types of tumors, such as colon and breast cancer. In particular, it suppresses the proliferation of MO-MDSCs and induces the apoptosis of PMN-MDSCs. Nevertheless, MDSCs acquire resistance to sunitinib by granulocyte-macrophage colony-stimulating factor (GM-CSF) obtained from tumor tissue in a STAT5-dependent manner *in vitro* and *in vivo*. Furthermore, whereas sunitinib remarkably reduces human MDSCs (CD33⁺CD15⁺HLA-DR⁻ and CD15⁻CD33⁺HLA-DR⁻) in the peripheral blood (PB) of primary tumor resected mRCC patients, MDSCs are restored in patients with primary tumors because of tumor-infiltrated MDSCs, which are not affected as much by sunitinib, leading to resistance to the treatment (Ko *et al.*, 2009, 2010; Finke *et al.*, 2011; Guislain *et al.*, 2015). CD40 is a member of the tumor necrosis factor receptor (TNFR) superfamily and plays a role in the activation of dendritic cells (DCs) and the induction of adaptive immunity. However, it also plays a role in promoting VEGF expression and MDSC function to induce T cell tolerance and Treg cell accumulation in the tumor (Flaxenburg *et al.*, 2004; Pan *et al.*, 2010; Vonderheide and Glennie, 2013). Although immunotherapy strategies that target CD40 have antitumor effects, these strategies only show quite a limited efficacy due to the immunosuppressive cells in the tumor. The combination treatment of an anti-CD40 antibody with sunitinib induces CD11c⁺ DC activation and reduces the number of CD11b⁺Gr1⁺ MDSCs in the tumor-draining lymph node and leads to the promotion of therapeutic efficacy in both B16F10 and T241 tumor-bearing mice (van Hooren *et al.*, 2016). In another combination therapy, co-treatment of sunitinib with viral vector-based cancer vaccine, which contains Semliki Forest virus replicon particles and encodes the oncoproteins E6 and E7 (SFVeE6,7) of human papilloma virus (HPV), increases the ratio of E7-specific cytotoxic T lymphocytes (CTLs) to MDSCs in the blood and tumors of TC-1 tumor-bearing mice. This combination therapy exerts its antitumoral effects by changing the immunological balance of the tumor (Draghiciu *et al.*, 2015). Although several reports have shown an inhibitory effect of sunitinib on MDSCs in some tumor models, it has also been reported that the monocytic CD14⁺HLA-DR^{-low} MDSCs that are differentiated from mature human monocytes by activated hepatic stellate cells (HSCs) were not affected by sunitinib. Other TKIs, including dasatinib, nilotinib and sorafenib, inhibit MDSC differentiation under the same conditions, but they do not affect differentiated MDSCs. Consistent with these results, the suppressive activity of MDSCs on CD8⁺ T cells is

reduced by treatment with sorafenib, nilotinib or dasatinib, but the activity is not reduced by treatment with sunitinib (Heine *et al.*, 2016). Sorafenib, a TKI inhibitor targeting multiple kinases, has been known as the first-line therapy for patients with advanced liver cancer as well as several other types of cancer. While sorafenib does not affect CD4⁺CD25⁺FOXP3⁺ Tregs and CD11b⁺Gr-1⁺MDSCs in normal healthy mice, it does reduce the population of Tregs and CD11b⁺Gr-1⁺ MDSCs in both the bone marrow and the spleens of liver hepatoma tumor-bearing mice (Cao *et al.*, 2011). However, it has also been reported that the proportion and suppressive activity of tumor-infiltrated PMN-MDSCs are increased by sorafenib in a mouse orthotopic liver tumor model, although they are decreased by sorafenib in a subcutaneous mouse model. In addition, the expression of the IL-6 receptor is increased in PMN-MDSCs from an orthotopic mouse model; this leads to an increase in STAT3 signaling, which is a crucial mechanism for its suppressive activity (Chang *et al.*, 2018). Axitinib, a selective small molecule TKI of VEGF1, 2 and 3 signaling, reduces the suppressive activity of MO-MDSCs and induces tumor-infiltrated immune cells, including CD3⁺CD8⁺ T cells and CD11b⁺ cells, in a mouse melanoma model *in vivo* (Zhang *et al.*, 2014; Du Four *et al.*, 2015). AZD4547, a fibroblast growth factor receptor (FGFR)-targeted small-molecule TKI, is being tested in a phase II clinical study of breast cancer patients. AZD4547 suppresses breast tumor progression *in vitro* and *in vivo* in a mouse model and, consistent with the inhibition of tumor progression, reduces tumor-infiltrated CD11b⁺Gr-1⁺ MDSCs as well as MDSCs in the lung tissue, peripheral blood and spleen (Liu *et al.*, 2014). SAR131675 has been known as a specific TKI against VEGFR-3, suppresses the circulating MDSCs, promotes the proportion of M1-type TAMs and inhibits tumor progression in the 4T1 tumor model. Moreover, it blocks the number of MDSCs expressing VEGFR3 in the blood and spleen of 4T1 tumor-bearing mice (Espagnollet *et al.*, 2014). In summary, these reports suggest that the most of TKIs inhibit MDSCs in several diseases models, whereas some of TKIs show controversial effect on MDSC. Therefore, the exact mechanisms by which each TKI exerts therapeutic efficacy in MDSCs should be further verified in specific disease model.

Tofacitinib (inhibitor of the enzyme janus kinase 1 (JAK1) and janus kinase 3 (JAK 3))

The janus kinase (JAK) family, which includes JNK1, JAK2, JAK3 and tyrosine kinase 2 in mammals, is a receptor-associated tyrosine kinase. The JAKs are activated by binding to the intracellular domains of type I and type II cytokine receptors and induce signaling pathways related to diverse cytokines and growth factors. JAK-dependent cytokines have diverse roles in immunopathology by regulating inflammatory-related genes (Changelian *et al.*, 2003; Nishimura *et al.*, 2015; Schwartz *et al.*, 2017). A small-molecule JAK inhibitor, tofacitinib, has been shown to inhibit JAK1 and JAK3 (Changelian *et al.*, 2003). It has been reported that PMN-MDSC accumulation is elevated in the bone marrow of tofacitinib-treated SKG mice and that tofacitinib induces the differentiation and expansion of MDSCs *in vitro*. Therefore, it ameliorates rheumatoid arthritis (RA) in an animal model, and the effect is abolished by treatment with anti-Gr-1 mAb. Moreover, another JAK1 selective inhibitor, GLPG0634, and the JAK3 selective inhibitor, PF-956980, have also been found to induce the differentiation of MDSCs *in vitro* (Nishimura *et al.*, 2015). Although STAT3

inhibition generally hampers MDSCs differentiation and proliferation, some of JAK inhibitors show opposite results. Given that JAK has several family members and each JAK might influence different functions of MDSCs, it is important to investigate the specific function of MDSCs controlled by JAKs.

AZD1480 (inhibitor of JAK1/2 kinase)

AZD1480, a competitive small-molecule inhibitor of JAK1/2, has antitumor effects, such as tumor growth inhibition in a STAT3-dependent manner *in vitro* and *in vivo* (Scuto *et al.*, 2011). It has been reported that AZD1480 also regulates MDSCs in a melanoma mouse model. Although the MDSC population (MO-MDSCs; CD11b⁺Ly6C⁺Ly6G⁻ and PMN-MDSCs; CD11b⁺Ly6C^{low}Ly6G⁺) from tumor bearing mice is reduced by the oral administration of AZD1480, the suppressive effect of MDSCs on T-cell proliferation is increased by AZD1480 in a similar way to that of tofacitinib (Maenhout *et al.*, 2014).

Cyclin-dependent kinase (CDK) inhibitor

p16^{Ink4a} and p21^{Cip1/Waf1} are tumor suppressors that regulate cellular senescence through CDK inhibition (Hara *et al.*, 1996; Okuma *et al.*, 2017). However, it has been reported that MO-MDSCs express p16^{Ink4a} and p21^{Cip1/Waf1}, and PMN-MDSCs only express p16^{Ink4a} but do not show cellular senescence features. In addition, although the immunosuppressive effects of MDSCs on T cell proliferation are not changed by p16/p21-DKO, the number of intratumoral MO-MDSCs is reduced in tumor-bearing p16/p21-DKO mice. These effects originate from the blocking of CX3CL1/CX3CR1 axis-dependent chemotaxis in p16/p21-DKO mice and CX3CL1 expression in MO-MDSCs is reduced by CDK. Furthermore, MO-MDSCs treated with the pan-CDK inhibitor flavopiridol, which is known as a potential antitumor agent especially in leukemia, have shown elevated levels of *Cx3cr1* expression, and the accumulation of MO-MDSCs in tumor sites has been observed after treatment; this leads to the acceleration of tumor growth *in vivo* (Arguello *et al.*, 1998; Okuma *et al.*, 2017).

Phosphoinositide 3-kinase (PI3K) inhibitor

PI3Ks have a crucial role in diverse cellular signaling pathways by mediating signals from cell surface receptors to downstream pathways. PI3Ks are divided into three different classes based on amino acid sequence and functional homology. Among them, the class I PI3Ks are divided into two different subsets. The class IA PI3Ks are heterodimeric and are composed of a regulatory subunit (p85) with catalytic subunits (p110 α , p110 β and p110 δ) and class IB is composed of regulatory subunits (p101 and p84) with the catalytic subunit p110 γ . PI3Ks have been demonstrated to play important roles in hematopoietic cell biology and in the activation of Gr1⁺/CD11b⁺ myeloid cells (Schmid *et al.*, 2011; Cushing *et al.*, 2012; Winkler *et al.*, 2013; Kim *et al.*, 2014). J32, a PI3K p110 α (PI3K α)-specific inhibitor, has shown cytotoxicity to PMN-MDSCs compared with CD8⁺ T cells with lower concentrations, and the combination treatment of J32 with immune checkpoint blockade antibodies, such as anti-PD-1 and anti-CTLA-4, into 4T1 tumor-bearing mice has shown remarkably reduced numbers of PMN-MDSCs and 4T1 tumors (Mandelker *et al.*, 2009; Kim *et al.*, 2014). IPI-145 (also known as INK-1197), a PI3K isoform p110 δ (PI3K δ) and p110 γ (PI3K γ)-specific inhibitor, has been clinically evaluated for the treatment of multiple inflammatory, autoimmune and hematologic diseases. It has been reported

that the inactivation of PI3K δ reduces tumor growth and metastasis, Treg cell function and PMN-MDSC number and function in PI3K δ^{D910A} mice that have inactivated p110 δ . In accordance, IPI-145 treatment into tumor infiltrating PMN-MDSCs leads to a reduction in the arginase 1 (Arg1), NOS2 mRNA levels and immune suppressive effect on T cells, whereas tumor-infiltrating MDSCs and primary tumor growth are not significantly changed by treatment with IPI-145. However, the combination treatment of IPI-145 with the immune checkpoint inhibitor PD-L1 mAb significantly reduces the suppressive activity of PMN-MDSCs and reduces the primary tumor growth; in addition, this strategy improves the CD8 $^+$ T cell activation and survival of murine oral cancer (MOC) tumor-bearing mice. (Winkler *et al.*, 2013; Ali *et al.*, 2014; Davis *et al.*, 2017).

Casein kinase 2 (CK2) inhibitor

Casein kinase 2 (CK2), a serine/threonine protein kinase, plays a role in tumor progression and is involved in diverse signaling pathways related to self-renewal, cell proliferation, migration and hematopoietic cell survival and function (Piazza *et al.*, 2012). Potent and selective inhibitors of CK2, including BMS-595, BMS-699 and BMS-211, can reduce the cell proliferation of various mouse cell lines and human colorectal and lung cancer cell lines. They show a synergistic effect on cell proliferation inhibition with the anti-CTLA-4-mIgG2a antibody, an immune checkpoint inhibitor. The CK2 inhibitor BMS-595 reduces the population of CD11b $^+$ Ly6G $^+$ Ly6C lo PMN-MDSCs and CD11b $^+$ Ly6G $^+$ F4/80 hi macrophages in the spleens of LLC tumor-bearing mice, although the CD11b $^+$ Ly6G $^+$ Ly6C hi MO-MDSC and CD11c $^+$ I-Ab hi DC populations are not altered. However, while the presence of CD11b $^+$ Ly6G $^+$ Ly6C lo PMN-MDSCs is not changed at the tumor site, the populations of TAMs and DCs are decreased. The alteration of the immune cell population by CK2 inhibition is caused by the change of differentiation through the regulation of the function of C/EBP α , but not by the change of the apoptosis or proliferation of myeloid cells (Hashimoto *et al.*, 2018).

