Molecular Basis of Overcoming Immune Checkpoint Inhibitor Resistance by Targeting Myeloid Derived Suppressor Cells

Writing Assignment

Under supervision of Marjolein Stip and Danielle Krijgsman
Jeanette Leusen Research group
University Medical Center Utrecht

Fleur van Oosterom

17-11-2022

Layman's Summary

Kanker is nog steeds één van de meest voorkomende doodsoorzaken wereldwijd. Kankerpatiënten worden nog vaak behandeld door middel van chemotherapie of bestraling, deze blijken echter minder effectief wanneer de diagnose later gesteld wordt. Daarom wordt er intensief onderzoek gedaan naar nieuwe medicijnen die de overlevingskans verhogen. Eén van deze technieken die de laatste jaren steeds vaker gebruikt wordt, is immunotherapie met behulp van immuun checkpoint inhibitors (ICI). De functie van ICIs is om een immuunreactie te remmen en daarmee auto immuunziektes te voorkomen. Bij ICI therapie wordt gebruik gemaakt van antilichamen die de remmende werking van ICIs opheffen. Dit betekent dat het eigen immuunsysteem kankercellen leert herkennen en zo de groei van tumoren kan remmen en zelfs kan doden. Het gebruik van ICI therapie heeft de overlevingskans voor veel patiënten enorm verbeterd.

Het blijkt echter dat ICI therapie niet effectief is in alle patiënten. Of patiënten reageren op ICI therapie is afhankelijk van verschillende factoren, zoals kankersoort, het stadium van de tumor, maar ook de omgeving om de tumor heen, ook wel het tumor micromilieu (TME) genoemd. Het TME is uniek voor elke tumor. In latere stadia wordt het immuunsysteem geremd door cellen in het tumor micromileu. Dit zorgt ervoor dat tumorcellen niet gedood kunnen worden, waardoor de tumor verder kan groeien en ook kan verspreiden naar ander plekken. Cellen die een grote rol spelen in het remmen van het immuunsysteem rond de tumor, zijn myeloïde-afstammende suppressieve cellen (MDSCs). Deze cellen worden zo genoemd, omdat ze komen van een stamcel die we een myeloïde cel noemen. Deze bevindt zich in het beenmerg en is verantwoordelijk voor de aanmaak van nieuwe immuuncellen. MDSCs zijn dus een type immuun cellen die door de tumor een remmende werking krijgen op het immuunsysteem van de patiënt. In gezonde individuen komen deze cellen bijna niet voor in de circulatie, maar in kankerpatiënten worden deze cellen in hogere concentraties gevonden.

MDSCs worden nu ook steeds vaker in verband gebracht met het ondermijnen van de werking van ICI therapie. ICI therapie probeert het immuunsysteem te activeren. MDSCs werken deze activatie tegen door het immuunsysteem te remmen. In kankerpatiënten die niet reageerden op ICI therapie, werden gemiddeld hogere aantallen MDSCs gevonden. Daarom wordt er steeds meer onderzoek gedaan naar een manier om deze MDSCs te verwijderen uit het tumor micromilieu of ervoor te zorgen dat de immuun remmende eigenschap van deze cellen verloren gaat. Er zijn al verschillende medicijnen ontwikkeld om dit te bereiken. Sommige van deze medicijnen zijn al goedgekeurd voor gebruik in verschillende soorten kanker, zoals het medicijn ATRA dat goedgekeurd is voor behandeling van acute leukemie.

Hier bekijken wij de optie om deze medicijnen gericht tegen MDSCs te combineren met ICI therapie om resistentie tegen ICI therapie tegen te gaan. Daarom hebben wij studies samengevat waarbij onderzoekers deze combinatie van medicijnen uitgeprobeerd hebben in cellen, muizen en in klinische studies in mensen. Vervolgens gebruiken wij deze samenvatting om onze visie te geven op het combineren van medicijnen tegen MDSCs en ICI therapie.

Molecular Basis of Overcoming Immune Checkpoint Inhibitor Resistance by Targeting Myeloid Derived Suppressor Cells

In the last decades, new strategies such as Immune Checkpoint Inhibitor (ICI) therapy have increased survival chances for cancer patients considerably and have become one of the leading immunotherapies to combat cancer. Immune Checkpoint Proteins are expressed to maintain immune homeostasis and prevent autoimmune disease. Using ICI therapy, activation of cytotoxic T cells is promoted with monoclonal antibodies. However, responses to ICI therapy are highly dependent upon the (immunosuppressive) tumor microenvironment (TME). One of the major drivers in creating this immunosuppressive environment are Myeloid Derived Suppressor Cells (MDSCs). MDSCs are a heterogenous population of immature myeloid cells that inhibit T cell proliferation, differentiation and activation. Thereby, MDSCs play a major role in the development of resistance to ICI therapy. In this paper, we review different MDSC targeting strategies, such as depletion, inhibition of immunosuppressive characteristics, and promoting maturation. Furthermore, we assess the potential to overcome ICI resistance by combining ICI therapy with targeting of MDSCs. Studies combining FDA approved anti-CTLA-4, anti-PD-1, and anti-PD-L1 ICI therapies in combination with MDSC targets show reduced MDSC levels and enhances cytotoxic T cell infiltration in the TME of preclinical mouse models, as well as in clinical trials. Therefore, we conclude that targeting important molecular pathways in MDSCs can be used to overcome ICI resistance. However, since the TME is unique for every tumor, more research into molecular pathways is necessary to pave the way towards personalized medicine.

Keywords: ICI therapy; TME; MDSCs; Combination therapy; Resistance

1. INTRODUCTION

In 2018, cancer led to approximately 9,6 million deaths according to the World Health Organization (WHO), making it one of the leading causes of death worldwide. To date, cancer is predominantly treated with conventional therapies, including surgery, radiation, and chemotherapy. However, these conventional therapies show reduced efficacy in metastatic disease (1). Therefore, new therapies with higher efficacy are required. Recently, immunotherapy has gained popularity in the field of oncolytic therapy.

The immune system is a double edged sword in cancer progression. On the one hand, activation of innate and adaptive immunity leads to eradication of tumor cells. On the other hand, the immune system can also promote tumor growth (2). The dual role of the immune system is called immunoediting and consists of three phases: elimination, equilibrium and escape (3,4). In the last decades, studying immune escape strategies employed by the tumor has propelled the field of immunotherapy. Different strategies are now being employed to (re-)activate the immune system against tumor cells: 1) Adoptive cellular therapies; 2) Oncolytic viruses; 3) Cytokine therapy; 4) Cancer vaccines; and 5) Monoclonal antibody therapy, which are antibodies designed to bind to specific targets on cancer cells or immune cells for immune activation. Some monoclonal antibodies bind to cytotoxic T cells directly to induce activation, while others bind to cancer cells to mark the cells for immune recognition (**Figure 1**) (5). In this review, we focus on Immune Checkpoint Inhibitors (ICIs). ICIs are part of the monoclonal antibody therapy designed to activate T cells to fight cancer cells (5).

Checkpoint proteins are expressed on the surface of immune cells to ensure immune tolerance, which is required to tone down the immune response, thereby maintaining immune homeostasis and preventing autoimmune diseases (6,7). Under normal conditions, immune inhibitory and immune activating signals are balanced (8). However, tumor cells can exploit this system by overexpressing inhibiting proteins on the cell surface, thereby suppressing an immune response and promoting tumor growth and metastasis (7,9). Monoclonal antibodies used to block checkpoint proteins, such as Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4), Programmed cell Death protein 1 (PD-1) and its ligand (PD-L1), have demonstrated high efficacy in clinical trials, resulting in FDA approval (10–13). Recently, new ICI targets have been discovered, such as T cell Immunoglobulin and Mucin domain-containing protein 3 (TIM3), Cluster of Differentiation 47 (CD47), CD38, CD39, Lymphocyte-Activation Gene 3 (LAG3), and T cell Immunoreceptor with Ig and ITIM domains (TIGIT) (14).

In this review, we only focus on the FDA approved ICI targets, anti-CTLA-4, anti-PD-1, and anti-PD-L1, because they have already been used for treatment of different types of cancers.

Despite the promising results in clinical trials and FDA approval, responses to ICI therapy vary per patient and per cancer type (15). Mathematical models estimate that less than 20% of all cancer patients respond to ICI therapy (16,17). ICI resistance is mediated by for example acidity, hypoxia or loss of nutrients from the tumor microenvironment (TME) (18–23). Particularly, immune cells residing in the TME propelled research into ICI resistance (24). The TME is a dynamic and complex network of immunosuppressive cytokines, chemokines, immune cells, stromal cells (fibroblasts, endothelial, and mesenchymal cells), and the extracellular matrix (ECM), which help the tumor proliferate, progress and metastasize (25). One of the major drivers of creating an immunosuppressive environment are Myeloid Derived Suppressor Cells (MDSCs) (26). MDSCs are myeloid progenitor cells that have the inherent ability to suppress T cells, leading to decreased T cell proliferation and expansion of the regulatory T cell (Treg) population (27,28). Furthermore, MDSCs facilitate tumor angiogenesis and dampen the overall immune response by secretion of immunosuppressive cytokines, such as IL-10 and TGFβ (29,30). Attracting MDSCs into the TME is an immune escape strategy achieved by the secretion of chemokines, including CXCL1, CXCL2, and CXCL5 by cancer cells (31). The population of MDSCs in the TME negatively correlates with ICI efficacy in different cancer types (32).

Moreover, Sun *et al.* studied how MDSC levels correlated with the clinical response of advanced melanoma patients to ICI therapy. They found higher levels of MDSCs in melanoma patients compared to healthy individuals, which increased after ICI therapy. The increase in MDSCs levels reached a plateau in ICI responders, whereas MDSC populations continued to rise in patients showing disease progression under ICI treatment (33). These results are in line with a study by Meyer *et al.* who observed lower levels of MDSCs in melanoma patients responding to ICI therapy compared to non-responders (34). Additionally, Weber *et al.* have shown that higher levels of MDSCs correspond with poorer clinical outcomes in patients with metastatic melanoma receiving ICI therapy (35).

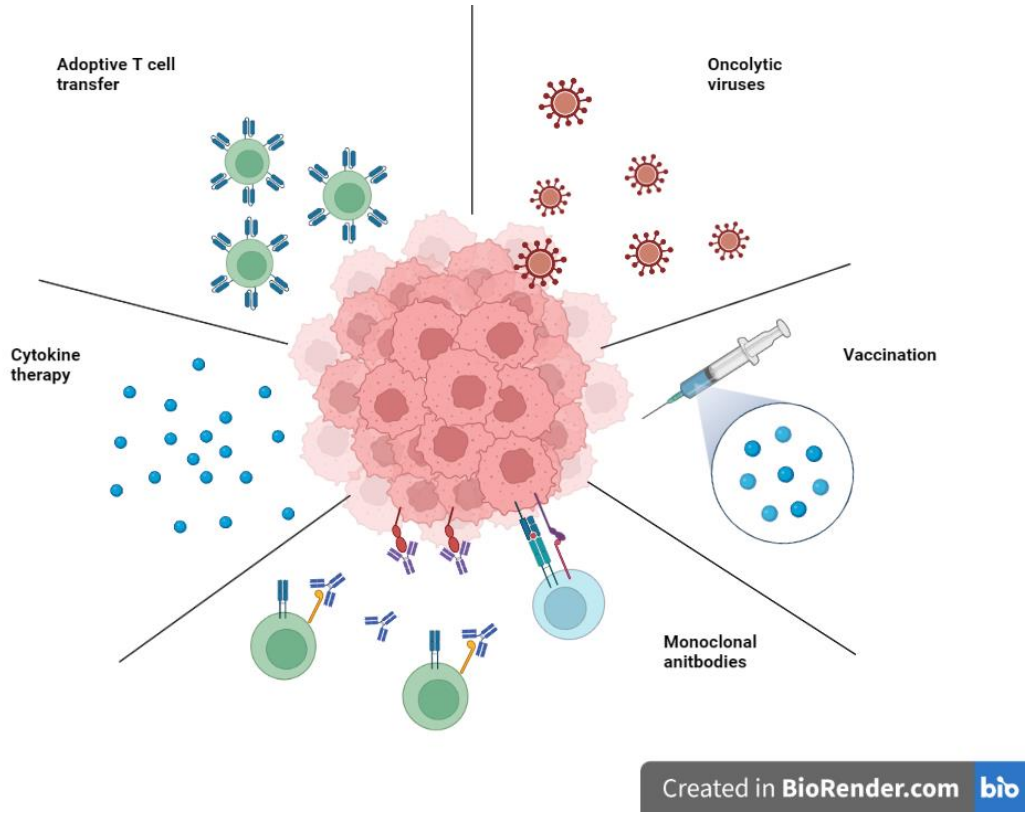

Figure 1: Overview of the different types of immunotherapies that are being studied. 1) Adoptive T cell Transfer; 2) Oncolytic viruses; 3) Cytokine Therapy; 4) Cancer vaccines; and 5) Monoclonal antibodies, including ICI therapy.

Thus, MDSC infiltration into the TME is associated with ICI resistance and poorer clinical outcomes in many cancer patients. Reducing MDSC populations in the TME could abolish immunosuppressive characteristics, resulting in abrogation of $CD8^+$ T cell inhibition, and thereby stimulation of T cell activation. As mentioned before, ICI therapy with CTLA-4, PD-1, and PD-L1 relies on T cell activation. Therefore, targeting of MDSCs in combination with ICI therapy is currently being studied. In this review, we aim to summarize molecular mechanisms for overcoming ICI resistance by targeting MDSCs in the TME. Therefore, we first explain the mechanism for T cell activation that CTLA-4, PD-1, and PD-L1 rely on, before focusing on MDSC targets and molecular pathways active in combining targeting MDSCs and ICI therapy. We summarize *in vitro*, *in vivo,* and clinical data to demonstrate the potential of combining these two strategies before suggesting future research directions.

2. IMMUNE CHECKPOINT INHIBITORS

In 2018, the Nobel Prize for Medicine was awarded to Allison and Honjo for the discovery of immune checkpoint proteins CTLA-4 and PD-1/PD-L1 (36). The discovery of these proteins has led to the development of many different antibody therapies. **Table 1** shows an overview of the FDA approved ICI therapies against these three targets. ICI therapy relies on T cell activation which is only achieved in the presence of three signals. The first signal is T cell receptor (TCR) recognition of antigens presented by major histocompatibility complex (MHC). The second signal is co-stimulation by binding of B7 expressed on dendritic cells (DCs) to CD28 on T cells. Finally, the third signal is binding of the pro-inflammatory cytokine IL-7 to mediate differentiation and T cell expansion (37). However, even with these three signals present, T cell activation can be blocked by ICI proteins (8).

2.1 CTLA-4

Before its role in cancer was discovered, mutations in the *CTLA-4* gene were recognized as major players in the development of auto-immune diseases (38). CTLA-4 is a homolog of CD28, the co-stimulatory receptor needed for T cell activation. CTLA-4 competes with CD28 for the same ligands, CD80 (B7.1) and CD86 (B7.2). However, CTLA-4 has a higher affinity for B7.1 and B7.2 (39). Therefore CTLA-4 is more likely to bind compared to CD28. Binding of CTLA-4 to its ligand reduces production of IL-2 and the α-chain of the IL-2 receptor (40), while IL-2 signaling has been identified as a key event in T cell activation an proliferation (41,42). IL-2 binding to cell cycle inhibitors induces T cell proliferation by activation of Cyclin Dependent Kinases (CDKs). Blocking IL-2-signaling through CTLA-4 activation leads to inhibition of T cell proliferation by reducing cyclin D3, cdk4, and cdk6 (43). In 1996, Leach *et al.* observed that blocking CTLA-4 with a monoclonal antibody resulted in inhibition of colon tumor growth, as well as clearance of established colorectal tumors in mice (44). This led to the development of the first ICI, Ipilimumab, which was approved for treatment of metastatic melanoma in 2014 (45). However, in a long-term survival study with melanoma patients, only 20% demonstrates a durable response 5-10 years after starting CTLA-4 treatment (46).

2.2 PD-1/PD-L1

Similar to CTLA-4, mutations in PD-1 were associated with the development of autoimmune diseases before their role in cancer biology was established (47,48). Under steady state conditions, immune cells express low levels of PD-1, but expression increases on T cells, B cells, NK cells, DCs and macrophages upon activation (49). PD-1 has two main ligands, PD-L1 and PD-L2. PD-L1 is mainly expressed on T cells, endothelial cells, fibroblasts, and tumor cells, whereas PD-L2 is expressed on monocytes, macrophages, DCs, and B cells (50). Interestingly, activated MDSCs have been shown to express high levels of PD-L1 (51). Interaction between PD-1 on T cells, and PD-L1 leads to T cell inhibition by inducing immunosuppressive interleukin 10 (IL-10) expression, exhaustion, anergy, and apoptosis of T cells (52). To evade a cytotoxic T cell-driven immune response, many human tumors express PD-L1, including lung, kidney and melanoma (53). Blocking the PD-1/PD-L1 interaction has been shown to lead to more active, tumor-specific $CD8^+$ T cells in the TME (54). Nowadays, anti-PD-1/PD-L1 monoclonal antibodies, including Nivolumab and Pembrolizumab to target PD-1, and Atezolizumab targeting PD-L1, are one of the most prescribed anticancer therapies (55). In melanoma patients treated with Pembrolizumab, 33% showed a clinical response after 3 years, while 70% of the patients initially responded to the treatment (56).

Table 1: FDA approval of ICIs. A list of FDA-approved ICI therapies targeting CTLA-4, PD-1, and PD-L1 (57–59).

Target	Brandname	Approval	Indication
CTLA-4	Ipilimumab (Yervoy®)	2011	Melanoma
		2018	Colorectal Cancer
		2018	Renal Cell Carcinoma
	YH001	2022	Alveolar Soft Part Sarcoma and chondrosarcoma
PD-1	Nivolumab (Opdivo®)	2014	Melanoma
		2016	Hodgkin's Lymphoma
		2017	Colorectal cancer
		2018	Small cell lung cancer
		2018	Renal cell carcinoma
		2020	Hepatocellular carcinoma
		2020	Squamous cell carcinoma
		2021	Urothelial cancer
		2022	Non-small cell lung cancer
	Pembrolizumab (Keytruda®)	2014	Melanoma
		2017	Gastric Cancer
		2018	Diffuse large B-cell Lymphoma
		2018	Hepatocellular carcinoma
		2018	Merkel cell carcinoma
		2019	Non-small cell lung cancer
		2019	Small cell lung cancer
		2020	Squamous cell carcinoma
		2020	Colorectal cancer
		2020	Hodgkin's Lymphoma
		2021	Renal cell carcinoma
		2021	Esophageal cancer
		2021	Urothelial cancer
		2021	Cervical cancer
	Cemiplimab (Libtayo®)	2018	Cutaneous squamous cell carcinoma
	Dostarlimab (Jemperli®)	2021	Endometrial cancer
PD-L1	Atezolizumab (Tecentriq®)	2016	Non-small cell lung cancer
	Avelumab (Bavencio®)	2017	Merkel cell carcinoma
		2019	Renal cell carcinoma
		2020	Urothelial cancer
	Durvalumab (Imfinizi®)	2017	Bladder cancer
		2022	Non-small cell lung cancer

3. MYELOID DERIVED SUPPRESSOR CELLS

As highlighted before, resistance to ICI therapy is often associated with accumulation of MDSCs in the TME (32). Under normal conditions, myeloid progenitor cells differentiate in the bone marrow from multipotent hematopoietic stem cells (HSCs) and mature into DCs, macrophages or granulocytes (60). However, during cancer development, granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) are secreted by tumor cells, leading to increased myelopoiesis while maturation is inhibited (61,62). Reduced maturation of myeloid progenitor cells leads to accumulation of a large population of immature myeloid derived cells (63). In healthy individuals, GM-CSF and G-CSF levels are generally low (64). Therefore, MDSCs populations are almost absent in healthy individuals, but accumulation of MDSCs has been observed both in inflammatory diseases and cancer (65–67).