INHIBITION OF MDSCS BY STAT3, PDE5 AND γ -SECRETASE INHIBITORS

STAT3 inhibitor

It has been identified that there are 7 members of the STAT family in mammals. STATs are latent cytoplasmic transcription factors and are activated by several cytokines and growth factors. They are involved in diverse signaling pathways related to the development, immunity and malignant properties of human cancers (Yu and Jove, 2004; Ko and Kim, 2016). Therefore, the regulation of the STAT family in various diseases, including cancer and immune response-mediated diseases, has been broadly studied. Among the several STATs, STAT3 signaling is known as the novel factor responsible for the expansion and activation of MDSCs by regulating the pro-inflammatory, pro-proliferation or anti-apoptotic proteins (Nefedova *et al.*, 2005; Gabrilovich *et al.*, 2012). STAT3 also induces MDSC migration and accumulation by regulating pro-inflammatory proteins, such as S100A8/A9 (Cheng *et al.*, 2008; Sinha *et al.*, 2008). For that reason, several small molecule inhibitors of STAT3 have been evaluated in preclinical and clinical studies. In fact, it has been documented that STAT3 inhibition by the pSTAT3-specific small molecule inhibi-

tor STATTIC (6-nitro-1-benzothiophene 1,1-dioxide) or siRNA reduces the expression of the MO-MDSC activity marker Arg1 in MO-MDSCs derived from patients with ankylosing spondylitis (Liu *et al.*, 2018). STATTIC not only reduces the activity of MO-MDSCs but also increases the pro-apoptotic pathway in MDSCs. A recent study has shown that STATTIC and another STAT3 inhibitor, BBI608 (napabucasin), induce apoptosis in liver tumor MDSCs in a bax-dependent manner. STAT3 inhibition by these inhibitors in liver-associated MDSCs (L-MDSCs) activates both the intrinsic and extrinsic pathways of apoptosis and induces the enhancement of the antitumor activities of chimeric antigen receptor T cells (CAR-T) in a murine liver metastasis (LM) model (Guha *et al.*, 2019). S3I-201, a tolerable selective inhibitor of STAT3, decreases the population of MDSCs in a genetically defined mouse model with head and neck squamous cell carcinoma. The number of MDSCs is increased in tumor-bearing mice, but it is diminished after treatment with S3I-201 (Bu *et al.*, 2016). In addition, the STAT3 selective inhibitor JSI-124 induces the differentiation of Gr-1 $^+$ CD11b $^+$ immature myeloid cells, which represent MDSCs, into mature DCs and macrophages (Nefedova *et al.*, 2005). Interleukin-10 (IL-10) is known to be an anti-inflammatory cytokine, but it has been verified that IL-10 has a role in STAT3 signaling by regulating IL-6. Interestingly, it has been shown that pSTAT3 is elevated in MDSCs obtained from IL-10-deficient mice compared with that of wild-type mice, and the elevated tumor infiltration of MDSCs in IL-10-deficient mice is abolished by an IL-6 blockade (Lee *et al.*, 2016). In fact, several studies have reported MDSC suppression by STAT3 inhibition leading to alleviation of tumor development. Thereby, these findings suggest that STAT3-mediated signaling could be an ideal therapeutic target for patients with abnormal MDSC accumulation.

PDE5 inhibitor

Tadalafil is a potent and selective inhibitor of cyclic GMP-specific phosphodiesterase type 5 (PDE5) and has been utilized for erectile dysfunction and pulmonary arterial hypertension (PAH) (Ghofrani *et al.*, 2004). However, recent reports have revealed that PDE5 inhibition by tadalafil and another PDE5 inhibitor, sildenafil, induces antitumor effects by regulating immune cells (Serafini *et al.*, 2006; Noonan *et al.*, 2014; Califano *et al.*, 2015; Weed *et al.*, 2015). Tadalafil and sildenafil delay primary tumor growth in immuno-competent mice and sildenafil decreases the nitric oxide synthase-2 (NOS2) and MDSC maker IL-4R α as well as its downstream Arg1, thereby decreasing the suppressive effect of MDSCs on T cell proliferation. Therefore, PDE5 inhibition prevents tumor-induced immune dysfunction in mouse tumor models and restores the proliferation of peripheral blood lymphocytes (PBLs) from myeloma and head and neck cancer patients (Serafini *et al.*, 2006). In accordance with sildenafil, tadalafil has also shown a clinical response in patients with end-stage relapsed/refractory multiple myeloma and head and neck squamous cell carcinoma (HNSCC) by reducing MDSC function. It downregulates the mRNA expression of inducible nitric oxide synthase (iNOS) and Arg1, IL-4R α and reactive oxygen species (ROS) levels that are related to MDSC activity. Moreover, tadalafil can reduce the Treg cell concentration in the blood and tumor and can induce the expression of the TCR- ζ chain and the production of IFN γ in bone marrow-infiltrating CD8 $^+$ T cells from patients (Noonan *et al.*, 2014; Califano *et al.*, 2015; Weed *et al.*, 2015).

γ -Secretase inhibitor

Notch signaling has an important role in the development and differentiation of stem/progenitor cells and diseases, including several cancers. The effect of Notch signaling on the activity and differentiation of MDSCs has also been reported (Wang *et al.*, 2016; Hossain *et al.*, 2018). Recombination signal binding protein-J κ (RBP-J) is a transcription factor mediating signaling from all four mammalian Notch receptors, which are related to γ -secretase-mediated proteolytic mechanisms. The blockade of Notch-RBP-J signaling reduces the immunosuppressive activity of MDSCs on T cell proliferation, DC maturation and antigen presentation, leading to the suppression of tumor growth (Wang *et al.*, 2016). MDSCs, TAMs and Tregs have been implicated and have a crucial role in the head and neck squamous cell carcinoma (HNSCC) tumor microenvironment (Azoury *et al.*, 2016). Notch 1 signaling is activated in *Tgfb1/pten* double conditional knockout HNSCC mouse models, and Notch1/HES1 inhibition by DAPT, a γ -secretase inhibitor, reduces both the populations of PMN-MDSCs and MO-MDSCs, as well as the levels of TAM, Tregs and immune checkpoint molecules (PD1, CTLA4, TIM3 and LAG3) in HNSCC tumor-bearing mice. Interestingly, HES1 expression reveals a significant positive correlation with TAM and MDSC markers, immune checkpoint and Foxp3 expression (Mao *et al.*, 2018).

INHIBITION OF MDSCS BY AMPK ACTIVATOR

In general, AMPK activation stimulates energy production through glucose and fatty acid oxidation, whereas it inhibits anabolic processes, such as glycogen synthesis, gluconeogenesis, fatty acid synthesis and cholesterol synthesis. There is also convincing evidence that AMPK activation prevents inflammatory responses through the inhibition of pro-inflammatory signaling pathways (O'Neill and Hardie, 2013). Interestingly, in a few studies, it has been demonstrated that AMPK activators can inhibit the function of MDSCs and can elicit anti-tumor activities in various types of cancers (Trikha *et al.*, 2016; Kim *et al.*, 2017; Qin *et al.*, 2018). AMPK activators reduce the numbers of MDSCs in both the spleens and tumors of carcinoma-bearing mice. The administration of the AMPK activator increases the numbers of T cells and NK cells in the spleens and tumors of tumor-bearing mice. Moreover, treatment with metformin, an AMPK activator, reduced the accumulation of MDSCs into esophageal tumors by inhibiting NF- κ B signaling in an AMPK-dependent manner (Qin *et al.*, 2018). In addition, it has recently been reported that metformin-induced AMPK activation can reduce the expression of CD39 and CD73 in the MDSCs of ovarian cancer patients and can consequently attenuate the immunosuppressive activity of MDSCs (Li *et al.*, 2018b). These findings suggest that AMPK activators could be promising drug candidates for the future treatment of cancers through the improvement of antitumor immunity by inhibiting MDSC-mediated immunosuppression.

MDSC ACTIVATION OR EXPANSION BY IMMUNE MODULATORS THAT REGULATE INFLAMMATORY RESPONSES

Dexamethasone

Dexamethasone (Dex), one of the glucocorticoid family members, is a classic immune regulatory drug that has been extensively used for patients with inflammatory disorders (Abraham *et al.*, 2006). Dex induces CD11b⁺Gr-1^{int/low} MDSCs by treatment with GM-CSF, whereas the CD11b⁺Gr-1^{high} population is not changed by Dex *in vitro*. It also promotes MDSC-mediated T cell suppression through the iNOS pathway *in vitro*. GM-CSF plus Dex-induced MDSCs (MDSC-Dex) induces cardiac allograft survival after transferring allogeneic cardiac transplants into mice. In addition, Dex increases the infiltration of MDSCs into allografts depending on glucocorticoid receptor (GR) signaling and MDSC-Dex increases the proportion of CD4⁺Foxp3⁺ Tregs in MDSC-receiving mice (Zhao *et al.*, 2018b). Patients with immune thrombocytopenia (ITP) often show a reduced number of MDSCs with immune suppressive function. High doses of dexamethasone increase the number of CD11b⁺CD33⁺HLA-Dr^{low} MDSCs in ITP patients and enhance MDSC function through the transcription factor *Ets1 in vitro*. Therefore, the immunopathogenesis of ITP can be alleviated by Dex-modulated MDSCs that are transferred into a murine severe ITP model (Hou *et al.*, 2016). As mentioned earlier, miR-21 and miR-155 induce the expansion of MDSCs. Interestingly, Dex increases the levels of miR-21 and miR-155. In addition, it promotes MDSC expansion and suppressive activity of MDSCs and promotes the mRNA levels of iNOS and Arg1 by co-treatment with GM-CSF and IL-6 (Li *et al.*, 2014).

Glucocorticoids (GCs)

Glucocorticoids (GCs) have been utilized as anti-inflammatory drugs for patients with organ transplantation or inflammatory disorders but their uses have been limited by potential side effects, such as hepatic steatosis induction (Schacke *et al.*, 2002). Dex-treated mice show increased numbers of PMN-MDSCs and increased levels of hepatomegaly with the upregulation of triglyceride, cholesterol and free fatty acids by a deficiency of activating transcription factor 3 (ATF3). Moreover, mice transplanted with PMN-MDSCs from Dex-treated WT or ATF3^{-/-} mice exhibits an increase in liver weight and lipid contents in a GR-dependent manner. GR reduces the expression of ATF3 in myeloid cells by binding to the negative GR-response elements (nGREs) of the ATF3 promoter region, leading to the upregulation of S100A8 and S100A9 expression; this causes the induction of MDSC development and activation. As a result, GC-induced hepatic steatosis can occur (Liu *et al.*, 2016).

mTOR inhibitor

The mammalian target of rapamycin (mTOR) plays a crucial role in cellular processes, including cell growth, metabolism, differentiation and autophagy, as well as immune responses. The mTOR complex is divided into mTORC1 and mTORC2, and rapamycin (RAPA) is a specific inhibitor of mTORC1 (Engelman, 2009). Recently, the role of the mTOR pathway has been described in MDSCs. RAPA has been found to induce the expansion and function of PMN-MDSCs *in vitro* as well as *in vivo*, such as in an acute graft-versus-host disease (aGVHD) mouse model through the upregulation of Arg1 and

iNOS (Lin *et al.*, 2018). In line with these observations, RAPA promotes the accumulation of PMN-MDSCs into the injured kidney through CXCL1,2/CXCR2 interaction-induced migration in a mouse acute kidney injury (AKI) model. The administration of RAPA-treated MDSCs to AKI mice alleviates renal function and reduces the proportion of CD4⁺ T cells. Importantly, while MO-MDSCs are reduced by RAPA, the mRNA levels of the activation markers iNOS and Arg1 are increased (Zhang *et al.*, 2017). Furthermore, RAPA increases the number of iNOS-expressing MDSCs in the allograft vessels in a murine cardiac transplantation (CTx) model and leads to an increase in the graft survival rate. In addition, RAPA promotes the suppressive activity of both PMN-MDSCs and MO-MDSCs, and MDSCs derived from rapamycin-treated CTx mice induce CD3⁺CD4⁺Foxp3⁺ Tregs and allograft survival. However, the mitogen-activated protein kinase kinase (MEK) inhibitor trametinib abolishes the induction of RAPA-mediated MDSC expansion and allograft survival (Nakamura *et al.*, 2015). It has also been reported that the tumor-infiltrating MO-MDSCs reveal stronger suppressive activities than those of the splenic MO-MDSCs via higher glycolysis, and the inhibition of glycolysis by RAPA results in the reduction of MO-MDSC function in tumor-bearing mice (Deng *et al.*, 2018). Interestingly, the nanomicelle-modified RAPA (RAPA nanomicelle ophthalmic solution), which is the modified, amphiphilic form (RAPA is insoluble in water), increases the ability of RAPA to accumulate MDSCs and prolongs corneal allograft survival in a mouse model. MDSCs derived from RAPA nanomicelle ophthalmic solution-treated mice exhibit an increased ability of MDSCs to suppress T cell proliferation (Wei *et al.*, 2018). Another mTOR inhibitor INK128, an oral mTORC1/2 dual inhibitor, also reduces DSS-induced colitis and the differentiation of MDSCs into macrophages leads to the expansion of Tregs in a mouse experimental colitis model (Shi *et al.*, 2019).

HMG-CoA inhibitor

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a rate-limiting enzyme that converts HMG-CoA into mevalonate in the cholesterol synthesis pathway. Statins, also known as HMG-CoA reductase inhibitors, have diverse roles in immunomodulation and there are various types of statins prescribed, including atorvastatin (Lipitor; Pfizer), simvastatin (Zocor; Merck & Co., Kenilworth, NJ, USA), lovastatin (Mevacor; Merck & Co. and Altprev; Andrx Labs, Fort Lauderdale, FL, USA) and rosuvastatin (Crestor; AstraZeneca, Cambridge, UK) (Greenwood *et al.*, 2006). Recent studies have demonstrated an interesting relationship between statins and MDSCs. Atorvastatin induces the expansion of PMN-MDSCs *in vitro* and *in vivo* through the inhibition of the mevalonate pathway and the suppressive activity of MDSCs is promoted by atorvastatin *in vivo* in a NO-dependent manner, even though MDSCs from the control mouse spleen do not show immunosuppressive activity. Atorvastatin alleviates DSS-induced colitis and increases PMN-MDSCs. Moreover, it has been demonstrated that while MDSCs are increased in a mouse DSS-induced acute and chronic colitis model, the symptoms are reduced by transferring atorvastatin-derived PMN-MDSCs into colitis mice (Lei *et al.*, 2016). In line with this observation, we have also found that simvastatin disrupts IRF4 expression, which is correlated with the inhibition of MDSC differentiation and function and the differentiation of PMN-MDSCs derived from BM cells is increased by treat-

ment with simvastatin. Furthermore, simvastatin can induce the suppressive activity of MDSCs on CD8⁺ T cell proliferation via promoting IL-10 and ROS production (Nam *et al.*, 2016).

Prostaglandin E2 (PGE2)

PGE2 is released from various tumor cells and plays an important role in the tumor microenvironment by promoting tumor progression and inflammation and regulating several signaling pathways (Nandi *et al.*, 2017). MDSCs from tumor-bearing mice express all four E-type prostanoid receptors, which are composed of EP1, EP2, EP3 and EP4. In particular, the PGE2 or EP2 agonist butaprost induces the differentiation of BM cells into MDSCs, and these effects are abolished by the EP1 and EP2 antagonist AH6809EP4 and/or antagonist AH23848 *in vitro*. Furthermore, the accumulation and suppressive activity of MDSCs are decreased in tumor-bearing EP2^{-/-} mice when compared with that of wild-type BALB/c mice and these EP2^{-/-} mice develop primary tumors with slower growth rates compared with those in the WT mice. In addition, the COX-2 inhibitor SC58236 that inhibits PGE2 biosynthesis suppresses the growth of 4T1 primary tumors and reduces MDSC accumulation (Sinha *et al.*, 2007).

Finasteride (FIN), an FDA-approved drug for patients with benign prostatic hyperplasia (BPH), has been found to induce HLA-DR^{-low}CD11b⁺CD33⁺ MO-MDSCs derived from fresh human peripheral blood mononuclear cells (PBMCs), and FIN-induced MDSCs exhibit suppressive activity on CD4⁺ and CD8⁺ T cells. FIN also increases the expression of Arg1 followed by the activation of the COX-2/PGE2 pathway. Interestingly, the effect of FIN on MDSC generation is abolished by the COX1/2-specific inhibitor indomethacin and the PGE2-neutralizing antibody (Zhang *et al.*, 2016).

INHIBITION OF THE IMMUNOSUPPRESSIVE FUNCTIONS OF MDSCS BY COX-2, IDO1, NOS, DNMT AND HDAC INHIBITORS

Cyclooxygenases-2 (COX-2) inhibitor

COX-2 has enzymatic activity in the biosynthesis of prostaglandins and plays diverse roles in cancer. It has been reported that COX-2 induces Arg1 expression in myeloid suppressor cells (MDSCs) associated with lung carcinoma (Rodriguez *et al.*, 2005). The COX-2 inhibitor celecoxib inhibits prostaglandin E2 (PGE2), which is a COX-2 downstream molecule, both *in vitro* and *in vivo*. Interestingly, the oral administration of celecoxib reduces the PMN-MDSC population in the spleens of mice inoculated with the mesothelioma AB1 cell line. ROS production, which inhibits the ζ-chain on T cells and leads to an increase in T cell tolerance, is reduced through COX-2 inhibition in all MDSC subsets obtained from celecoxib-treated mice. Moreover, tumor-infiltrated MDSCs are decreased by celecoxib, which suppresses tumor-derived PGE2 and NO levels in tumor-bearing mice (Veltman *et al.*, 2010).

Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor

IDO1 is an intracellular enzyme related to the kynurenine pathway and plays a crucial role in the inflammatory environment, including the tumor microenvironment (TME) (Moon *et al.*, 2015). IDO1 is highly expressed in F4/80⁺Gr1^{int}CD11b⁺ MDSCs but is only weakly expressed in GR1^{int}CD11b⁺ MDSCs. Moreover, the proportion of F4/80⁺Gr1^{int}CD11b⁺ MDSCs

with IDO1⁺ is increased in a mouse anti-PD1-resistant lung cancer model (344SQ_R) compared with that in the tumor-bearing mice of its parent model 344SQ_P. In contrast, the population of F4/80⁺Gr1^{int}CD11b⁺ MDSCs is reduced by treatment with the IDO1 inhibitor INCB023843. Importantly, the proportion of CD8⁺ T cells is reduced in the 344SQ_R mice and the percentage of the Treg cell population is not altered compared with the proportion and percentage in 344SQ_P mice (Li *et al.*, 2018a). Therefore, inhibitors targeting IDO1 in MDSCs provide an opportunity for development of a new strategy for anti-PD1 therapy-resistant cancers.

Nitric-oxide synthase (NOS) inhibitor

Nitric oxide (NO) is released from endothelial cells or cancer cells as well as from MDSCs by NOS and plays an important role in diverse physiological and pathological processes including cell differentiation, tumor progression and inflammation. Moreover, NO induces immunosuppressive markers of MDSCs through COX-2/PGE2 axis (Groth *et al.*, 2018). For that reason, various inhibitors targeting NO have been evaluated for several cancers and immune-related disorders. N(G)-nitro-L-arginine methyl ester (L-NAME) is a NOS inhibitor and reduces NO production and tumor growth. As mentioned earlier, the PDE5 inhibitor sildenafil delays primary tumor growth and inhibits MDSC function and reduces the expression of Arg1 and NOS2 in mouse tumor models and patients with myeloma or head and neck cancer (Orucevic *et al.*, 1999; Serafini *et al.*, 2006). Tumor growth is delayed by both L-NAME and sildenafil in CT26GM-bearing mice, which is a GM-CSF-secreting CT26 tumor model with a more aggressive phenotype in comparison with that of the controls, whereas L-NAME only reduces tumor volume in Rauscher virus-induced T lymphoma (RMA)-implanted mice. L-NAME and sildenafil do not alter the proportion of CD11b⁺Gr1⁺ and CD11b⁺Gr1⁻ cells in the spleens of CT26GM tumor-bearing mice, but CD11b⁺Gr1⁺ cells in the blood are reduced by these inhibitors. Moreover, sildenafil alone reduces CD11b⁺Gr1⁻ cells, which have a suppressive effect on CD8⁺ T cells, in the blood obtained from both mouse models (Capuano *et al.*, 2009).

5-Azacytidine

DNA methyltransferase inhibitors (DNMTis), which are epigenetic change-modulating cytostatic agents, including 5-azacytidine (5AC) and 2'-deoxy-5-azacytidine (DAC), have been utilized in patients with myelodysplastic syndrome, preleukemic hematological disease and established leukemia (Cashen *et al.*, 2010). The DNA-alkylating agent cyclophosphamide (CY), an anti-neoplastic drug, has been found to induce tumor-infiltrated MDSCs, while tumor growth is reduced by CY *in vivo*. 5AC also reduces tumor-induced MDSCs (TU-MDSCs) and CY-induced MDSCs (CY-MDSCs) as well as the expression of Arg1 in the spleens and tumor sites of mice. Thus, the combination treatment of CY and 5AC shows the highest therapeutic efficacy against tumor growth without the elevation of the MDSC population compared to the efficacy of the monotherapy of CY in two different tumor-bearing mouse models. Moreover, while CY-MDSCs do not exhibit suppressive activity on CD8⁺ T cells, 5AC blocks the suppressive activities of TU-MDSCs and CY-MDSCs on CD4⁺ and CD8⁺ T cells (Mikyskova *et al.*, 2014).

Selective class I histone deacetylase inhibitor entinostat

Entinostat is a class I-specific histone deacetylase inhibitor (HDACi) and plays a role in blocking the interaction between TME and the host immune system (Shen *et al.*, 2016). The combination therapy of entinostat and the mouse checkpoint inhibitor anti-PD-1 effectively reduces tumor growth and induces an increase in the overall survival in lung (LLC) and renal cell (RENCA) carcinoma syngeneic mouse models. Entinostat does not exhibit additional effects on PD-1-mediated changes in the Tregs or macrophages of TME. However, although it induces tumor-infiltrated PMN-MDSCs in both mouse tumor models, both entinostat alone and combination therapy remarkably induce the suppressive activities of PMN-MDSCs and MO-MDSCs on CD8⁺ T cell proliferation through NOS2, Arg1 and COX-2 inhibition. The combination therapy induces the alteration of the host environment and TME, leading to the enhancement of the anti-PD-1-mediated antitumor effect (Orillion *et al.*, 2017).

MDSC MODULATION BY HISTAMINE OR HISTAMINE RECEPTOR ANTAGONIST

Histamine

Histamine is secreted from mast cells and plays various roles in cell growth, motility and signaling mechanisms via binding to one of the four histamine receptors (HRs) expressed on diverse cell types (Borriello *et al.*, 2017). HR1-3 are expressed on MDSCs whose proliferation can be enhanced by histamine. HR1 or 2 antagonists, cetirizine (CT) and cimetidine (CIM), decrease PMN-MDSC functions related to *Nippostrongylus brasiliensis* (Nb) clearance in mice, an important function of PMN-MDSCs; CIM reduces lung metastasis in B16 melanoma-bearing mice. In addition, an increased level of MDSCs is often found in patients with allergies (Martin *et al.*, 2014). Histamine dihydrochloride (HDC) is a histamine salt that dissociates into histamine in an aqueous solution. Interestingly, HDC suppresses tumor growth through the inhibition of NADPH oxidase (NOX2)-positive MDSCs in EL-4 lymphoma and 4T1 mammary carcinoma mouse models. GR1⁺ cells from HDC-treated EL-4-bearing mice have shown less suppressive activity on T cells than those from the control mice. HLA-DR⁻ is a common phenotype of human MDSCs and HDC blocks the IL-6 and GM-CSF-induced depletion of HLA-DR in human monocytes. Moreover, the co-treatment of HDC with a low dose of IL-2 reduces MO-MDSCs in the blood of AML patients. In addition, it is worth noting that, although HDC does not affect the expression of PD-L1 on MDSCs or PD-1 on CD8⁺ T cells in mice, the combination therapy of HDC and α -PD-1/ α -PD-L1 antibodies significantly suppresses tumor progression in both EL-4 and MC-38 mouse tumor models (Grauers Wiktorin *et al.*, 2018).

Cimetidine

Cimetidine (CIM) is the oldest antagonist of histamine type-2 receptor and has been used for patients with gastro-esophageal reflux diseases and gastric ulcers (Freston, 1982). It has also been involved in several types of tumor suppression. CIM suppresses the accumulation of MDSCs in the spleen, blood and tumor sites of 3LL tumor-bearing mice and reduces suppressive effect of MDSCs on T-cell proliferation *in vitro*. Moreover, although CIM does not alter the differentiation of

MDSCs, it promotes the apoptosis of MDSCs via the induction of Fas and FasL expression, but it does not promote the activation of the H2 receptor by histamine (Zheng *et al.*, 2013).

INHIBITION OF MDSC ACTIVITY BY SMALL MOLECULES WITH OTHER ANTI-TUMOR PROPERTIES

All-trans retinoic acid (ATRA)

All-trans retinoic acid, an active vitamin A metabolite, has been used to induce the differentiation of leukemic blasts into mature promyelocytic leukemia. It is also able to induce the differentiation of MDSCs into mature cells and to improve the therapeutic effect of antiangiogenic therapies (AAT) by blocking the antiangiogenic therapy-induced expansion of MDSCs secreting high levels of vessel-destabilizing S100A8 in breast cancer (Nefedova *et al.*, 2007; Bauer *et al.*, 2018). In addition, it has been shown that the treatment of sarcoma-bearing mice with ATRA largely eradicates MO-MDSCs and diminishes the suppressive capacity of PMN-MDSCs. Therefore, combined therapy using disialoganglioside (GD2)-chimeric antigen receptor (CAR) T cells plus ATRA significantly enhances the antitumor efficacy of CAR against sarcoma xenografts (Long *et al.*, 2016). Intriguingly, in addition to the inhibition of the immunosuppressive function and gene expression of MDSCs, ATRA also significantly reduces the frequency of circulating MDSCs compared to treatment with anti-CTLA-4 antibody alone in advanced-stage melanoma patients, indicating that targeting MDSCs, such as treatment with ATRA, may augment immunotherapeutic efficiency in cancer patients (Tobin *et al.*, 2018). Moreover, the combination of therapeutic tumor vaccination using antigen/CpG/ α GC with delayed ATRA administration significantly enhances the antitumor effect in those animals that initially did not respond to therapeutic tumor vaccination by suppressing MDSC function (Heine *et al.*, 2017). Similarly, vaccination with tumor-exosome-loaded DC in combination with ATRA, which prevents MDSC maturation and activation, prolongs the survival of pancreatic cancer-bearing mice and induces the strong inhibition of the metastatic spread of cancer cells (Xiao *et al.*, 2017). Together, concomitant treatment with ATRA may hold the potential to improve immunotherapeutic efficiency in various tumor types.