Originally, MDSCs were discovered in tumor-bearing mice (68). In mice, identification of MDSCs is based on the expression of CD11b and Gr-1 (69). Murine MDSCs are further classified into granulocytic (G-MDSCs) or monocytic (M-MDSCs) by expression of Ly6G or Ly6C, respectively (70). In humans, MDSC-specific markers are lacking, making identification challenging. However, some common expression patterns have been identified. Human MDSCs are classified into three subtypes: early-immature MDSCs (eMDSCs), monocytic-MDSCs (M-MDSCs), and granulocytic-MDSCs (G-MDSCs). eMDSCs have been described more recently and can be identified by $CD33^+$ $CD11b^+$ $CD14^-CD15^-$ HLA^-DR^- expression (71). M-MDSCs are characterized by expression of surface markers $CD33^+CD11b^+CD14^+CD15^-HLA\text{-}DR^{lo/-}$, whereas G-MDSCs express $CD33^+CD11b^+CD14^-CD15^+$ (or $CD66^+$) (72). However, since these surface markers are not specific for MDSCs, but can also be found on other immune cells, the immunosuppressive activity should be shown in addition. Therefore, assays measuring T cell proliferation and activation have been developed (73). Only when both markers and immunosuppressive features have been observed, cells are identified as MDSCs.

3.1 Immunosuppressive features of MDSCs

MDSCs can inhibit both innate and adaptive immune responses (60) (**Figure 2**). The innate immune response is inhibited by secretion of inhibitory cytokines, including IL-10 and TGF-β (74). These cytokines negatively affect NK cell maturation and drive polarization of macrophages towards the immunosuppressive M2 phenotype instead of the immune activating M1 phenotype, thereby promoting tumor progression (75).

MDSCs inhibit the adaptive immune response predominantly by suppression of T cells. They are infamous for depletion of essential amino acids including L-arginine and L-cystine required for T cell proliferation and activation from the TME. Depletion of L-arginine is achieved by elevated Arginase-1 (ARG1) secretion by MDSCs (76), which degrades L-arginine into L-ornithine and urea. Additionally, inducible nitric oxide synthase (iNOS) upregulation by MDSCs leads to metabolization of L-arginine into nitric oxide (NO) and L-citrulline (77). Consequently, the lack of L-arginine in the TME leads to loss of the TCRζ chain, resulting in decreased T cell proliferation and differentiation (78). Elevated levels of L-cystine uptake and metabolization by MDSCs result in a deficiency in both L-cystine and L-cysteine in the TME, since MDSCs are unable to transport L-cysteine back into the extracellular environment (79). A lack of L-cysteine and L-cystine in the TME results in decreased proliferation and activation of T cells (80,81).

Moreover, MDSCs produce nitric oxide (NO), reactive oxygen (ROS) and reactive nitrogen species (RNS) (82). As mentioned before, MDSCs secretion of iNOS leads to the production of NO, which has been shown to block T cell function by interference with IL-2R signaling, thereby inhibiting T cell proliferation (77). In addition, NO leads to nitration of the TCR, thereby preventing binding of the TCR to the antigen presenting MHC molecule (83). ROS production is achieved by upregulation of NADPH-oxidase isoform (NOX2) and is associated with catalyzing nitration of the TCR, as well as inducing T cell apoptosis (84). Additionally, a chemical reaction can take place when NO and ROS are in close proximity, generating RNS. Similar to NO, RNS also lead to nitration of the TCR preventing antigen presentation (83). Additionally, RNS-mediated nitration of the cytotoxic T cell chemoattractant CCL2 has been observed, reducing infiltration of cytotoxic T cells into the TME (85).

Furthermore, cytokines and chemokines, including IL-10 and TGFβ, secreted by MDSCs induce regulatory T cell (Treg) proliferation, leading to accumulation of Treg cells (86). A complex interplay between MDSCs and Tregs is subsequently established in which MDSCs induce Treg polarization, while Tregs promote survival and proliferation of MDSC (28). This was shown in an ex-vivo experiment in which co-incubation of murine MDSCs with Tregs increased the population of MDSCs significantly (28). However, the exact molecular mechanism by which Tregs affect MDSC survival and proliferation is not known yet.

Next to these immunosuppressive functions, MDSCs also have tumor promoting abilities that do not rely on immune inhibition, including promotion of neo-angiogenesis by exploiting vascular endothelial growth factor (VEGF). Tumor cells secrete the VEGF growth factor, which leads to induction of neo-angiogenesis and, ultimately, metastasis (87). VEGF works as a chemoattractant for MDSCs, promoting migration of MDSCs from the bone marrow towards cancer sites (88). There, binding of VEGF to the VEGF receptor expressed on MDSCs induces a positive feedback loop in which activation of JAK/STAT3 signaling results in the production of more VEGF (89,90). This mechanism promotes neo-angiogenesis and maintains MDSC populations in the TME (91).

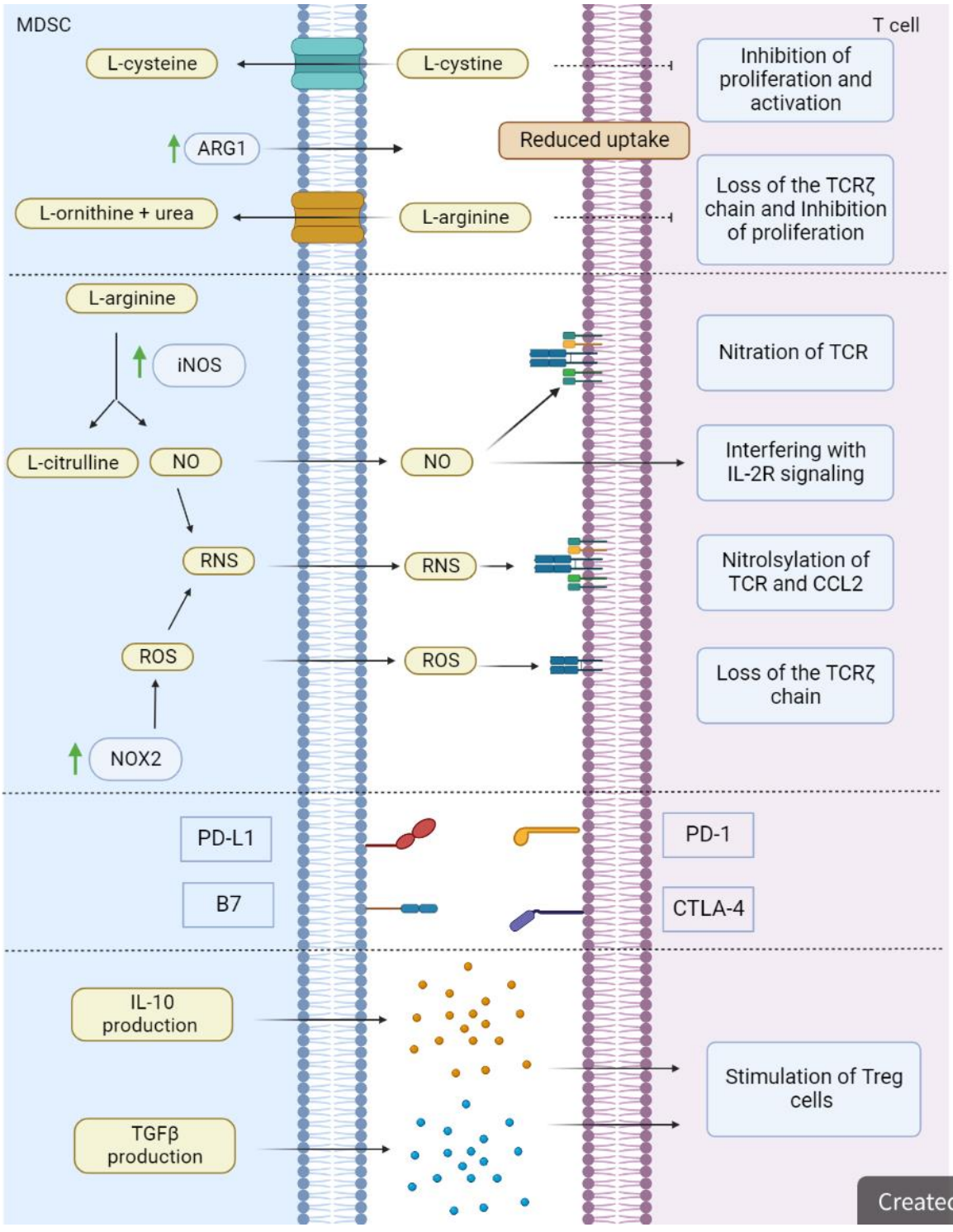

Figure 2: Immunosuppressive effects of MDSCs on T cells. MDSCs deplete the TME from amino acids necessary for T cell functioning. This is achieved by internalization of L-cystine, which is metabolized to L-cysteine and cannot be transported out of MDSCs. Furthermore, ARG1 overexpression and increased L-arginine uptake result in depletion of -arginine out of the TME. L-arginine is subsequently metabolized into L-ornithine and urea. However, upregulation of iNOS can also result in metabolization of L-arginine into L-citrulline and NO, which leads to nitration of the TCR and interferes with IL-2R signaling. NOX2 upregulation leads to increased ROS production, which by itself leads to loss of the TCRζ chain. In addition, ROS and NO can form RNS which leads to nitrosylation of the TCR and CCL2. Moreover, MDSCs also express inhibiting ligands, for example PD-L1 and B7. Finally, MDSCs produce immunosuppressive cytokines, thereby increasing Tregs in the TME.

3.2 Targeting MDSCs

Recent understanding of MDSC-mediated T cell suppression, has led to a shift in research focus. By inhibiting T cells, MDSCs overrule the ICI-mediated T cell activation (92). Three different approaches are being studied to neutralize the immunosuppressive effect of MDSCs; 1) Depletion of MDSCs in the TME; 2) Reducing the immunosuppressive effect of MDSCs; 3) promoting maturation of MDSCs.

3.2.1 Depletion of MDSCs

In a murine lung cancer model, MDSC depletion increased APCs in the TME, which enhanced the activity of cytotoxic T cells and reduced tumor growth (93). Next to that, a reduction in MDSCs in the TME, has been shown to correlate with overcoming ICI resistance in metastatic melanoma patients (94). Therefore, depleting MDSCs from the TME is one of the major strategies to enhance immune responses in cancer patients.