Gemcitabine

Gemcitabine, an antitumor drug targeting DNA replication and telomerase activity, is broadly used for patients with advanced pancreatic cancers as a standard chemotherapy. It has also been reported that gemcitabine reduces B cells and CD11b⁺Gr-1⁺ cells without affecting CD4⁺ and CD8⁺ T cells in tumor-bearing mice (Suzuki, 2005; Yoyen-Ermis *et al.*, 2018). The levels of both human PMN-MDSCs (CD11b⁺CD14⁻CD33⁺HLA-DR⁻) and MO-MDSCs (CD11b⁺CD14⁺CD33⁺HLA-DR⁻) are increased in pancreatic cancer patients compared to the levels of healthy donors, and PMN-MDSCs are markedly decreased after treatment with gemcitabine, while it does not affect MO-MDSCs. Gemcitabine does not alter the levels of IL-6-induced STAT3 phosphorylation in MiaPaCa2 and Panc01 pancreatic cancer cell lines. Nevertheless, it indirectly reduces STAT3 phosphorylation in myeloid cells. Furthermore, gemcitabine elicits a mild inhibition of Tregs in patients even though it has been known to not affect the lymphocyte popu-

lation (Eriksson *et al.*, 2016). It has also been reported that tumor-infiltrated MDSCs are decreased by the co-treatment of gemcitabine with superoxide dismutase (SOD) mimetics in LLC tumor-bearing mice. The combination therapy suppresses ROS levels and ROS-related immune cells, including tumor-associated macrophages (TAM) and tumor-associated neutrophils (TAN), in the mouse model. The combination therapy also induces the population of memory CD8⁺ T cells through the alteration of MDSC infiltration and MDSC-associated ROS, thereby, increasing the survival rate of tumor-bearing mice (Sawant *et al.*, 2013). In a recent study, the efficacy of gemcitabine in a mouse pancreatic cancer model was evaluated by using a carrier system with gemcitabine-loaded polyethylene glycol (PEG)-cored poly (amidoamine) (PAMAM) dendrimers decorated with an anti-Flt1 antibody (D- α Flt1-Gem). D- α Flt1-Gem increases the effect of gemcitabine on the reduction of cancer-induced myeloid cells, including CD11b⁺Ly6G⁻Ly6C⁺ monocytes, and reduces the pancreatic tumor mass (Yoyen-Ermis *et al.*, 2018).

Amino-bisphosphonate

Amino-bisphosphonates, analogues of the naturally occurring compound PPi (P-O-P), have been known to have anti-tumor effects especially on bone metastasis (Clézardin *et al.*, 2005). Pamidronate and zoledronate are able to reduce tumor progression in a BALB-neu T mouse model, a spontaneous mammary tumor model. Zoledronate decreases pro-MMP-9 and VEGF in the serum and tumor infiltration of MDSCs and F4/80-positive inflammatory cells via inhibiting the expansion of BM progenitor cells in tumor-bearing mice. Therefore, zoledronate decreases the effects of dysregulated MMP-9 at the tumor site, leading to the reduction of MDSC expansion, although it does not affect normal hematopoiesis (Melani *et al.*, 2007).

RPN13/ADRM1 inhibitor

The bis(benzylidene)-piperidone RA190 is a proteasome inhibitor that is capable of binding to the ubiquitin receptor RPN13/ADRM1; in addition, it causes endoplasmic reticulum (ER) stress and the induction of apoptosis in several cancer types, such as bortezomib-resistant multiple myeloma (Anchoori *et al.*, 2013). RA190 decreases the expression of total STAT3 and leads to the downregulation of the suppressive molecules IL-10, arginase and iNOS in MDSCs *in vitro* and in tumor-bearing mice. This leads to a reduction in MDSC-induced T cell suppression. Moreover, RPN13 knockdown shows similar effects on MDSCs by regulating STAT3, IL-10, arginase and iNOS. Together, RA190 suppresses tumor growth and prolongs mouse survival (Soong *et al.*, 2016).

MDSC MODULATION BY SEX HORMONES OR COMPOUNDS THAT ARE NATURALLY FOUND IN PLANTS

17 β -Estradiol

Recently, it has been found that a number of MDSCs are induced in the peripheral blood and in the cord blood of healthy pregnant women to prevent immunologic fetal rejection, and therefore, MDSCs play an important role in the maternal immune system in maintaining maternal-fetal tolerance (Köstlin *et al.*, 2014). The induction of HLA-DR^{-low}CD11b^{high}CD33^{high}

Table 1. Clinical trials of immune checkpoint inhibitors (ICI) in combination with agents targeting myeloid derived suppressor cells (MDSCs)

	Official Title	Condition or disease	Intervention/ treatment	Phase	ClinicalTrials.gov Identifier
1	Depletion of Myeloid Derived Suppressor Cells to Enhance Anti PD-1 Therapy	· Non-Small Cell Lung Cancer · Stage IIIB	Nivolumab+ Gemcitabine	Phase 2	NCT03302247
2	Ipilimumab and All-Trans Retinoic Acid Combination Treatment of Advanced Melanoma	· Melanoma	Ipilimumab+ VESANOID (other Names: ATRA, Tretinoin)	Phase 2	NCT02403778
3	A Phase 1, Open-Label, Dose-Escalation With Expansion Study of SX-682 in Subjects With Metastatic Melanoma Concurrently Treated With Pembrolizumab	· Melanoma Stage iii and iv	SX-682+ Pembrolizumab	Phase 1	NCT03161431
4	Phase II Study Evaluating the Influence of LV5FU2 Bevacizumab Plus Anakinra Association on Vascularization of Liver Metastases of Metastatic Colorectal Cancer: Proof of Concept Study	· Metastatic Colorectal Cancer	Anakinra+ LV5FU2/ Bevacizumab	Phase 2	NCT02090101
5	A Phase 1 Study of RGX-104, a Small Molecule LXR Agonist, With or Without Nivolumab in Patients With Advanced Solid Malignancies and Lymphoma With an Expansion in Select Malignancies	· Malignant Neoplasms	RGX-104+ Nivolumab	Phase 1	NCT02922764
6	A Phase 1/1b First-In-Human, Dose-Escalation Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of IPI-549 Monotherapy and in Combination With Nivolumab in Subjects With Advanced Solid Tumors	· Advanced Solid Tumors · Non-small Cell Lung Cancer · Melanoma · Squamous Cell Cancer of the Head and Neck · Triple Negative Breast Cancer · Adrenocortical Carcinoma · Mesothelioma · High-circulating Myeloid-derived Suppressor Cells	IPI-549+ Nivolumab	Phase 1	NCT02637531
7	Open Label Phase II Trial to Evaluate Safety and Efficacy of Vinorelbine With Metronomic Administration in Combination With Atezolizumab as Second-line Treatment for Patients With Stage IV Non-small Cell Lung Cancer	· Non-small Cell Lung Cancer	Atezolizumab+ Vinorelbine	Phase 2	NCT03801304
8	A Phase I/II Trial of Pazopanib Alternating With Bevacizumab in Treatment-Naive Metastatic Clear Cell Renal Cell Carcinoma Patients	· Clear Cell Renal Cell Carcinoma · Stage IV Renal Cell Cancer	Pazopanib hydrochloride+ Bevacizumab	Phase 1 Phase 2	NCT01684397
9	Pembrolizumab and All-Trans Retinoic Acid in Combination Treatment of Advanced Melanoma	· Stage IV Melanoma · Stage III Melanoma · Advanced Melanoma	Pembrolizumab +VESANOID	Phase 1 Phase 2	NCT03200847
10	A Phase 1b Study of the Anti-PD1 Antibody Pembrolizumab in Combination With the Histone Deacetylase Inhibitor, Entinostat for Treatment of Patients With Myelodysplastic Syndromes After DNA Methyltransferase Inhibitor Therapy Failure	· Blasts 21-30 Percent of Bone Marrow Nucleated Cells · Myelodysplastic Syndrome · Previously Treated Myelodysplastic Syndrome	Entinostat+ Pembrolizumab	Phase 1	NCT02936752

Table 1. Continued

	Official Title	Condition or disease	Intervention/treatment	Phase	ClinicalTrials.gov Identifier
11	Profiling and Reversing Metabolic Insufficiency in the Tumor Microenvironment in Advanced Melanoma: A Trial of Pembrolizumab and Metformin Versus Pembrolizumab Alone in Advanced Melanoma	· Advanced Melanoma	Pembrolizumab Injection [Keytruda]+ Metformin	Phase 1	NCT03311308
12	A Phase 1 Study Evaluating Safety, Tolerability, and Preliminary Antitumor Activity of Entinostat and Nivolumab With or Without Ipilimumab in Advanced Solid Tumors	· Breast Adenocarcinoma · HER2/Neu Negative · Invasive Breast Carcinoma · Metastatic Breast Carcinoma · Metastatic Malignant Solid Neoplasm · Stage III Breast Cancer AJCC v7 · Stage IIIA Breast Cancer AJCC v7 · Stage IIIB Breast Cancer AJCC v7 · Stage IIIC Breast Cancer AJCC v7 · Stage IV Breast Cancer AJCC v6 and v7 · Unresectable Solid Neoplasm	Entinostat+ Ipilimumab+ Nivolumab	Phase 1	NCT02453620
13	An Open-label, Multicenter, Dose-escalation, Phase 1b/2 Study of the Safety, Efficacy, Pharmacodynamics, and Pharmacokinetics of RTA 408 in Combination With Ipilimumab or Nivolumab in the Treatment of Patients With Unresectable or Metastatic Melanoma	· Melanoma · Unresectable (Stage III) · Melanoma · Metastatic (Stage IV) · Melanoma	Omaveloxolone (RTA 408) Capsules+ Ipilimumab or Nivolumab	Phase 1 Phase 2	NCT02259231
14	A Phase 1 Study of TRAIL-R2 (DR5) Antibody DS-8273a Administered in Combination With Nivolumab in Subjects With Advanced Colorectal Cancer	· Colorectal Neoplasm	DS-8273a+ Nivolumab	Phase 1	NCT02991196 *Terminated

MO-MDSCs is found in the peripheral blood of healthy pregnant women when compared to healthy nonpregnant and postpartum women, and these MDSCs strongly suppress CD4⁺ and CD8⁺ T cell proliferation in an ROS-dependent manner. Interestingly, there has been a positive correlation between MO-MDSCs and 17 β -estradiol (E2) through Pearson's correlation analysis. E2 induces the differentiation of MDSCs *in vitro* through STAT3 activation, and the expression of S100A8 and S100A9, which are related to MDSC expansion, is upregulated by E2. Moreover, E2-induced MDSCs exhibit an increased ability to suppress T cells and to induce ROS production (Pan *et al.*, 2016).

Resveratrol

Resveratrol (RSV; 3,5,4'-trihydroxy-trans-stilbene), a pleiotropic phytochemical, is a natural compound found in grapes, peanuts and chocolates. It is known to play diverse roles in cellular processes, including inflammation, apoptosis and oxi-

dative stress (Li *et al.*, 2017). It has been reported that RSV prolongs the survival of LLC tumor-bearing mice and decreases the population of total- and PMN-MDSCs, although MO-MDSCs are not affected by RSV. In contrast to MDSCs, the number of CD8⁺ T cells is increased at the tumor site, spleen and draining lymph nodes (dLN). Moreover, RSV induces the apoptosis of PMN-MDSCs and indirectly blocks the migration of PMN-MDSCs through the reduction of HMGB1 expression, which is one of the candidates capable of promoting MDSC expansion. The suppressive effect of MDSCs on CD8⁺ T cells is also decreased by RSV. In addition, RSV induces the differentiation of MO-MDSCs into more mature myeloid cells by expressing CD11c and F4/80 (Zhao *et al.*, 2018a). However, contrary to this finding, it has also been reported that RSV induces MDSCs in a chronic colitis mouse model. RSV blocks the loss of body weight and decreases the serum amyloid A (SAA) concentration in IL-10^{-/-} mice with chronic colitis, whereas it does not affect chronic colitis in normal BL/6 WT

mice. The population of MDSCs is reduced in RSV-treated IL-10^{-/-} mice with chronic colitis, but MDSCs are not altered in a BL/6 mouse model (Singh *et al.*, 2012).

Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs), which are fatty acids, including omega-3 (*n*-3) and omega-6 (*n*-6), have various roles in the immune response, inflammation and lipid metabolism (Kremmyda *et al.*, 2011). The differentiation and suppressive functions of PMN-MDSCs are increased by α -linolenic acid (ALA) (*n*-3 PUFA) and LA (*n*-6 PUFA) *in vitro* and in a mouse model *in vivo* in an ROS-dependent manner, but the differentiation of MO-MDSCs is not affected by PUFAs. PUFAs also promote STAT3 activation and the expression of S100A8 and S100A9 in MDSCs. Furthermore, consistent with tumor-free mice, the oral administration of PUFAs induces the population of MDSCs and tumor progression in CT26 and LLC tumor-bearing mice (Yan *et al.*, 2013).