First of all, MDSC development and expansion can be inhibited by targeting GM-CSF and G-CSF, which are the major drivers of accumulation of MDSCs in humans (95). *In vitro* studies showed G-CSF-mediated promotion of MDSC survival and activation through signal transducer and activator of transcription (STAT3) signaling (96). Moreover, preclinical studies in a mouse colitis-associated cancer model have reported that blocking GM-CSF leads to decreased levels of MDSCs, promoting an antitumor immune response (96). These findings are in line with a clinical study performed in recurrent prostate cancer patients where administration of recombinant human GM-CSF increased MDSC levels in the blood (95). Some chemotherapeutics indirectly target these growth factors. For example, using Paclitaxel in a low, non-toxic dose decreases GM-CSF secretion, thereby reducing accumulation of MDSCs in the TME in a melanoma mouse model (97).

As mentioned before, VEGF secreted by tumor cells is an important chemo-attractant for MDSCs (98). Besides reducing MDSCs, targeting VEGF has the extra therapeutic potential of inhibiting neo-angiogenesis and tumor progression (99). Bevacizumab is an antibody binding to VEGF, blocking interaction between VEGF and its receptors (100). In non-small cell lung cancer (NSCLC) patients, bevacizumab treatment reduced numbers of circulating MDSCs (101). Additionally, bevacizumab has been used in a clinical trial in combination with a low dose of chemotherapeutic Capecitabine to treat recurrent glioblastoma. Results showed a decrease in MDSCs and an increase in tumor-specific cytotoxic T cells in the TME (102). However, in renal cell carcinoma (RCC) patients, administration of bevacizumab did not lead to a significant reduction in MDSCs in peripheral blood (103). Yet, MDSC populations in the TME have not been studied in RCC patients, leading to a measuring discrepancy between the two studies. Moreover, cancer type and stage can influence results, since not all cancer types utilize VEGF signaling to the same extent (104).

In addition, recruitment of MDSCs by blocking chemokine signaling can be exploited to deplete MDSCs from the TME. MDSCs have multiple chemokine receptors, such as CCR2, CCR4, and CCR5 (105). Chemokine CCL2, which interacts with CCR2 and CCR4, has been described to promote prostate cancer tumorigenesis in humans by recruiting MDSCs (106). In numerous preclinical models, including murine glioma and melanoma models, blocking CCL2 signaling by using monoclonal antibodies together with radiation or immunotherapy, resulted in a significant reduction in MDSCs (94,107,108). Monoclonal antibodies neutralizing CCR5 ligands reduce migration of MDSCs into the TME and improve survival of mice with melanoma (109). Additionally, CCR5 blockades inhibited growth of prostate, breast and pancreatic tumors in different mouse models (110,111). In colorectal cancer patients with liver metastases, blockade of CCR5 resulted in decreased levels of MDSCs and inhibited tumor progression (112).

As previously mentioned, MDSCs are hard to identify due to lack of specific markers and heterogeneity within populations. Recently Lectin-type Oxidized LDL receptor 1 (LOX-1), CD10 and CD33 overexpression has been observed on MDSC populations (113–115). However, overexpression of LOX-1 and CD10 is subtype-specific (113,114). Therefore, clinical agents against these two targets have not been developed yet. CD33 overexpression has been shown to overlap between MDSC subtypes (116). In fact, CD33 is currently being evaluated as a potential target to directly deplete MDSCs from the TME (117). A monoclonal antibody called BI 836858 depletes MDSCs from the TME of patients with myelodyplastic syndrome though antibody-dependent cell-mediated cytotoxicity (ADCC) (118). Additionally, another CD33 specific antibody, called Gemtuzumab

ozogamicin, reactivates T cells in the TME by depleting MDSCs observed in clinical trials acute myeloid leukemia (AML) patients (117). Therefore, Gemtuzumab ozogamicin has been FDA approved for treatment of AML patients.

Lastly, it has been observed that some chemotherapeutics and other anti-cancer drugs result in a reduction of MDSCs in the circulation of tumor-bearing individuals. For example, clinical data obtained from Gemcitabine and 5-Fluorouacil (5-FU) treatment in patients with different tumors specifically result in the depletion of MDSCs without negatively affecting T cells, B cells, NK cells, and DCs (119,120). Mechanistically, a single dose of Gemcitabine promotes apoptosis and necrosis of splenocytes in mice bearing TC-1 tumors, thereby reducing the immunosuppressive effect and MDSC populations significantly (119). Results for 5-FU treatment are in line with Gemcitabine treatment. However, when comparing Gemcitabine and 5-FU treatment in mice with colorectal tumors, higher efficacy was reported using 5-FU (120). 5-FU treatment enhanced IFN-γ production by $CD8^+$ T cells, as well as significantly increased numbers of infiltrating T cells into the TME.

3.2.2 Inhibition of the immunosuppressive mechanisms exploited by MDSCs

The second strategy focusses on neutralization of the immunosuppressive mechanisms MDSCs use to inhibit $CD8^+$ T cells activation and proliferation. The aforementioned enzymes ARG1, NOX2 and iNOS are key driver of the immunosuppressive characteristics carried out by MDSCs (76,77), as well as immunosuppressive cytokines secreted by MDSCs (86). Therefore, pathways underlying upregulation of these enzymes and cytokines are being studied.

As described before, MDSCs upregulate ARG1 secretion, which depletes the TME of L-arginine (81). Decreasing ARG1 expression can be achieved by targeting the cyclooxygenase 2 (COX-2)/Prostaglandin E2 (PGE2) pathway. COX-2 is an enzyme that catalyzes the production of PGE2 from arachidonic acid. Its expression is regulated and induced under tumorigenic and inflammatory conditions (121). PGE2 interacts with receptors from the E prostanoid (EP) family, which has 4 different subtypes, EP1-4. Prostate cancer cells have been shown to upregulate expression of EP2 and EP4 (122). The COX-2 pathway promotes MDSC accumulation and maintains the suppressive function of MDSCs (123–125). Both COX-2 and PGE2 overexpression are associated with immunosuppression, reduced cancer cell apoptosis and increased neo-angiogenesis (126). In MDSCs, COX2 and PGE2 overexpression were shown to play a critical role in differentiation of MDSCs in murine bone marrow (127). Furthermore, M-MDSC were shown to exhibit their suppressive activities after PGE2 signaling, which was observed by upregulation of ARG1 secretion and increased NO production (128). Blocking the COX-2 pathway with specific inhibitors leads to adverse effects (129). Therefore, research focusses on inhibitors that are activated later in the pathway, for example by inhibiting EP4 (130).

Furthermore, Histone deacetylase (HDAC) inhibitors (HDACIs) have been shown to reduce expression of COX-2, ARG1 and iNOS significantly (131,132). HDACs are enzymes that remove acetyl groups from N-acetyl lysine on histones, thereby regulating gene expression (133). The enzymes are subdivided in four different classes based on their sequence similarity, which are HDAC class I-IV (134). Interestingly, HDACIs have also been shown to regulate immune cells in the TME, although the exact mechanism is not understood yet (135). HDACI Entinostat reduced the immunosuppressive activity of G-MDSCs significantly in mice with EL4 lymphoma and Lewis Lung Carcinoma (LLC), whereas HDAC inhibitor Ricolinostat decreased the suppressive activity of M-MDSCs (136).

Moreover, phosphodiesterase-5 inhibitors, like Sildenafil and Tadalafil, have been associated with a reduction in MDSC immunosuppressive activity in preclinical mouse models, as well as in patients that were treated for benign prostatic hyperplasia and pulmonary hypertension (137). It has been shown that administration of Sildenafil in mice with melanoma downregulates the production of ROS and RNS and decreases ARG1 secretion by inhibition of STAT6 signaling (127). Additionally, higher levels of intracellular cyclic guanosine monophosphate (cGMP) have been reported in MDSCs after Sildenafil treatment (138). Increased cGMP levels lead to destabilization of iNOS mRNA, resulting in decreased NO secretion and a decrease in RNS in the TME (139). Furthermore, treatment of metastatic melanoma patients with Tadalafil resulted in a significant decrease in MDSCs in metastatic lesions in patient (140). In patients with head and neck squamous cell carcinoma (HNSCC),

Tadalafil treatment did not only reduce levels of Tregs and MDSCs, but it was also shown to decrease both ARG1 and iNOS secretion (141,142).

Recently, targeting the STAT3 pathway has gained interest to target for blocking MDSC immunosuppressive effects (143,144). STAT3 is part of the STAT protein family which consists out of 7 different proteins numbered from 1 to 7. Janus kinase (JAK) tyrosine kinases are responsible for phosphorylation of STAT proteins, which are activated by binding of cytokines or growth factors to the receptor. Phosphorylated STAT proteins travel to the nucleus to bind to STAT inducible genes (145). STAT3 signaling is directly responsible for transcription of *ARG1* and *NOX2* (146). In murine cancer models, blocking of STAT3 signaling in MDSCs resulted in activation of an anti-tumor response and reduction of MDSCs in the TME(147). Furthermore, a STAT3 antisense oligonucleotide inhibitor, called AZD1950, inhibited tumor growth in a phase I clinical trial in patients with lymphoma and lung cancer (148).

3.2.3 Maturation of MDSCs

The last strategy is maturation of MDSCs into mature neutrophils or macrophages without suppressive activity. Most of the studies focus on M-MDSC maturation, since the possibility of maturation of G-MDSCs into mature neutrophils is still under discussion (149).

DNA methyltransferase inhibitors, including Decitabine, induce maturation of MDSCs into APCs by reactivating epigenetically silenced tumor suppressor genes (150). In addition, blocking of retinoic acid signal transduction using all-trans retinoic acid (ATRA), a vitamin A metabolite, leads to differentiation of MDSCs into DCs and macrophages (151). ATRA upregulates glutathione synthase (GSH) expression, thereby leading to reduced ROS production, which has been associated with maturation of MDSCs (151). ATRA enhances IFNγ production by T cells in patients with metastatic RCC and late stage small cell lung cancer (SCLC) (105). Lastly, Docetaxel, an anti-microtubule drug used as cancer therapy, promoted differentiation of MDSCs into M1 macrophages in a mouse mammary carcinoma model by indirectly reducing STAT3 activation (152).

4. TARGETING MDSCS IN COMBINATION WITH ICI THERAPY

The exact mechanism behind ICI therapy resistance is still poorly understood. Considering the immunosuppressive effect MDSCs have on T cells, studies started investigating combining ICI therapy with targeting of MDSCs. As previously described, there are different methods to target MDSCs. Here, we subdivide studies into the mechanism that is used to target MDSCs. **Table 2** shows an overview of the different agents and most important outcomes of the different murine studies. In addition, **Table 3** presents the most important outcomes in human studies.

4.1 JAK/STAT3 signaling inhibition

Inhibitors of the aforementioned STAT3 pathway have been investigated extensively over the past years due to the important role STAT3 signaling plays in maintaining MDSC immunosuppressive activity (143,144). Moreover, the JAK/STAT3 pathway can regulate expression of PD-L1 and PD-L2 on tumor cells, as well as on MDSCs (153). APT_{STAT3}-9R is a STAT3 inhibitor that is currently being studied in clinical trials for treatment of $BRAF^{V600E}$-mutant melanoma which is resistant to BRAF inhibitor Vemurafenib (154). APT_{STAT3} was found to be a high affinity peptide binder for STAT3 which inhibits phosphorylation of STAT3, and thereby halts the STAT3 signaling pathway (155). APT_{STAT3} was then modified with a 9-arginine motif to penetrate cancer cells more easily (156). In a mouse model, APT_{STAT3}-9R was shown to remodel the TME by significantly reducing MDSCs and TAMs while increasing the $CD8^+/CD4^+$ T cell ratio (157). The increase in $CD8^+$ T cells led to the hypothesis that a combination with anti-PD-1 would results in a higher anti-tumor efficacy. The combination between APT_{STAT3}-9R and anti-PD-1 therapy resulted in a significant inhibition of tumor growth and longer overall survival compared to the groups that received a monotherapy (157).

Furthermore, 3,3'-Diindolylmethane (DIM) is a naturally occurring component that has been used in traditional Chinese medicine and exhibits anti-cancer activity by inhibition of cancer cell proliferation and promotion of cancer cell apoptosis (158). *In vitro*, MDSCs lost the immunosuppressive characteristics after DIM

treatment, which was observed by a decrease in ARG1 and NOX2 mRNA, as well as abrogation of the inhibitive effect from MDSCs on T cell proliferation (159). This effect was achieved by suppression of STAT3 activation (159). In a 4T1 breast cancer mouse model, a combination of DIM and anti-PD-1 provided a significant synergistic effect on the reduction of tumor growth compared to single treatment groups. Results from the group receiving combination therapy showed elevated IFNγ levels, an increase in both $CD4^+$ and $CD8^+$ T cells, and a decrease in the ratio of MDSCs from bone marrow, spleen, blood and in tumor tissue (159).

4.2 Histone Deacetylase inhibitors

Many different HDACIs have been developed which target different HDAC classes. Trichostatin A (TSA) is a panHDACI, which induces apoptosis in Eppstein-Bar virus associated tumor cells (160). After TSA treatment, a reduction of MDSCs in CT26/Her2 tumor-bearing mice was observed (161). Another HDACI is valproic acid (VPA), which inhibits HDAC class I enzymes. After VPA monotherapy, Xie Z *et al.* observed a reduction of the proportion of G-MDSCs, as well as a reduction in the level of ARG1 in mice injected with B16F10 melanoma cells (162). Furthermore, VPA reduces MDSC accumulation and leads to maturation of MDSCs into mature antigen presenting DCs (163). Remarkably, combining VPA with anti-PD-L1 in the same melanoma mouse model as described before, led to a significant reduction in MDSCs, as well as complete abrogation of the immunosuppressive characteristics of MDSCs (162).

Mocetinostat is a class I/IV HDACI involved in epigenetic silencing of immunoregulatory genes of immune cells and tumor cells (135). Interestingly, Mocetinostat treatment has been shown to upregulate PD-L1 *in vitro* (164). Therefore, the combination between Mocetinostat and anti-PD-L1 was tested in mice with NSCLC, showing an increase in tumor specific $CD8^+$ T cells, a decrease in MDSC population and an increase in pro-inflammatory factors, including CXCR6 and CCL5 (164). Moreover, class I HDACI Entinostat was shown to significantly reduce MDSC cell viability and thereby, sensitized tumor cells to anti-PD-1 treatment in lung and RCC mouse models (165). Additionally, in a study using pancreatic and breast cancer mouse models, Entinostat synergistically enhanced the effect of anti-PD-1 and anti-CTLA-4 therapy (165), an effect that was also observed using VPA in a melanoma mouse model (166).

After promising preclinical data was obtained using HDACIs to overcome ICI resistance, clinical trials were started. Mocetinostat has been tested in combination with Nivolumab and Ipilimumab in 10 unresectable melanoma patients (167). Of these 10 patients, 8 responded to the treatment, marked by inhibition of tumor progression and a significant decrease in MDSC levels in patient blood. However, toxic effects were observed in all patients (167). Therefore, other HDACIs were being tested in combination with ICI therapy. Entinostat treatment in combination with Nivolumab and Ipilimumab was tested in 33 patients with advanced solid tumors (168). Response rates in this clinical trial were 16% and patients that responded to the treatment had a higher $CD8^+$ T cell/Treg ratio (168). Patients with NSCLC treated with HDACI Vorinostat in combination with Pembrolizumab showed a 13% response rate (169). Strikingly, NSCLC patients with 'cold' tumors, which are tumors that show low cytotoxic T cell infiltration, benefited the most of Vorinostat and Pembrolizumab combination therapy due to enhanced cytotoxic T cells infiltration. With Pembrolizumab monotherapy, the response rate was 33%, while the response rate was 66% using combination therapy, which was associated with a lower number of MDSCs in the TME (170). These data support the development of HDACIs in combination with ICI therapy.

4.3 Tyrosine kinases inhibitors

Tyrosine kinases have been associated with trafficking, maturation and function of myeloid cells (171,172). AXL is a tyrosine kinase that is important for many signaling pathways, ranging from cell differentiation, cell proliferation to cell survival (173). Overexpression of AXL is observed in many human cancers and leads to immunotherapy resistance and a poorer prognosis. Strikingly, MDSCs were also shown to upregulate AXL expression significantly (174). In AXL knockout mice, the MDSC suppressive phenotype was abolished as measured using T cell proliferation assays (175). Consequently, tyrosine kinase inhibitors (TKIs) were developed, including AXL inhibitor SKI-G-801 (176). Mice injected with murine breast cancer cell line 4T1 were treated with SKI-G-108, anti-PD1, or a combination (175). Remarkably, combination therapy resulted in curing 2 out of 5 mice

and longer overall survival than either of the therapies alone. Immunohistochemistry analysis showed increased levels of $CD8^+$ T cells, decreased levels of MDSCs, and higher expression of PD-L1 on tumor cells, giving insight into the mechanism by which AXL inhibitor SKI-G-801 has a synergistic effect with anti-PD-1 ICI therapy (175).

In addition, Cabozantinib is a TKI that targets multiple tyrosine kinases at once: VEGFR-2; MET; AXL, and KIT (98). in a murine castrate resistant prostate cancer model, Cabozantinib treatment resulted in tumor clearance, mature neutrophil infiltration into the TME and a reduction MDSC populations, as well as a reduction in their ability to suppress T cells (177,178). When combined with anti-CTLA-4/anti-PD-1 ICI therapy, the castrate resistant prostate tumors growth and formation of metastases was synergistically reduced (178). Additionally, Cabozantinib treatment in a murine colon cancer model, resulted in $CD8^+$ T cell infiltration (179). These preclinical models were translated into clinical trials treating patients with genitourinary tumors. From the 64 patients that received Cabozantinib and Nivolumab treatment with or without ipilimumab, there was a 36% response rate, which was still ongoing after 2 years for 70% of the responders (180). These studies laid the groundwork for FDA approval of using Cabozantinib and Nivolumab as first-line treatment for patients with advanced RCC (181).

Furthermore, Sorafenib and Lenvatinib are TKIs which have shown to result in a significant reduction in MDSCs in the TME and increased infiltration of $CD4^+$ and $CD8^+$ T cells (182,183). Sorafenib and Lenvatinib have recently been approved for use as hepatocellular carcinoma (HCC) therapy (184). Lenvatinib in combination with anti-PD-1 antibody nivolumab showed high efficacy in a phase 1 trial which included 30 HCC patients (185). Lenvatinib has also been studied in combination with Pembrolizumab, showing little toxicity and a high response rate in treated HCC patients (186). Lee *et al.* tested different combinations of TKIs and ICIs in 33 patients which received different treatment combinations: 8 patients were treated with Sorafenib and Nivolumab; 4 with Sorafenib and Pembrolizumab treatment; 11 with Lenvatinib and Nivolumab treatment; and 10 with Lenvatinib Pembrolizumab treatment (187). Patients treated with combination therapy including Lenvatinib had the highest objective response rate (ORR): Lenvatinib/Pembrolizumab resulted in 70% ORR and Lenvatinib/Nivolumab in a 51,8% ORR, whereas the combination Sorafenib/Nivolumab resulted in a 25% ORR (192).

4.4 PI3K/AKT/mTOR signaling inhibition

The PI3K/AKT/mTOR pathway plays an important role in regulation of the cell cycle, stimulating cell proliferation after phosphorylation of PI3K (188). In addition, the PI3K signaling pathway has been shown to play an important role in myeloid cell function, chemotaxis, neutrophil apoptosis and MDSC proliferation and survival (189,190). MDSCs express high levels of PI3Kγ which promotes the production of inflammatory mediators and migration towards the TME (191). Enioutina *et al* showed the importance of PI3K signaling in the accumulation of MDSCs in aging mice (192).

In mouse models injected with murine cancers, including colorectal cancer cell lines CT26 and MC38, and breast cancer cell line 4T1, PI3Kγ inhibition with small molecule inhibitor IPI-549 reduced tumor growth and lung metastasis (193). Furthermore, IPI-549 treatment increased infiltration of $CD8^+$ T cell into the tumor bed (193). Combination of IPI-549 and either anti-CTLA-4 or anti-PD-1 led to a significant reduction in tumor growth using LLC and 4T1 murine cancer cell lines (193). These findings are in line with previous research by Kaneda *et al.* where a synergistic reduction of murine HPV^- HNSCC tumor growth was observed, as well as tumor regression of established tumors using a PI3Kγ small molecule inhibitor in combination with anti-PD-1 (194).