Korean red ginseng

Korean red ginseng (KRG) is made by undergoing several steps of steaming and drying using *Panax ginseng* C.A. Meyer, a highly precious plant among herbs. Ginseng has been used for a long time as a traditional medicinal herb in Asia, especially in Korea, and has been known to have various pharmacological activities (Yun, 2001). Although KRG does not affect tumor progression and MDSC accumulation, it does reduce the expression of iNOS and IL-10, immune suppression-related markers and NO production in MDSCs. Together, KRG increases the secretion of IL-2 and IFN- γ , which are essential factors for the growth, survival and differentiation of T cells by inhibiting the immunosuppressive activity of MDSCs (Jeon *et al.*, 2011).

CONCLUDING REMARKS

Given that MDSCs play a key role in the maintenance of immunosuppression in conditions of chronic inflammation, the suppression of MDSC expansion and activation by MDSC-targeting agents might increase the efficiency of the immune system. For instance, immune checkpoint inhibitors (ICI) for cancer therapy are already approved or are being clinically tested for the treatment of various types of cancers, including melanoma, NSCLC, head and neck carcinoma, hepatocellular carcinoma, colorectal carcinoma, renal cell carcinoma, bladder cancer, gastric cancer and B-cell lymphoma. However, accumulating evidence suggests that only a fraction of cancer patients benefit from ICI due to resistance to ICI after an initial response or a total lack of response. Resistance can be mediated by MDSCs, which makes these cells a promising target for combination therapy. Since it is suggested that targeting MDSCs would be beneficial for the treatment of cancer patients, some strategies modulating the MDSC numbers and immunosuppressive functions are being tested in various clinical trials in combination with ICI (Table 1). To date, fourteen clinical trials are being run to improve the efficacy of ICI treatment in cancer patients by reducing MDSC-mediated immunosuppression. Fortunately, given that there are a number of MDSC-targeting agents available on the market and some of them are already U.S. Food and Drug Administration (FDA)-approved for use in patients, their implementation in bi-

or multi-therapy regimens that include cancer immunotherapy is warranted. In various preclinical and clinical models, it has been reported that the use of MDSC-targeting agents potentiates the effects of ICI and leads to a significantly increased survival and even to the complete regression of tumors.

On the other hand, it is worth noting that most of our knowledge about MDSCs is based on tumor models and cancer patients. However, the relevance of these cells should be equally considered in non-cancer situations where overactivated immune responses are observed in acute or chronic inflammatory conditions and where autoimmune diseases need to be suppressed; in both cases, MDSCs may prove to be a boon for maintaining homeostasis in immune regulation. Therefore, in addition to agents targeting the harmful effects of MDSCs, agents supporting the beneficial roles of MDSCs in host protection are expected to emerge in a few years. For that to happen, a better understanding of MDSC regulation is needed to explain both the beneficial and detrimental effects of MDSCs.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

ACKNOWLEDGMENTS

This research was supported by grants from the National Research Foundation of Korea (NRF), which is funded by the Ministry of Science, ICT & Future Planning, Republic of Korea (NRF-2016R1A5A1011974) and from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), which is funded by the Ministry of Health & Welfare, Republic of Korea (Grant number: H18C1244).

REFERENCES

- Abraham, S. M., Lawrence, T., Kleiman, A., Warden, P., Medghalchi, M., Tuckermann, J., Saklatvala, J. and Clark, A. R. (2006) Anti-inflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J. Exp. Med.* **203**, 1883-1889.
- Ali, K., Soond, D. R., Pineiro, R., Hagemann, T., Pearce, W., Lim, E. L., Bouabe, H., Scudamore, C. L., Hancox, T., Maecker, H., Friedman, L., Turner, M., Okkenhaug, K. and Vanhaesebroeck, B. (2014) Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer. *Nature* **510**, 407-411.
- Anchoori, R. K., Karanam, B., Peng, S., Wang, J. W., Jiang, R., Tanno, T., Orlowski, R. Z., Matsui, W., Zhao, M., Rudek, M. A., Hung, C. F., Chen, X., Walters, K. J. and Roden, R. B. (2013) A bis-benzylidene piperidone targeting proteasome ubiquitin receptor RPN13/ADRM1 as a therapy for cancer. *Cancer Cell* **24**, 791-805.
- Arguello, F., Alexander, M., Sterry, J. A., Tudor, G., Smith, E. M., Kallavar, N. T., Greene, J. F., Jr., Koss, W., Morgan, C. D., Stinson, S. F., Siford, T. J., Alvord, W. G., Klabansky, R. L. and Sausville, E. A. (1998) Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity *In vivo* against human leukemia and lymphoma xenografts. *Blood* **91**, 2482-2490.
- Azoury, S. C., Gilmore, R. C. and Shukla, V. (2016) Molecularly targeted agents and immunotherapy for the treatment of head and neck squamous cell cancer (HNSCC). *Discov. Med.* **21**, 507-516.
- Bauer, R., Udonta, F., Wroblewski, M., Ben-Batalla, I., Santos, I. M.,

- Taverna, F., Kuhlencord, M., Gensch, V., Päsler, S., Vinckier, S., Brandner, J. M., Pantel, K., Bokemeyer, C., Vogl, T., Roth, J., Carmeliet, P. and Loges, S. (2018) Blockade of myeloid-derived suppressor cell expansion with all-trans retinoic acid increases the efficacy of anti-angiogenic therapy. *Cancer Res.* **78**, 3230-3232.
- Borriello, F., Iannone, R. and Marone, G. (2017) Histamine release from mast cells and basophils. *Handb. Exp. Pharmacol.* **241**, 121-139.
- Bu, L. L., Yu, G. T., Deng, W. W., Mao, L., Liu, J. F., Ma, S. R., Fan, T. F., Hall, B., Kulkarni, A. B., Zhang, W. F. and Sun, Z. J. (2016) Targeting STAT3 signaling reduces immunosuppressive myeloid cells in head and neck squamous cell carcinoma. *Oncoimmunology* **5**, e1130206.
- Califano, J. A., Khan, Z., Noonan, K. A., Rudraraju, L., Zhang, Z., Wang, H., Goodman, S., Gourin, C. G., Ha, P. K., Fakhry, C., Saunders, J., Levine, M., Tang, M., Neuner, G., Richmon, J. D., Blanco, R., Agrawal, N., Koch, W. M., Marur, S., Weed, D. T., Serafini, P. and Borrello, I. (2015) Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clin. Cancer Res.* **21**, 30-38.
- Cantoni, C., Cignarella, F., Ghezzi, L., Mikesell, B., Bollman, B., Berrien-Elliott, M. M., Ireland, A. R., Fehniger, T. A., Wu, G. F. and Piccio, L. (2017) Mir-223 regulates the number and function of myeloid-derived suppressor cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Acta Neuropathol.* **133**, 61-77.
- Cao, M. D., Xu, Y. L., Youn, J. I., Cabrera, R., Zhang, X. K., Gabrilovich, D., Nelson, D. R. and Liu, C. (2011) Kinase inhibitor Sorafenib modulates immunosuppressive cell populations in a murine liver cancer model. *Lab. Invest.* **91**, 598-608.
- Capuano, G., Rigamonti, N., Grioni, M., Freschi, M. and Bellone, M. (2009) Modulators of arginine metabolism support cancer immunosurveillance. *BMC Immunol.* **10**, 1.
- Cashen, A. F., Schiller, G. J., O'Donnell, M. R. and DiPersio, J. F. (2010) Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. *J. Clin. Oncol.* **28**, 556-561.
- Chang, C. J., Yang, Y. H., Chiu, C. J., Lu, L. C., Liao, C. C., Liang, C. W., Hsu, C. H. and Cheng, A. L. (2018) Targeting tumor-infiltrating Ly6G(+) myeloid cells improves sorafenib efficacy in mouse orthotopic hepatocellular carcinoma. *Int. J. Cancer* **142**, 1878-1889.
- Changelian, P. S., Flanagan, M. E., Ball, D. J., Kent, C. R., Magnuson, K. S., Martin, W. H., Rizzuti, B. J., Sawyer, P. S., Perry, B. D., Brissette, W. H., McCurdy, S. P., Kudlacz, E. M., Conklyn, M. J., Elliott, E. A., Koslov, E. R., Fisher, M. B., Strelevitz, T. J., Yoon, K., Whipple, D. A., Sun, J., Munchhof, M. J., Doty, J. L., Casavant, J. M., Blumenkopf, T. A., Hines, M., Brown, M. F., Lillie, B. M., Subramanyam, C., Shang-Poa, C., Milici, A. J., Beckius, G. E., Moyer, J. D., Su, C., Woodworth, T. G., Gaweco, A. S., Beals, C. R., Littman, B. H., Fisher, D. A., Smith, J. F., Zagouras, P., Magna, H. A., Saltarelli, M. J., Johnson, K. S., Nelms, L. F., Des Etages, S. G., Hayes, L. S., Kawabata, T. T., Finco-Kent, D., Baker, D. L., Larson, M., Si, M. S., Paniagua, R., Higgins, J., Holm, B., Reitz, B., Zhou, Y. J., Morris, R. E., O'Shea, J. J. and Borie, D. C. (2003) Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. *Science* **302**, 875-878.
- Chen, J., Deng, C. Y., Shi, Q. M., Jiang, J., Zhang, Y. B., Shan, W. and Sun, W. M. (2013) CpG oligodeoxynucleotide induces bone marrow precursor cells into myeloid-derived suppressor cells. *Mol. Med. Rep.* **8**, 1149-1154.
- Chen, S., Huang, A., Chen, H., Yang, Y., Xia, F., Jin, L. and Zhang, J. (2016) miR-34a inhibits the apoptosis of MDSCs by suppressing the expression of N-myc. *Immunol. Cell Biol.* **94**, 563-572.
- Chen, S., Zhang, Y., Kuzel, T. M. and Zhang, B. (2015a) Regulating tumor myeloid-derived suppressor cells by microRNAs. *Cancer Cell Microenviron.* **2**, e637.
- Chen, S. Q., Wang, L., Fan, J., Ye, C., Dominguez, D., Zhang, Y., Curiel, T. J., Fang, D. Y., Kuzel, T. M. and Zhang, B. (2015b) Host miR155 promotes tumor growth through a myeloid-derived suppressor cell-dependent mechanism. *Cancer Res.* **75**, 519-531.
- Cheng, P., Corzo, C. A., Luetteke, N., Yu, B., Nagaraj, S., Bui, M. M., Ortiz, M., Nacken, W., Sorg, C., Vogl, T., Roth, J. and Gabrilovich, D. I. (2008) Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J. Exp. Med.* **205**, 2235-2249.
- Clézardin, P., Ebetino, F. H. and Fournier, P. G. J. (2005) Bisphosphonates and cancer-induced bone disease: beyond their antiresorptive activity. *Cancer Res.* **65**, 4971-4974.
- Cushing, T. D., Metz, D. P., Whittington, D. A. and McGee, L. R. (2012) PI3Kdelta and PI3Kgamma as targets for autoimmune and inflammatory diseases. *J. Med. Chem.* **55**, 8559-8581.
- Davis, R. J., Moore, E. C., Clavijo, P. E., Friedman, J., Cash, H., Chen, Z., Silvín, C., Van Waes, C. and Allen, C. (2017) Anti-PD-L1 efficacy can be enhanced by inhibition of myeloid-derived suppressor cells with a selective inhibitor of PI3K delta/gamma. *Cancer Res.* **77**, 2607-2619.
- Deng, Y. T., Yang, J., Luo, F. F., Qian, J., Liu, R. H., Zhang, D., Yu, H. X. and Chu, Y. W. (2018) mTOR-mediated glycolysis contributes to the enhanced suppressive function of murine tumor-infiltrating monocytic myeloid-derived suppressor cells. *Cancer Immunol. Immunother.* **67**, 1355-1364.
- Doedens, A. L., Stockmann, C., Rubinstein, M. P., Liao, D., Zhang, N., DeNardo, D. G., Coussens, L. M., Karin, M., Goldrath, A. W. and Johnson, R. S. (2010) Macrophage expression of hypoxia-inducible factor-1 alpha suppresses T-cell function and promotes tumor progression. *Cancer Res.* **70**, 7465-7475.
- Draghiciu, O., Nijman, H. W., Hoogeboom, B. N., Meijerhof, T. and Daemen, T. (2015) Sunitinib depletes myeloid-derived suppressor cells and synergizes with a cancer vaccine to enhance antigen-specific immune responses and tumor eradication. *Oncoimmunology* **4**, e989764.
- Du Four, S., Maenhout, S. K., De Pierre, K., Renmans, D., Niclou, S. P., Thielemans, K., Neyns, B. and Aerts, J. L. (2015) Axitinib increases the infiltration of immune cells and reduces the suppressive capacity of monocytic MDSCs in an intracranial mouse melanoma model. *Oncoimmunology* **4**, e998107.
- El Gazzar, M. (2014) microRNAs as potential regulators of myeloid-derived suppressor cell expansion. *Innate Immunity* **20**, 227-238.
- Engelman, J. A. (2009) Targeting PI3K signalling in cancer: opportunities, challenges, and limitations. *Nat. Rev. Cancer* **9**, 550-562.
- Eriksson, E., Wenthe, J., Irenaeus, S., Loskog, A. and Ullenhag, G. (2016) Gemcitabine reduces MDSCs, tregs and TGF beta-1 while restoring the teff/treg ratio in patients with pancreatic cancer. *J. Transl. Med.* **14**, 282.
- Espagnolle, N., Barron, P., Mandron, M., Blanc, I., Bonnin, J., Agnel, M., Kerbelec, E., Heralut, J. P., Savi, P., Bono, F. and Alam, A. (2014) Specific inhibition of the VEGFR-3 tyrosine kinase by SAR131675 reduces peripheral and tumor associated immunosuppressive myeloid cells. *Cancers (Base)* **6**, 472-490.
- Finke, J., Ko, J., Rini, B., Rayman, P., Ireland, J. and Cohen, P. (2011) MDSC as a mechanism of tumor escape from sunitinib mediated anti-angiogenic therapy. *Int. Immunopharm.* **11**, 856-861.
- Flaxenburg, J. A., Melder, M., Lapchak, P. H., Briscoe, D. M. and Pal, S. (2004) The CD40-induced signaling pathway in endothelial cells resulting in the overexpression of vascular endothelial growth factor involves Ras and phosphatidylinositol 3-kinase. *J. Immunol.* **172**, 7503-7509.
- Freston, J. W. (1982) Cimetidine. I. Developments, pharmacology, and efficacy. *Ann. Intern. Med.* **97**, 573-580.
- Gabrilovich, D. I. and Nagaraj, S. (2009) Myeloid-derived suppressor cells as regulators of the immune system. *Nat. Rev. Immunol.* **9**, 162-174.
- Gabrilovich, D. I., Ostrand-Rosenberg, S. and Bronte, V. (2012) Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253-268.
- Ghofrani, H. A., Voswinckel, R., Reichenberger, F., Olschewski, H., Haredza, P., Karadas, B., Schermuly, R. T., Weissmann, N., Seeger, W. and Grimminger, F. (2004) Differences in hemodynamic and oxygenation responses to three different phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension: a randomized prospective study. *J. Am. Coll. Cardiol.* **44**, 1488-1496.
- Grauers Wiktorin, H., Nilsson, M. S., Kiffin, R., Sander, F. E., Lenox, B., Rydstrom, A., Hellstrand, K. and Martner, A. (2018) Histamine targets myeloid-derived suppressor cells and improves the anti-tumor efficacy of PD-1/PD-L1 checkpoint blockade. *Cancer Immunol. Im-*