Recently, ARTs (effective antimalarial drugs) have also been reported to exhibit immunomodulatory and antitumor properties (195,196). In a mouse model injected with murine breast cancer cell line 4T1, a decline in the immunosuppressive effects of MDSCs was observed after ART therapy, marked by reduced ARG1 and iNOS secretion (197). To determine the mechanism behind ART antitumor effects, murine melanoma cell line B16F10 and murine HCC cell line Hepa1-6 were injected into mice (198). ART was shown to polarize MDSCs from an immunosuppressive phenotype towards an antitumoral phenotype through inhibition of PI3K/AKT/mTOR signaling, resulting in expansion of $CD8^+$ T cells and inhibition of tumor growth. Moreover, ART therapy reduced levels of Tregs and MDSCs without altering levels of $CD4^+$ T cells, B cells, NK cells, DCs and macrophages compared to vehicle treated mice. Interestingly, combining ART and anti-PD-L1 immunotherapy resulted in a striking delay in tumor growth, a reduction in the levels of MDSCs and increased levels of $CD8^+$ specific cells (198).

Table 2: An overview of agents targeting MDSCs and the most important *in vivo* outcomes

MDSC target	ICI	Cancer	Effect on MDSCs	Outcome	Ref.
DIM	Anti-PD-1	Breast cancer mouse model	- Neutralization of the immunosuppressive effect of MDSCs	- Inhibition of tumor growth - Increase in $CD8^+$ T cells in the TME	(159)
APT_{STAT3}-9R	Anti-PD-1	Melanoma mouse model	- Reduction of MDSCs in the TME	- Inhibition of tumor growth - Longer overall survival	(157)
Entinostat	Anti-PD-1	Lung and renal cancer mouse models	- Reduce MDSC viability	- Inhibition of tumor growth	(165)
	Anti-PD-1/anti-CTLA-4	Pancreatic and breast cancer mouse models	- Reduce MDSC viability	- Inhibition of tumor growth	(165)
VPA	Anti-PD-1/anti-CTLA-4	Melanoma mouse model	- Reduce MDSC viability	- Inhibition of tumor growth -	(166)
SKI-G-801	Anti-PD-1	Breast cancer mouse model	- Reduction of MDSCs in the TME	- Inhibition of tumor growth - Increase in $CD8^+$ T cells in the TME - Long-term curing	(175)
Cabozantinib	Anti-PD-1/ anti-CTLA-4	Colon, renal and prostate cancer mouse model	- Reduction of MDSCs in the TME - Neutralization of the immunosuppressive effect of MDSCs	- Increase in $CD8^+$ T cells in the TME - Tumor clearance - Mature neutrophil infiltration	(178)
Gemcitabine	Anti-PD-1 and Anti-PD-L1	Hepatocellular carcinoma mouse model	- Reduction of MDSCs in the TME	- Increase in $CD8^+$ T cells in the TME - Tumor clearance - Inhibition of tumor growth	(199)
IPI-549	Anti-CTLA-4 or anti-PD-1	Lung and breast cancer mouse model	- Inhibition of MDSC recruitment to the TME	- Inhibition of tumor growth - Increase in $CD8^+$ T cells in the TME	(193)
ART	Anti-PD-L1	Breast cancer and melanoma mouse model	- Maturation of MDSCs	- Inhibition of tumor growth - Increase in $CD8^+$ T cells in the TME	(198)
YY001	Anti-PD-1	Prostate cancer mouse model	- Inhibition of MDSC recruitment to the TME	- Increase in $CD8^+$ T cells in the TME - Inhibition of tumor growth	(200)

					-		
E7046	Anti-PD-1 and Anti-PD-L1	Melanoma mouse model	-	Inhibition of MDSC recruitment to the TME	- -	Inhibition of tumor growth Increase in $CD8^+$ T cells in the TME	(130), (201)
	Anti-CTLA-4	Colon cancer mouse model	-	Inhibition of MDSC recruitment to the TME	- -	Inhibition of tumor growth Increase in $CD8^+$ T cells in the TME	(201)
HDC	Anti-PD-1 and anti-PD-L1	Mouse models for lymphoma and colon adenocarinoma	- -	Maturation of MDSCs Neutralization of the immunosuppressive effect of MDSCs	- - -	Inhibition of tumor growth Improves the antitumor efficacy of ICI therapy	(202)
ATRA	Anti-PD-L1	Mouse model of cervical tumors	-	Neutralization of the immunosuppressive effect of MDSCs	- -	Inhibition of tumor growth Increase in $CD8^+$ T cells in the TME	(203)
	Anti-CTLA-4	mesothelioma mouse model	- -	Maturation of MDSCs Neutralization of the immunosuppressive effect of MDSC	- -	Long-term curing Decreased levels of ROS	(204)
Trametinib	Anti-PD-1	Head and neck cancer and colon mouse model	-	Reduction of MDSCs in the TME	-	Inhibition of tumor growth	(205)
Anti-CXCR2	Anti-PD-1	Mouse model for rhabdomyosarcoma	- -	Reprogramming of of macrophages to an M1 phenotype Inhibition of MDSC recruitment to the TME	-	Decrease in tumor area	(206)
Anti-Bv8	Anti-PD-1	Breast cancer mouse model	-	Inhibition of MDSC recruitment to the TME	-	Inhibition of tumor growth Increase in $CD8^+$ T cells in the TME	(207)

4.5 COX-2/PGE2 signaling

As highlighted before, COX-2/PGE2 signaling has been linked to development of MDSCs and Tregs (208). Blocking COX-2 resulted in considerable toxic effects in patients (209). Therefore, research started looking into a strategy downstream. YY001, an EP4 antagonist blocking PGE-2/EP4 signaling, strongly synergizes with anti-PD-1 antibodies in a prostate cancer mouse model (200). Synergy was achieved by reversing the inhibition of IFNγ secretion by T cells and promoting T cell proliferation and expansion, resulting in more tumor specific $CD8^+$ T cells and significantly reduced MDSC infiltration into the TME (200). These findings are similar to studies performed with the first small-molecule EP4 inhibitor, E7046, which inhibited tumor growth, reduced infiltration of MDSCs and increased $CD8^+$ T cell activity in various mouse cancer models (130). Furthermore in a mouse melanoma model, higher efficacy was observed in combination treatment using anti-PD-1/anti-PD-L1 and E7046 therapy than either of the two therapies alone (201). Moreover, E7046 therapy in combination with anti-CTLA4 also showed increased efficacy in a mouse CT26 colon cancer model (201). Therefore, EP4 inhibitors are now being studied in clinical trials.

4.6 ROS signaling

Myeloid cells express an NADPH oxidase (NOX2), which plays an important role in defending host cells against bacterial infections by ROS production (210). However, ROS in extracellular spaces leads to apoptosis or dysfunction of neighboring T cells (211). MDSCs upregulate NOX2 to increase ROS production (212). Administration of histamine dihydrochloride (HDC) *in vitro* and *in vivo* inhibits NOX2 expression leading to differentiation of MDSCs into mature DCs (213). Use of HDC in combination with IL-2 is approved in Europe in patients that recovered from AML for relapse prevention (214). In murine models with EL-4 lymphoma or MC38 colon adenocarcinoma, HDC was combined with anti-PD-1 and anti-PD-L1 which improved efficacy of these ICIs by neutralization of the immunosuppressive effect from MDSCs (202).

Metabolic derivatives of retinoic acid (vitamin A) have been highlighted before due to their promoting effect on MDSC maturation, as well as abolishment of the immunosuppressive nature (151). Upregulation of GSH synthase is observed after ATRA treatment, leading to decreased ROS production and differentiation into mature myeloid cells (151). Consequently, the suppressive activity of MDSCs was abrogated, leading to increased frequency of $CD8^+$ T cells in mice with cervical tumors and reduced tumor growth (203). This effect was enhanced using anti-PD-L1 treatment, showing synergy between ATRA treatment and anti-PD-L1 (203). Similarly, in a bilateral subcutaneous mesothelioma mouse model, significantly higher efficacy with long-term curing of mice was observed using combination therapy of ATRA and anti-CTLA4 therapy compared to either one alone (204). ATRA is now FDA approved for treatment of acute promyelocytic leukemia (215).

4.7 MAPK/MEK/ERK signaling inhibition

The Mitogen-Associated Protein Kinase (MAPK) pathway is often mutated in human cancers, leading to overactivation of MAPK/ERK Kinase (MEK)/ Extracellular signal-Regulated Kinase (ERK) signaling (216). MEK/ERK signaling promotes cell survival, proliferation, and tumor metastasis (217–219). Therefore, reducing MAPK pathway activation is an interesting strategy to inhibit tumor growth. Interestingly, MEK activation in tumor cells supports the accumulation of MDSCs into the TME by secretion of CXCL1/2 (220). MEK1/2 inhibitors do not only inhibit tumor cell proliferation directly, but also affect the cell heterogeneity of the TME, for example by reducing MDSC populations (221–223).

Trametinib is a MEK1/2 inhibitor approved for treatment of NSCLC and MAPK kinase driven melanoma (224). Additionally, Trametinib treatment also promotes antitumor activity in HNSCC patients (225). In a HNSCC mouse model, Trametinib treatment upregulated expression of PD-L1 on tumor cells and induced the influx of $CD8^+$ T cells into the TME (226). Consequently, more studies have been performed combining MEK inhibitors with ICI therapy. In a mouse model with CT26 induced colorectal cancer, Trametinib in combination with different ICIs controlled tumor growth more efficiently than either of the therapies alone (227). Mechanistically, Trametinib treatment leads to reduction of MDSCs in the TME by downregulating colony-stimulating factor 1 (CSF1) secretion by tumor cells. To create a synergistic effect between anti-PD-1 and Trametinib, timing of the anti-PD-1 antibody treatment is important. The optimal window to administer anti-PD-1 is when trametinib treatment has lowered the CSF-1 secretion and increased PD-L1 expression on tumor cells (205). This is one of the first studies that includes a time-dependent synergistic effect of two different oncolytic therapies.