- munother.* **68**, 163-174.
- Greenwood, J., Steinman, L. and Zamvil, S. S. (2006) Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. *Nat. Rev. Immunol.* **6**, 358-370.
- Groth, C., Hu, X., Weber, R., Fleming, V., Altevogt, P., Utikal, J. and Umansky, V. (2018) Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br. J. Cancer* **120**, 16-25.
- Guha, P., Gardell, J., Darpolor, J., Cunetta, M., Lima, M., Miller, G., Esparat, N. J., Junghans, R. P. and Katz, S. C. (2019) STAT3 inhibition induces Bax-dependent apoptosis in liver tumor myeloid-derived suppressor cells. *Oncogene* **38**, 533-548.
- Guislain, A., Gadiot, J., Kaiser, A., Jordanova, E. S., Broeks, A., Sanders, J., van Boven, H., de Gruijij, T. D., Haanen, J. B., Bex, A. and Blank, C. U. (2015) Sunitinib pretreatment improves tumor-infiltrating lymphocyte expansion by reduction in intratumoral content of myeloid-derived suppressor cells in human renal cell carcinoma. *Cancer Immunol. Immunother.* **64**, 1241-1250.
- Guo, X., Qiu, W., Wang, J., Liu, Q., Qian, M., Wang, S., Zhang, Z., Gao, X., Chen, Z., Guo, Q., Xu, J., Xue, H. and Li, G. (2018a) Glioma exosomes mediate the expansion and function of myeloid-derived suppressor cells through microRNA-29a/Hbp1 and microRNA-92a/Prkar1a pathways. *Int. J. Cancer* **144**, 3111-3126.
- Guo, X. F., Qiu, W., Liu, Q. L., Qian, M. Y., Wang, S. B., Zhang, Z. P., Gao, X., Chen, Z. H., Xue, H. and Li, G. (2018b) Immunosuppressive effects of hypoxia-induced glioma exosomes through myeloid-derived suppressor cells via the miR-10a/Rora and miR-21/Pten Pathways. *Oncogene* **37**, 4239-4259.
- Hara, E., Smith, R., Parry, D., Tahara, H., Stone, S. and Peters, G. (1996) Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. *Mol. Cell. Biol.* **16**, 859-867.
- Hashimoto, A., Gao, C., Mastio, J., Kossenkov, A., Abrams, S. I., Purandare, A. V., Desilva, H., Wee, S., Hunt, J., Jure-Kunkel, M. and Gabrilovich, D. I. (2018) Inhibition of casein kinase 2 disrupts differentiation of myeloid cells in cancer and enhances the efficacy of immunotherapy in mice. *Cancer Res.* **78**, 5644-5655.
- Hegde, V. L., Tomar, S., Jackson, A., Rao, R., Yang, X., Singh, U. P., Singh, N. P., Nagarkatti, P. S. and Nagarkatti, M. (2013) Distinct microRNA expression profile and targeted biological pathways in functional myeloid-derived suppressor cells induced by delta9-tetrahydrocannabinol *in vivo*: regulation of CCAAT/enhancer-binding protein alpha by microRNA-690. *J. Biol. Chem.* **288**, 36810-36826.
- Heine, A., Flores, C., Gevensleben, H., Diehl, L., Heikenwalder, M., Ringelhan, M., Janssen, K.-P., Nitsche, U., Garbi, N., Brossart, P., Knolle, P. A., Kurts, C. and Höchst, B. (2017) Targeting myeloid derived suppressor cells with all-trans retinoic acid is highly time-dependent in therapeutic tumor vaccination. *Oncoimmunology* **6**, e1338995.
- Heine, A., Schilling, J., Grünwald, B., Krüger, A., Gevensleben, H., Held, S. A. E., Garbi, N., Kurts, C., Brossart, P., Knolle, P., Diehl, L. and Höchst, B. (2016) The induction of human myeloid derived suppressor cells through hepatic stellate cells is dose-dependently inhibited by the tyrosine kinase inhibitors nilotinib, dasatinib and sorafenib, but not sunitinib. *Cancer Immunol. Immunother.* **65**, 273-282.
- Hojjat-Farsangi, M. (2014) Small-molecule inhibitors of the receptor tyrosine kinases: promising tools for targeted cancer therapies. *Int. J. Mol. Sci.* **15**, 13768-13801.
- Hossain, F., Majumder, S., Ucar, D. A., Rodriguez, P. C., Golde, T. E., Minter, L. M., Osborne, B. A. and Miele, L. (2018) Notch signaling in myeloid cells as a regulator of tumor immune responses. *Front. Immunol.* **9**, 1288.
- Hou, Y., Feng, Q., Xu, M., Li, G. S., Liu, X. N., Sheng, Z., Zhou, H., Ma, J., Wei, Y., Sun, Y. X., Yu, Y. Y., Qiu, J. H., Shao, L. L., Liu, X. G., Hou, M. and Peng, J. (2016) High-dose dexamethasone corrects impaired myeloid-derived suppressor cell function via Ets1 in immune thrombocytopenia. *Blood* **127**, 1587-1597.
- Huang, A. F., Zhang, H. T., Chen, S., Xia, F., Yang, Y., Dong, F. L., Sun, D., Xiong, S. D. and Zhang, J. P. (2014) miR-34a expands myeloid-derived suppressor cells via apoptosis inhibition. *Exp. Cell Res.* **326**, 259-266.
- Huber, V., Vallacchi, V., Fleming, V., Hu, X., Cova, A., Dugo, M., Shah, E., Sulisenti, R., Vergani, E., Filipazzi, P., De Laurentis, A., Lalli, L., Di Guardo, L., Patuzzo, R., Vergani, B., Casiraghi, E., Cossa, M., Gualeni, A., Bollati, V., Arienti, F., De Braud, F., Mariani, L., Villa, A., Altevogt, P., Umansky, V., Rodolfo, M. and Rivoltini, L. (2018) Tumor-derived microRNAs induce myeloid suppressor cells and predict immunotherapy resistance in melanoma. *J. Clin. Invest.* **128**, 5505-5516.
- Jeon, C., Kang, S., Park, S., Lim, K., Hwang, K. W. and Min, H. (2011) T cell stimulatory effects of Korean red ginseng through modulation of myeloid-derived suppressor cells. *J. Ginseng Res.* **35**, 462-470.
- Köstlin, N., Kugel, H., Spring, B., Leiber, A., Marmé, A., Henes, M., Rieber, N., Hartl, D., Poets, C. F. and Gille, C. (2014) Granulocytic myeloid derived suppressor cells expand in human pregnancy and modulate T-cell responses. *Eur. J. Immunol.* **44**, 2582-2591.
- Kanasty, R., Dorkin, J. R., Vegas, A. and Anderson, D. (2013) Delivery materials for siRNA therapeutics. *Nat. Mater.* **12**, 967-977.
- Kim, K., Skora, A. D., Li, Z. B., Liu, Q., Tam, A. J., Blosser, R. L., Diaz, L. A., Papadopoulos, N., Kinzler, K. W., Vogelstein, B. and Zhou, S. B. (2014) Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 11774-11779.
- Kim, S. H., Li, M., Trousil, S., Zhang, Y., Pasca di Magliano, M., Swanson, K. D. and Zheng, B. (2017) Phenformin inhibits myeloid-derived suppressor cells and enhances the anti-tumor activity of PD-1 blockade in melanoma. *J. Invest. Dermatol.* **137**, 1740-1748.
- Klinman, D. M. (2004) Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nat. Rev. Immunol.* **4**, 249-259.
- Ko, H. J. and Kim, Y. J. (2016) Signal transducer and activator of transcription proteins: regulators of myeloid-derived suppressor cell-mediated immunosuppression in cancer. *Arch. Pharm. Res.* **39**, 1597-1608.
- Ko, J. S., Rayman, P., Ireland, J., Swaidani, S., Li, G. Q., Bunting, K. D., Rini, B., Finke, J. H. and Cohen, P. A. (2010) Direct and differential suppression of myeloid-derived suppressor cell subsets by sunitinib is compartmentally constrained. *Cancer Res.* **70**, 3526-3536.
- Ko, J. S., Zea, A. H., Rin, B. I., Ireland, J. L., Elson, P., Cohen, P., Golshayan, A., Rayman, P. A., Wood, L., Garcia, J., Dreicer, R., Bukowski, R. and Finke, J. H. (2009) Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin. Cancer Res.* **15**, 2148-2157.
- Kremmyda, L.-S., Trzicka, E., Stankova, B. and Zak, A. (2011) Fatty acids as biocompounds: their role in human metabolism, health and disease: a review. part 2: fatty acid physiological roles and applications in human health and disease. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* **155**, 195-218.
- Kuchen, S., Resch, W., Yamane, A., Kuo, N., Li, Z., Chakraborty, T., Wei, L., Laurence, A., Yasuda, T., Peng, S., Hu-Li, J., Lu, K., Dubois, W., Kitamura, Y., Charles, N., Sun, H. W., Muljo, S., Schwartzberg, P. L., Paul, W. E., O'Shea, J., Rajewsky, K. and Casellas, R. (2010) Regulation of microRNA expression and abundance during lymphopoiesis. *Immunity* **32**, 828-839.
- Ledo, A. M., Sasso, M. S., Bronte, V., Marigo, I., Boyd, B. J., Garcia-Fuentes, M. and Alonso, M. J. (2018) Co-delivery of RNAi and chemokine by polyarginine nanocapsules enables the modulation of myeloid-derived suppressor cells. *J. Control. Release* **295**, 60-73.
- Lee, B. R., Kwon, B. E., Hong, E. H., Shim, A., Song, J. H., Kim, H. M., Chang, S. Y., Kim, Y. J., Kweon, M. N., Youn, J. I. and Ko, H. J. (2016) Interleukin-10 attenuates tumour growth by inhibiting interleukin-6/signal transducer and activator of transcription 3 signalling in myeloid-derived suppressor cells. *Cancer Lett.* **381**, 156-164.
- Lei, A., Yang, Q., Li, X., Chen, H., Shi, M., Xiao, Q., Cao, Y., He, Y. and Zhou, J. (2016) Atorvastatin promotes the expansion of myeloid-derived suppressor cells and attenuates murine colitis. *Immunology* **149**, 432-446.
- Li, A., Barsoumian, H. B., Schoenhals, J. E., Cushman, T. R., Caetano, M. S., Wang, X., Valdecana, D. R., Niknam, S., Younes, A. I., Li, G., Woodward, W. A., Cortez, M. A. and Welsh, J. W. (2018a) Indoleamine 2,3-dioxygenase 1 inhibition targets anti-PD1-resistant lung tumors by blocking myeloid-derived suppressor cells. *Cancer Lett.* **431**, 54-63.
- Li, J., Yu, S., Ying, J., Shi, T. and Wang, P. (2017) Resveratrol prevents

- ROS-induced apoptosis in high glucose-treated retinal capillary endothelial cells via the activation of AMPK/Sirt1/PGC-1 α pathway. *Oxid. Med. Cell. Longev.* **2017**, 7584691.
- Li, L., Wang, L., Li, J., Fan, Z., Yang, L., Zhang, Z., Zhang, C., Yue, D., Qin, G., Zhang, T., Li, F., Chen, X., Ping, Y., Wang, D., Gao, Q., He, Q., Huang, L., Li, H., Huang, J., Zhao, X., Xue, W., Sun, Z., Lu, J., Yu, J. J., Zhao, J., Zhang, B. and Zhang, Y. (2018b) Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian cancer. *Cancer Res.* **78**, 1779-1791.
- Li, L., Zhang, J., Diao, W., Wang, D., Wei, Y., Zhang, C. Y. and Zen, K. (2014) MicroRNA-155 and microRNA-21 promote the expansion of functional myeloid-derived suppressor cells. *J. Immunol.* **192**, 1034-1043.
- Lin, Y., Wang, B. S., Shan, W., Tan, Y. M., Feng, J. J., Xu, L., Wang, L. M. M., Han, B. Q., Zhang, M. M., Yu, J., Yu, X. H. and Huang, H. (2018) mTOR inhibitor rapamycin induce polymorphonuclear myeloid-derived suppressor cells mobilization and function in protecting against acute graft-versus-host disease after bone marrow transplantation. *Clin. Immunol.* **187**, 122-131.
- Liu, L., Ye, T. H., Han, Y. P., Song, H., Zhang, Y. K., Xia, Y., Wang, N. Y., Xiong, Y., Song, X. J., Zhu, Y. X., Li, D. L., Zeng, J., Ran, K., Peng, C. T., Wei, Y. Q. and Yu, L. T. (2014) Reductions in myeloid-derived suppressor cells and lung metastases using AZD4547 treatment of a metastatic murine breast tumor model. *Cell. Phys. Biochem.* **33**, 633-645.
- Liu, Q., Zhang, M., Jiang, X., Zhang, Z., Dai, L., Min, S., Wu, X., He, Q., Liu, J., Zhang, Y., Zhang, Z. and Yang, R. (2011) miR-223 suppresses differentiation of tumor-induced CD11b(+) Gr1(+) myeloid-derived suppressor cells from bone marrow cells. *Int. J. Cancer* **129**, 2662-2673.
- Liu, Y., Lai, L., Chen, Q., Song, Y., Xu, S., Ma, F., Wang, X., Wang, J., Yu, H., Cao, X. and Wang, Q. (2012) MicroRNA-494 is required for the accumulation and functions of tumor-expanded myeloid-derived suppressor cells via targeting of PTEN. *J. Immunol.* **188**, 5500-5510.
- Liu, Y. F., Wei, J. Y., Shi, M. H., Jiang, H. and Zhou, J. (2016) Glucocorticoid induces hepatic steatosis by inhibiting activating transcription factor 3 (ATF3)/S100A9 protein signaling in granulocytic myeloid-derived suppressor cells. *J. Biol. Chem.* **291**, 21771-21785.
- Liu, Y. F., Zhuang, K. H., Chen, B., Li, P. W., Zhou, X., Jiang, H., Zhong, L. M. and Liu, F. B. (2018) Expansion and activation of monocytic-myeloid-derived suppressor cell via STAT3/arginase-I signaling in patients with ankylosing spondylitis. *Arthritis Res. Ther.* **20**, 168.
- Long, A. H., Highfill, S. L., Cui, Y., Smith, J. P., Walker, A. J., Ramakrishna, S., El-Etriby, R., Galli, S., Tsokos, M. G., Orentas, R. J. and Mackall, C. L. (2016) Reduction of MDSCs with all-trans retinoic acid improves CAR therapy efficacy for sarcomas. *Cancer Immunol. Res.* **4**, 869-880.
- Maenhout, S. K., Du Four, S., Corthals, J., Neyns, B., Thielemans, K. and Aerts, J. L. (2014) AZD1480 delays tumor growth in a melanoma model while enhancing the suppressive activity of myeloid-derived suppressor cells. *Oncotarget* **5**, 6801-6815.
- Mandelker, D., Gabelli, S. B., Schmidt-Kittler, O., Zhu, J., Cheong, I., Huang, C. H., Kinzler, K. W., Vogelstein, B. and Amzel, L. M. (2009) A frequent kinase domain mutation that changes the interaction between PI3K α and the membrane. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16996-17001.
- Mao, L., Zhao, Z. L., Yu, G. T., Wu, L., Deng, W. W., Li, Y. C., Liu, J. F., Bu, L. L., Liu, B., Kulkarni, A. B., Zhang, W. F., Zhang, L. and Sun, Z. J. (2018) γ -Secretase inhibitor reduces immunosuppressive cells and enhances tumour immunity in head and neck squamous cell carcinoma. *Int. J. Cancer* **142**, 999-1009.
- Marigo, I., Bosio, E., Solito, S., Mesa, C., Fernandez, A., Dolcetti, L., Ugel, S., Sonda, N., Biccato, S., Falisi, E., Calabrese, F., Basso, G., Zanovello, P., Cozzi, E., Mandruzzato, S. and Bronte, V. (2010) Tumor-induced tolerance and immune suppression depend on the C/EBP β transcription factor. *Immunity* **32**, 790-802.
- Martin, R. K., Saleem, S. J., Folgosa, L., Zellner, H. B., Damle, S. R., Nguyen, G. K., Ryan, J. J., Bear, H. D., Irani, A. M. and Conrad, D. H. (2014) Mast cell histamine promotes the immunoregulatory activity of myeloid-derived suppressor cells. *J. Leukoc. Biol.* **96**, 151-159.
- McClure, C., Brudecki, L., Ferguson, D. A., Yao, Z. Q., Moorman, J. P., McCall, C. E. and El Gazzar, M. (2014) MicroRNA 21 (miR-21) and miR-181b couple with NF1-A to generate myeloid-derived suppressor cells and promote immunosuppression in late sepsis. *Infect. Immun.* **82**, 3816-3825.
- Melani, C., Sangaletti, S., Barazzetta, F. M., Werb, Z. and Colombo, M. P. (2007) Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma. *Cancer Res.* **67**, 11438-11446.
- Mikyskova, R., Indrova, M., Vlkova, V., Bieblova, J., Simova, J., Parackova, Z., Pajtasz-Piasecka, E., Rossowska, J. and Reinis, M. (2014) DNA demethylating agent 5-azacytidine inhibits myeloid-derived suppressor cells induced by tumor growth and cyclophosphamide treatment. *J. Leukoc. Biol.* **95**, 743-753.
- Moon, Y. W., Hajjar, J., Hwu, P. and Naing, A. (2015) Targeting the indoleamine 2,3-dioxygenase pathway in cancer. *J. Immunother. Cancer* **3**, 51.
- Motzer, R. J., Rini, B. I., Bukowski, R. M., Curti, B. D., George, D. J., Hudes, G. R., Redman, B. G., Margolin, K. A., Merchan, J. R., Wilding, G., Ginsberg, M. S., Bacik, J., Kim, S. T., Baum, C. M. and Michaelson, M. D. (2006) Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* **295**, 2516-2524.
- Nakamura, T., Nakao, T., Yoshimura, N. and Ashihara, E. (2015) Rapamycin prolongs cardiac allograft survival in a mouse model by inducing myeloid-derived suppressor cells. *Am. J. Transplant.* **15**, 2364-2377.
- Nam, S., Kang, K., Cha, J. S., Kim, J. W., Lee, H. G., Kim, Y., Yang, Y., Lee, M. S. and Lim, J. S. (2016) Interferon regulatory factor 4 (IRF4) controls myeloid-derived suppressor cell (MDSC) differentiation and function. *J. Leukoc. Biol.* **100**, 1273-1284.
- Nam, S., Lee, A., Lim, J. and Lim, J.-S. (2019) Analysis of the expression and regulation of PD-1 protein on the surface of myeloid-derived suppressor cells (MDSCs). *Biomol. Ther. (Seoul)* **27**, 63-70.
- Nandi, P., Girish, G. V., Majumder, M., Xin, X., Tutunea-Fatan, E. and Lala, P. K. (2017) PGE2 promotes breast cancer-associated lymphangiogenesis by activation of EP4 receptor on lymphatic endothelial cells. *BMC Cancer* **17**, 11.
- Nefedova, Y., Fishman, M., Sherman, S., Wang, X., Beg, A. A. and Gabrilovich, D. I. (2007) Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. *Cancer Res.* **67**, 11021-11028.
- Nefedova, Y., Nagaraj, S., Rosenbauer, A., Muro-Cacho, C., Sebt, S. M. and Gabrilovich, D. I. (2005) Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res.* **65**, 9525-9535.
- Nishimura, K., Saegusa, J., Matsuki, F., Akashi, K., Kageyama, G. and Morinobu, A. (2015) Tofacitinib facilitates the expansion of myeloid-derived suppressor cells and ameliorates arthritis in SKG mice. *Arthritis Rheumatol.* **67**, 893-902.
- Noman, M. Z., Janji, B., Hu, S., Wu, J. C., Martelli, F., Bronte, V. and Chouaib, S. (2015) Tumor-promoting effects of myeloid-derived suppressor cells are potentiated by hypoxia-induced expression of miR-210. *Cancer Res.* **75**, 3771-3787.
- Noonan, K. A., Ghosh, N., Rudraraju, L., Bui, M. and Borrello, I. (2014) Targeting immune suppression with PDE5 inhibition in end-stage multiple myeloma. *Cancer Immunol. Res.* **2**, 725-731.
- O'Neill, L. A. J. and Hardie, D. G. (2013) Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* **493**, 346-355.
- Okuma, A., Hanyu, A., Watanabe, S. and Hara, E. (2017) p16(Ink4a) and p21(Cip1/Waf1) promote tumour growth by enhancing myeloid-derived suppressor cells chemotaxis. *Nat. Commun.* **8**, 2050.
- Orillion, A., Hashimoto, A., Damayanti, N., Shen, L., Adelaiye-Ogala, R., Arisa, S., Chintala, S., Ordentlich, P., Kao, C., Elzey, B., Gabrilovich, D. and Pili, R. (2017) Entinostat neutralizes myeloid-derived suppressor cells and enhances the antitumor effect of PD-1 inhibition in murine models of lung and renal cell carcinoma. *Clin. Cancer Res.* **23**, 5187-5201.
- Orcucevic, A., Bechberger, J., Green, A. M., Shapiro, R. A., Billiar, T.