4.8 Others

As mentioned before, chemotherapeutics have been shown to affect MDSC populations, for example Gemcitabine (119). It was observed that Gemcitabine treatment correlates with overexpression of PD-L1 on tumor cells and PD-1 on T cells *in vivo*. A combination of the two therapies resulted in a significant number of mice being cured from their HCC tumors (199). This also correlated with higher numbers of circulating tumor specific $CD8^+$ T cells and lower numbers if MDSCs in the TME (199).

Moreover, antibodies blocking VEGF signaling to inhibit neo-angiogenesis and recruitment of MDSCs to the TME have also been studied to overcome ICI resistance. One of these antibodies is the aforementioned monoclonal antibody Bevacizumab, which has been FDA approved since 2009 for treatment of recurrent glioblastoma (228). The IMbrave150 study combined anti-PD-L1 antibody Atezolizumab and Bevacizumab. This combination has now been approved as a first-line treatment for advanced HCC (229). Ng *et al.* studied the effect of treatment with TKIs before ICIs or treatment with ICIs before TKIs in which 27 and 23 patients enrolled,

respectively (230). Results showed that treatment sequence does not result in a significant difference in response rates (230).

Overall, the studies reviewed here show similar outcomes: a reduction in MDSCs and an increase in tumor-specific $CD8^+$ T cells, which is the goal of this combination therapy. In addition, murine studies show reduced tumor growth and in some studies complete remission is observed. Therefore, the strategy to target MDSCs to overcome ICI resistance is highly promising. Nevertheless, different cancer cell lines seem to call for different targeting strategies.

Table 3: An overview of agents targeting MDSCs and the most important outcomes in human studies

MDSC target	ICI	Cancer	Effect on MDSCs	Outcome	Ref.
Entinostat	Anti-PD-1/anti-CTLA-4	Advanced solid tumor patients	- Reduce MDSC viability	- Inhibition of tumor growth - Increase in $CD8^+$ T cells in the TME	(168)
Mocetinostat	Anti-PD-1/anti-CTLA-4	Unresectable melanoma patients	- Reduction of MDSCs in the TME	- Inhibition of tumor growth	(167)
Vorinostat	Anti-PD-L1	Non-small lung cancer patients	- Reduction of MDSCs in the TME	- Inhibition of tumor growth	(169), (170)
Sorafenib	Anti-PD-1	Hepatocellular cancer patients	- Reduction of MDSCs in the TME	- Increase in $CD8^+$ T cells in the TME - Inhibition of tumor growth	
Lenvatinib	Anti-PD-1	Hepatocellular cancer patients	- Reduction of MDSCs in the TME	- Increase in $CD8^+$ T cells in the TME - Inhibition of tumor growth	(185)
	Anti-PD-L1	Hepatocellular cancer patients	- Reduction of MDSCs in the TME	- Inhibition of tumor growth	(186)
Bevacizumab	Anti-PD-L1	Hepatocellular cancer patients	- Reduction of MDSCs in the TME	- Inhibition of tumor growth	(230)

5. FUTURE DIRECTIONS

In this review, we have highlighted strategies to combine targeting of MDSCs with ICI therapy. Targeting of MDSCs focusses on three major strategies: 1) Depletion of MDSCs from the TME; 2) Inhibition of the immunosuppressive effects MDSCs exhibit; 3) Promoting maturation of MDSCs into APCs. To deplete MDSCs from the TME, anti-growth factors can be used, as well as reducing chemokines signals to inhibit recruitment of MDSCs into the TME (34,96). Both of these show a reduction in MDSCs in preclinical studies, which is associated with improved clinical outcomes (97,107,108,110,111). Furthermore, a general MDSC cell marker, CD33, is currently being studied as a target for direct depletion via ADCC with two different CD33 monoclonal antibodies (117,118). Nevertheless, targeting CD33 has not been tested in combination with ICI therapy yet. Inhibition of the immunosuppressive mechanisms of MDSCs focusses on inhibition of the pathways of key enzymes, like ARG1, iNOS, and NOX2. Using therapies, including STAT3 , HDAC and EP4 inhibitors, have shown decreased ROS, NO and RNS secretion, and additionally, reduced ARG1 and iNOS mRNA expression (131,143,148,231). To promote maturation in MDSCs, DNA methyltransferases, ATRA and docetaxel have been reported to result in differentiation of MDSCs into mature APC and macrophages (150–152). Combining these different targets with

ICI therapy in preclinical and clinical models, has shown reduced numbers of MDSCs in the TME, increased levels of tumor-specific cytotoxic T cells and beneficial clinical outcomes in preclinical models, as well as in clinical trials.

Targeting of the STAT3 pathway has recently gained interest because of the direct effect this pathway has on the immunosuppressive activity of MDSCs (146). Inhibition of STAT3 signaling using DIM or APT_{STAT3}-9R shows reduced levels of MDSC activity as measured by ARG1 and iNOS transcription (143). In mouse models, the combination of STAT3 targeting and ICI therapy resulted in increased cytotoxic T cell infiltration into the TME, reduced levels of MDSCs, and inhibition of tumor growth (157,158). However, clinical trials using these MDSCs targeting agents in combination with ICIs have not been finished yet. HDACIs are a promising anti-cancer method on their own, but have also shown to overcome ICI resistance in combination therapy (120,154,158–162). Nevertheless, high toxicity has been associated with the use of HDACIs. The most promising therapy seems to be the different TKIs, Cabozantinib, Sorafenib and Lenvatinib. Cabozantinib in combination with Nivolumab is already used as first-line treatment in patients with advanced RCC (181). Furthermore, clinical trials with Sorafenib and Lenvatinib in combination with Nivolumab and Pembrolizumab show high efficacy, little toxicity and a high response rate in HCC patients, for which limited therapies are available (185–187).

Other promising MDSC targets in combination with ICI therapy are still being investigated, for example blocking of MDSC recruitment. As described before, chemokines, cytokines and complement factors have been described to affect MDSC infiltration into the TME (232–234). From these different factors, chemokines have turned out to be the major player in recruitment of MDSCs to the TME (234). However, only a few studies have looked into blocking these different chemokines to enhance ICI therapy. One of these combinations is ICI therapy together with anti-CXCR2. CXCR2 is a chemokine receptor that predominantly interacts with CXCL1, CXCL2, and CXCL5 (235). In a colorectal cancer mouse model tumor-associated macrophages (TAMs) have been shown to secrete CXCL1, which leads to recruitment of $CXCR2^+$ MDSCs. These MDSCs ultimately promote liver metastasis (236). In a mouse model with established rhabdomyosarcoma (RMS) tumors, PD-1 blockade treatment was shown to have a low efficacy. However, combination therapy using anti-CXCR2 together with anti-PD-1 therapy led to a significant decrease in the tumor area compared to either anti-CXCR2 or anti-PD-1 alone (206). Furthermore, MDSCs secrete prokineticin (Bv8), which promotes neo-angiogenesis around the tumor (237). Blocking Bv8 with a monoclonal antibody leads to inhibition of tumor growth and reduced MDSC recruitment to the TME (238). In a preclinical mouse model injected with EMT6 breast carcinoma cells, it was demonstrated that Bv8 blockade sensitizes tumors to immunotherapy that showed resistance to anti-PD-1 without the use of Bv8 blockade (207). However, no clinical trials have been reported combining these two MDSC targets with ICI therapy. Additionally, the exact molecular effect on MDSCs has not been reported.

Interestingly, it has been hypothesized that pathways active in MDSCs, as well as the dominant MDSC subtype vary in different tissues and tumors. For example, in breast cancer, some studies report higher levels of G-MDSCs (239), while other studies report increased levels of M-MDSCs (240). Furthermore, HDACs have been shown to play an important role in breast cancer progression (241). MDSCs have the ability to differentiate into osteoclasts leading to metastasis to the bone (242), which seems to be mediated by differential HDACs expression (241). Therefore, Entinostat in combination with ICI therapy is now tested in clinical trials for the treatment of breast cancer (243). In colorectal cancer, chronic inflammation plays a major role in the accumulation of MDSCs (244,245). Instead of accumulation of M-MDSCs, G-MDSCs have been shown to accumulate driven by IL-17 secretion by tumor cells (246–248). IL-17 utilizes the CXCR2/CXCL5 pathway to enhance immunosuppressive characteristics of MDSCs (249). Therefore, an inhibitor for this pathway, could overcome ICI therapy in colorectal cancer patients. For advanced melanoma patients, both increased levels of G-MDSCs and M-MDSCs have been reported (250–252). In melanoma patients, overexpression of the COX-2/PGE2/EP4 pathway has been observed frequently (253). Consequently, melanoma studies focus on inhibiting this pathway in combination with ICI therapy. These studies show the importance for research into specific targets in different cancer types is necessary, touching upon personalized medicine.

Finally, a lack of specific markers for MDSCs has limited research possibilities. Recent studies have highlighted the possibility that MDSCs are mature myeloid cells in which activation markers have been lost due to extensive TLR signaling activation (254,255). Additionally, in response to hypoxia, which is a common feature of the TME, loss of mature cell markers has also been shown (256). Therefore, some researchers suggest that

MDSCs are monocytes or neutrophils in which immunosuppressive pathways are activated. However, the importance of targeting these cells does not change.

6. CONCLUSION

More and more studies highlight the correlation between higher levels of MDSCs in the TME and ICI therapy resistance. The studies reviewed here show the potential of targeting MDSCs in the TME in combination with ICI therapy to overcome resistance and enhance the efficacy of ICI therapy in different cancers. In the last few years, studies in this field have progressed considerably, leading to promising new therapies that are already used as a first-line of treatment in different cancer types, for example HDACIs. However, more information is needed on molecular mechanisms exploited by MDSCs to promote tumor growth and inhibit T cell activity. This molecular data can help explore targeting different pathways and use different combinations of MDSC targets and ICI therapy.