- R. and Lala, P. K. (1999) Nitric-oxide production by murine mammary adenocarcinoma cells promotes tumor-cell invasiveness. *Int. J. Cancer* **81**, 889-896.
- Pan, P. Y., Ma, G., Weber, K. J., Ozao-Choy, J., Wang, G., Yin, B., Divino, C. M. and Chen, S. H. (2010) Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res.* **70**, 99-108.
- Pan, T., Zhong, L., Wu, S., Cao, Y., Yang, Q., Cai, Z., Cai, X., Zhao, W., Ma, N., Zhang, W., Zhang, H. and Zhou, J. (2016) 17-Oestradiol enhances the expansion and activation of myeloid-derived suppressor cells via signal transducer and activator of transcription (STAT)-3 signalling in human pregnancy. *Clin. Exp. Immunol.* **185**, 86-97.
- Piazza, F., Manni, S., Ruzzene, M., Pinna, L. A., Gurrieri, C. and Semenzato, G. (2012) Protein kinase CK2 in hematologic malignancies: reliance on a pivotal cell survival regulator by oncogenic signaling pathways. *Leukemia* **26**, 1174-1179.
- Qin, G., Lian, J., Huang, L., Zhao, Q., Liu, S., Zhang, Z., Chen, X., Yue, D., Li, L., Li, F., Wang, L., Umansky, V., Zhang, B., Yang, S. and Zhang, Y. (2018) Metformin blocks myeloid-derived suppressor cell accumulation through AMPK-DACH1-CXCL1 axis. *Oncoimmunology* **7**, e1442167.
- Rodriguez, P. C., Hernandez, C. P., Quiceno, D., Dubinett, S. M., Zabaleta, J., Ochoa, J. B., Gilbert, J. and Ochoa, A. C. (2005) Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. *J. Exp. Med.* **202**, 931-939.
- Rong, Y., Yuan, C. H., Qu, Z., Zhou, H., Guan, Q., Yang, N., Leng, X. H., Bu, L., Wu, K. and Wang, F. B. (2016) Doxorubicin resistant cancer cells activate myeloid-derived suppressor cells by releasing PGE2. *Sci. Rep.* **6**, 23824.
- Roskoski, R., Jr. (2007) Sunitinib: a VEGF and PDGF receptor protein kinase and angiogenesis inhibitor. *Biochem. Biophys. Res. Commun.* **356**, 323-328.
- Sawant, A., Schafer, C. C., Jin, T. H., Zmijewski, J., Tse, H. M., Roth, J., Sun, Z. H., Siegal, G. P., Thannickal, V. J., Grant, S. C., Ponnazhagan, S. and Deshane, J. S. (2013) Enhancement of antitumor immunity in lung cancer by targeting myeloid-derived suppressor cell pathways. *Cancer Res.* **73**, 6609-6620.
- Schacke, H., Docke, W. D. and Asadullah, K. (2002) Mechanisms involved in the side effects of glucocorticoids. *Pharmacol. Ther.* **96**, 23-43.
- Schmid, M. C., Avraamides, C. J., Dippold, H. C., Franco, I., Foubert, P., Ellies, L. G., Acevedo, L. M., Manglicmot, J. R., Song, X., Wrasidlo, W., Blair, S. L., Ginsberg, M. H., Cheresch, D. A., Hirsch, E., Field, S. J. and Varner, J. A. (2011) Receptor tyrosine kinases and TLR/IL1Rs unexpectedly activate myeloid cell PI3kgamma, a single convergent point promoting tumor inflammation and progression. *Cancer Cell* **19**, 715-727.
- Schwartz, D. M., Kanno, Y., Villarino, A., Ward, M., Gadina, M. and O'Shea, J. J. (2017) JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat. Rev. Drug Discov.* **17**, 78.
- Scuto, A., Krejci, P., Popplewell, L., Wu, J., Wang, Y., Kujawski, M., Kowolik, C., Xin, H., Chen, L., Wang, Y., Kretzner, L., Yu, H., Wilcox, W. R., Yen, Y., Forman, S. and Jove, R. (2011) The novel JAK inhibitor AZD1480 blocks STAT3 and FGFR3 signaling, resulting in suppression of human myeloma cell growth and survival. *Leukemia* **25**, 538-550.
- Serafini, P., Meckel, K., Kelso, M., Noonan, K., Califano, J., Koch, W., Dolcetti, L., Bronte, V. and Borrello, I. (2006) Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J. Exp. Med.* **203**, 2691-2702.
- Setten, R. L., Rossi, J. J. and Han, S. P. (2019) The current state and future directions of RNAi-based therapeutics. *Nat. Rev. Drug Discov.* **18**, 421-446.
- Shen, L., Orillion, A. and Pili, R. (2016) Histone deacetylase inhibitors as immunomodulators in cancer therapeutics. *Epigenomics* **8**, 415-428.
- Shi, G., Li, D., Ren, J., Li, X., Wang, T., Dou, H. and Hou, Y. (2019) mTOR inhibitor INK128 attenuates dextran sodium sulfate-induced colitis by promotion of MDSCs on Treg cell expansion. *J. Cell. Physiol.* **234**, 1618-1629.
- Shirota, Y., Shirota, H. and Klinman, D. M. (2012) Intratumoral injection of CpG oligonucleotides induces the differentiation and reduces the immunosuppressive activity of myeloid-derived suppressor cells. *J. Immunol.* **188**, 1592-1599.
- Singh, U. P., Singh, N. P., Singh, B., Hofseth, L. J., Taub, D. D., Price, R. L., Nagarkatti, M. and Nagarkatti, P. S. (2012) Role of resveratrol-induced CD11b(+) Gr-1(+) myeloid derived suppressor cells (MDSCs) in the reduction of CXCR3(+) T cells and amelioration of chronic colitis in IL-10(-/-) mice. *Brain Behav. Immun.* **26**, 72-82.
- Sinha, P., Clements, V. K., Fulton, A. M. and Ostrand-Rosenberg, S. (2007) Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res.* **67**, 4507-4513.
- Sinha, P., Okoro, C., Foell, D., Freeze, H. H., Ostrand-Rosenberg, S. and Srikrishna, G. (2008) Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J. Immunol.* **181**, 4666-4675.
- Soong, R. S., Anchoori, R. K., Yang, B., Yang, A., Tseng, S. H., He, L. M., Tsai, Y. C., Roden, R. B. S. and Hung, C. F. (2016) RPN13/ADRM1 inhibitor reverses immunosuppression by myeloid-derived suppressor cells. *Oncotarget* **7**, 68489-68502.
- Suzuki, E. (2005) Gemcitabine selectively eliminates splenic Gr-1/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin. Cancer Res.* **11**, 6713-6721.
- Tobin, R. P., Jordan, K. R., Robinson, W. A., Davis, D., Borges, V. F., Gonzalez, R., Lewis, K. D. and McCarter, M. D. (2018) Targeting myeloid-derived suppressor cells using all-trans retinoic acid in melanoma patients treated with Ipilimumab. *Int. Immunopharm.* **63**, 282-291.
- Trikha, P., Plews, R. L., Stiff, A., Gautam, S., Hsu, V., Abood, D., Wesolowski, R., Landi, I., Mo, X., Phay, J., Chen, C.-S., Byrd, J., Caligiuri, M., Tridandapani, S. and Carson, W. E. (2016) Targeting myeloid-derived suppressor cells using a novel adenosine monophosphate-activated protein kinase (AMPK) activator. *Oncoimmunology* **5**, e1214787.
- van Hooren, L., Georganaki, M., Huang, H., Mangsbo, S. M. and Dimberg, A. (2016) Sunitinib enhances the antitumor responses of agonistic CD40-antibody by reducing MDSCs and synergistically improving endothelial activation and T-cell recruitment. *Oncotarget* **7**, 50277-50289.
- Veltman, J. D., Lambers, M. E. H., van Nimwegen, M., Hendriks, R. W., Hoogsteden, H. C., Aerts, J. G. J. V. and Hegmans, J. P. J. J. (2010) COX-2 inhibition improves immunotherapy and is associated with decreased numbers of myeloid-derived suppressor cells in mesothelioma. Celecoxib influences MDSC function. *BMC Cancer* **10**, 464.
- Vonderheide, R. H. and Glennie, M. J. (2013) Agonistic CD40 antibodies and cancer therapy. *Clin. Cancer Res.* **19**, 1035-1043.
- Wang, S. H., Lu, Q. Y., Guo, Y. H., Song, Y. Y., Liu, P. J. and Wang, Y. C. (2016) The blockage of Notch signalling promoted the generation of polymorphonuclear myeloid-derived suppressor cells with lower immunosuppression. *Eur. J. Cancer* **68**, 90-105.
- Weed, D. T., Vella, J. L., Reis, I. M., De la Fuente, A. C., Gomez, C., Sargi, Z., Nazarian, R., Califano, J., Borrello, I. and Serafini, P. (2015) Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. *Clin. Cancer Res.* **21**, 39-48.
- Wei, C., Wang, Y. X., Ma, L., Wang, X., Chi, H., Zhang, S., Liu, T., Li, Z. Y., Xiang, D. M., Dong, Y. L., Wu, X. G., Shi, W. Y. and Gao, H. (2018) Rapamycin nano-micelle ophthalmic solution reduces corneal allograft rejection by potentiating myeloid-derived suppressor cells' function. *Front. Immunol.* **9**, 2283.
- Winkler, D. G., Faia, K. L., DiNitto, J. P., Ali, J. A., White, K. F., Brophy, E. E., Pink, M. M., Proctor, J. L., Lussier, J., Martin, C. M., Hoyt, J. G., Tillotson, B., Murphy, E. L., Lim, A. R., Thomas, B. D., Macdougall, J. R., Ren, P., Liu, Y., Li, L. S., Jessen, K. A., Fritz, C. C., Dunbar, J. L., Porter, J. R., Rommel, C., Palombella, V. J., Changelian, P. S. and Kutok, J. L. (2013) PI3K-delta and PI3K-gamma inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models. *Chem. Biol.* **20**, 1364-1374.

- Xiao, L., Erb, U., Zhao, K., Hackert, T. and Zöller, M. (2017) Efficacy of vaccination with tumor-exosome loaded dendritic cells combined with cytotoxic drug treatment in pancreatic cancer. *Oncoimmunology* **6**, e1319044.
- Xu, Z., Ji, J. J., Xu, J. J., Li, D., Shi, G. P., Liu, F., Ding, L., Ren, J., Dou, H., Wang, T. T. and Hou, Y. Y. (2017) MiR-30a increases MDSC differentiation and immunosuppressive function by targeting SOCS3 in mice with B-cell lymphoma. *FEBS J.* **284**, 2410-2424.
- Yamaoka, T., Kusumoto, S., Ando, K., Ohba, M. and Ohmori, T. (2018) Receptor tyrosine kinase-targeted cancer therapy. *Int. J. Mol. Sci.* **19**, 3491.
- Yan, D. H., Yang, Q., Shi, M. H., Zhong, L. M., Wu, C. Y., Meng, T., Yin, H. Y. and Zhou, J. (2013) Polyunsaturated fatty acids promote the expansion of myeloid-derived suppressor cells by activating the JAK/STAT3 pathway. *Eur. J. Immunol.* **43**, 2943-2955.
- Yoyen-Ermis, D., Ozturk-Atar, K., Kursunel, M. A., Aydin, C., Ozkazanc, D., Gurbuz, M. U., Uner, A., Tulu, M., Calis, S. and Esendagli, G. (2018) Tumor-induced myeloid cells are reduced by gemcitabine-loaded PAMAM dendrimers decorated with anti-Flt1 antibody. *Mol. Pharm.* **15**, 1526-1533.
- Yu, H. and Jove, R. (2004) The STATs of cancer--new molecular targets come of age. *Nat. Rev. Cancer* **4**, 97-105.
- Yun, T. K. (2001) Brief introduction of Panax ginseng C.A. Meyer. *J. Kor. Med. Sci.* **16**, S3.
- Zhang, C., Wang, S., Li, J. W., Zhang, W. T., Zheng, L., Yang, C., Zhu, T. Y. and Rong, R. M. (2017) The mTOR signal regulates myeloid-derived suppressor cells differentiation and immunosuppressive function in acute kidney injury. *Cell Death Dis.* **8**, e2695.
- Zhang, M. M., Liu, Q. F., Mi, S. P., Liang, X., Zhang, Z. Q., Su, X. M., Liu, J. Y., Chen, Y. Y., Wang, M. M., Zhang, Y. A., Guo, F. H., Zhang, Z. J. and Yang, R. C. (2011) Both miR-17-5p and miR-20a alleviate suppressive potential of myeloid-derived suppressor cells by modulating STAT3 expression. *J. Immunol.* **186**, 4716-4724.
- Zhang, S., Wu, K., Liu, Y., Lin, Y., Zhang, X., Zhou, J., Zhang, H., Pan, T. and Fu, Y. (2016) Finasteride enhances the generation of human myeloid-derived suppressor cells by up-regulating the COX2/PGE2 pathway. *PLoS ONE* **11**, e0156549.
- Zhang, X. H., Fang, X. Y., Gao, Z. Z., Chen, W., Tao, F. F., Cai, P. F., Yuan, H. Q., Shu, Y. Q., Xu, Q., Sun, Y. and Gu, Y. H. (2014) Axitinib, a selective inhibitor of vascular endothelial growth factor receptor, exerts an anticancer effect in melanoma through promoting antitumor immunity. *Anticancer Drugs* **25**, 204-211.
- Zhao, Y., Shao, Q., Zhu, H., Xu, H., Long, W., Yu, B., Zhou, L., Xu, H., Wu, Y. and Su, Z. (2018a) Resveratrol ameliorates Lewis lung carcinoma-bearing mice development, decreases granulocytic myeloid-derived suppressor cell accumulation and impairs its suppressive ability. *Cancer Sci.* **109**, 2677-2686.
- Zhao, Y., Shen, X. F., Cao, K., Ding, J., Kang, X., Guan, W. X., Ding, Y. T., Liu, B. R. and Du, J. F. (2018b) Dexamethasone-induced myeloid-derived suppressor cells prolong allo cardiac graft survival through iNOS- and glucocorticoid receptor-dependent mechanism. *Front. Immunol.* **9**, 282.
- Zheng, Y. S., Xu, M., Li, X., Jia, J. P., Fan, K. X. and Lai, G. X. (2013) Cimetidine suppresses lung tumor growth in mice through proapoptosis of myeloid-derived suppressor cells. *Mol. Immunol.* **54**, 74-83.
- Zilio, S., Vella, J. L., De la Fuente, A. C., Daftarian, P. M., Weed, D. T., Kaifer, A., Marigo, I., Leone, K., Bronte, V. and Serafini, P. (2017) 4PD functionalized dendrimers: a flexible tool for *in vivo* gene silencing of tumor-educated myeloid cells. *J. Immunol.* **198**, 4166-4177.
- Zoglmeier, C., Bauer, H., Noerenberg, D., Wedekind, G., Bittner, P., Sandholzer, N., Rapp, M., Anz, D., Endres, S. and Bourquin, C. (2011) CpG blocks immunosuppression by myeloid-derived suppressor cells in tumor-bearing mice. *Clin. Cancer Res.* **17**, 1765-1775.