LITERATURE

1. Baskar R, Lee KA, Yeo R, Yeoh KW. Cancer and radiation therapy: Current advances and future directions. Vol. 9, International Journal of Medical Sciences. 2012. p. 193–9.
2. Bhatia A, Kumar Y. Cellular and molecular mechanisms in cancer immune escape: A comprehensive review. Vol. 10, Expert Review of Clinical Immunology. 2014. p. 41–62.
3. Yang Y. Cancer immunotherapy: Harnessing the immune system to battle cancer. Vol. 125, Journal of Clinical Investigation. American Society for Clinical Investigation; 2015. p. 3335–7.
4. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Vol. 22, Annual Review of Immunology. 2004. p. 329–60.
5. Mishra AK, Ali A, Dutta S, Banday S, Malonia SK. Emerging Trends in Immunotherapy for Cancer. Diseases [Internet]. 2022 Sep 6;10(3):60. Available from: https://www.mdpi.com/2079-9721/10/3/60
6. Smyth MJ, Dunn GP, Schreiber RD. Cancer Immunosurveillance and Immunoediting: The Roles of Immunity in Suppressing Tumor Development and Shaping Tumor Immunogenicity. Vol. 90, Advances in Immunology. 2006. p. 1–50.
7. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immuno-surveillance to tumor escape [Internet]. 2002. Available from: http://www.nature.com/natureimmunology
8. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64.
9. Mukherjee AG, Wanjari UR, Namachivayam A, Murali R, Prabakaran DS, Ganesan R, et al. Role of Immune Cells and Receptors in Cancer Treatment: An Immunotherapeutic Approach. Vaccines (Basel) [Internet]. 2022 Sep 7;10(9):1493. Available from: https://www.mdpi.com/2076-393X/10/9/1493
10. Kuzmanovszki D, Kiss N, Tóth B, Kerner T, Tóth V, Szakonyi J, et al. Anti-PD-1 Monotherapy in Advanced Melanoma—Real-World Data from a 77-Month-Long Retrospective Observational Study. Biomedicines. 2022 Jul 1;10(7).
11. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. New England Journal of Medicine. 2010 Aug 19;363(8):711–23.
12. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. New England Journal of Medicine. 2012 Jun 28;366(26):2443–54.
13. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Safety and Tumor Responses with Lambrolizumab (Anti–PD-1) in Melanoma. New England Journal of Medicine. 2013 Jul 11;369(2):134–44.
14. Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. J Hematol Oncol. 2021;14(1):1–29.
15. Schoenfeld AJ, Hellmann MD. Acquired resistance to immune checkpoint inhibitors. Cancer Cell. 2020;37(4):443–55.
16. Haslam A, Prasad V. Estimation of the percentage of US patients with cancer who are eligible for and respond to checkpoint inhibitor immunotherapy drugs. JAMA Netw Open. 2019;2(5):e192535–e192535.
17. Fares CM, van Allen EM, Drake CG, Allison JP, Hu-Lieskovan S. Mechanisms of resistance to immune checkpoint blockade: why does checkpoint inhibitor immunotherapy not work for all patients? American Society of Clinical Oncology Educational Book. 2019;39:147–64.
18. Guerra L, Bonetti L, Brenner D. Metabolic modulation of immunity: a new concept in cancer immunotherapy. Cell Rep. 2020;32(1):107848.
19. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. Cell. 2015;162(6):1229–41.
20. Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, Triulzi T, et al. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation. In: Seminars in cancer biology. Elsevier; 2017. p. 74–89.
21. Wang JX, Choi SYC, Niu X, Kang N, Xue H, Killam J, et al. Lactic acid and an acidic tumor microenvironment suppress anticancer immunity. Int J Mol Sci. 2020;21(21):8363.
22. Miranda-Galvis M, Teng Y. Targeting hypoxia-driven metabolic reprogramming to constrain tumor progression and metastasis. Int J Mol Sci. 2020;21(15):5487.
23. Li Y, Patel SP, Roszik J, Qin Y. Hypoxia-driven immunosuppressive metabolites in the tumor microenvironment: new approaches for combinational immunotherapy. Front Immunol. 2018;9:1591.
24. Li Y, Liu J, Gao L, Liu Y, Meng F, Li X, et al. Targeting the tumor microenvironment to overcome immune checkpoint blockade therapy resistance. Immunol Lett. 2020;220:88–96.
25. Hino C, Pham B, Park D, Yang C, Nguyen MHK, Kaur S, et al. Targeting the Tumor Microenvironment in Acute Myeloid Leukemia: The Future of Immunotherapy and Natural Products. Vol. 10, Biomedicines. MDPI; 2022.
26. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Vol. 19, Nature Medicine. 2013. p. 1423–37.

27. Singh B, Singh DK, Ganatra SR, Escobedo RA, Khader S, Schlesinger LS, et al. Myeloid-Derived Suppressor Cells Mediate T Cell Dysfunction in Nonhuman Primate TB Granulomas [Internet]. 2021. Available from: https://journals.asm.org/journal/mbio
28. Siret C, Collignon A, Silvy F, Robert S, Cheyrol T, André P, et al. Deciphering the Crosstalk Between Myeloid-Derived Suppressor Cells and Regulatory T Cells in Pancreatic Ductal Adenocarcinoma. Front Immunol. 2020 Jan 22;10.
29. Chikamatsu K, Sakakura K, Toyoda M, Takahashi K, Yamamoto T, Masuyama K. Immunosuppressive activity of CD 14+ HLA-DR− cells in squamous cell carcinoma of the head and neck. Cancer Sci. 2012;103(6):976–83.
30. Hart KM, Byrne KT, Molloy MJ, Usherwood EM, Berwin B. IL-10 immunomodulation of myeloid cells regulates a murine model of ovarian cancer. Front Immunol. 2011;2:29.
31. Mukherjee AG, Wanjari UR, Namachivayam A, Murali R, Prabakaran DS, Ganesan R, et al. Role of Immune Cells and Receptors in Cancer Treatment: An Immunotherapeutic Approach. Vaccines (Basel) [Internet]. 2022 Sep 7;10(9):1493. Available from: https://www.mdpi.com/2076-393X/10/9/1493
32. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. Vol. 9, Frontiers in Immunology. Frontiers Media S.A.; 2018.
33. Sun SH, Benner B, Savardekar H, Lapurga G, Good L, Abood D, et al. Effect of Immune Checkpoint Blockade on Myeloid-Derived Suppressor Cell Populations in Patients With Melanoma. Front Immunol. 2021;4239.
34. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, Leyvraz L, et al. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. Cancer Immunology, Immunotherapy. 2014;63(3):247–57.
35. Weber J, Gibney G, Kudchadkar R, Yu B, Cheng P, Martinez AJ, et al. Phase I/II study of metastatic melanoma patients treated with nivolumab who had progressed after ipilimumab. Cancer Immunol Res. 2016;4(4):345–53.
36. Ledford H, Else H, Warren M. Cancer immunologists scoop medicine Nobel prize. Nature [Internet]. 2018;562:20+. Available from: https://link.gale.com/apps/doc/A572995269/HRCA?u=anon~5f7480d7&sid=googleScholar&xid=36e1180f
37. Sckisel GD, Bouchlaka MN, Monjazeb AM, Crittenden M, Curti BD, Wilkins DEC, et al. Out-of-Sequence Signal 3 Paralyzes Primary CD4+ T-Cell-Dependent Immunity. Immunity. 2015 Aug 18;43(2):240–50.
38. Kristiansen OP, Larsen ZM, Pociot F. CTLA-4 in autoimmune diseases – a general susceptibility gene to autoimmunity? Genes Immun [Internet]. 2000;1(3):170–84. Available from: https://doi.org/10.1038/sj.gene.6363655
39. Collins A v, Brodie DW, Gilbert RJC, Iaboni A, Manso-Sancho R. The Interaction Properties of Costimulatory Molecules Revisited. Vol. 17, Immunity.
40. Krummel MF, Allison JP. CTLA-4 engagement inhibits IL-2 accumulation and cell cycle progression upon activation of resting T cells. J Exp Med. 1996;183(6):2533–40.
41. Nourse J, Firpo E, Flanagan WM, Coats S, Polyak K, Lee MH, et al. Interleukin-2-mediated elimination of the p27Kipl cyclin-dependent kinase inhibitor prevented by rapamycin. Nature. 1994;372(6506):570–3.
42. Firpo EJ, Koff A, Solomon MJ, Roberts JM. Inactivation of a Cdk2 inhibitor during interleukin 2-induced proliferation of human T lymphocytes. Mol Cell Biol. 1994;14(7):4889–901.
43. Brunner MC, Chambers CA, Chan FKM, Hanke J, Winoto A, Allison JP. CTLA-4-mediated inhibition of early events of T cell proliferation. The Journal of Immunology. 1999;162(10):5813–20.
44. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science (1979). 1996;271(5256):1734–6.
45. Korman AJ, Peggs KS, Allison JP. Checkpoint Blockade in Cancer Immunotherapy. Vol. 90, Advances in Immunology. 2006. p. 297–339.
46. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. Journal of clinical oncology. 2015;33(17):1889.
47. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. Vol. 27, Trends in Immunology. 2006. p. 195–201.
48. Prokunina L, Castillejo-López C, Öberg F, Gunnarsson I, Berg L, Magnusson V, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet. 2002 Dec 1;32(4):666–9.
49. Keir ME, Francisco LM, Sharpe AH. PD-1 and its ligands in T-cell immunity. Vol. 19, Current Opinion in Immunology. 2007. p. 309–14.
50. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations [Internet]. Available from: https://www.science.org
51. Noman MZ, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. Journal of Experimental Medicine. 2014;211(5):781–90.

52. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations [Internet]. Available from: https://www.science.org
53. Wang C, Thudium KB, Han M, Wang XT, Huang H, Feingersh D, et al. In vitro characterization of the anti-PD-1 antibody nivolumab, BMS-936558, and in vivo toxicology in non-human primates. Cancer Immunol Res. 2014 Sep 1;2(9):846–56.
54. Chen L, Han X. Anti-PD-1/PD-L1 therapy of human cancer: Past, present, and future. Vol. 125, Journal of Clinical Investigation. American Society for Clinical Investigation; 2015. p. 3384–91.
55. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Vol. 11, Nature Communications. Nature Research; 2020.
56. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA. 2016;315(15):1600–9.
57. Ganju V, Cooper A, Gao B, Wilkinson K, George S. A first-in-human phase I dose escalation of YH001, an anti-CTLA-4 monoclonal antibody (mAb), in combination with toripalimab (anti-PD-1 mAb) in patients with advanced solid tumors. 2022.
58. Costa B, Vale N. Dostarlimab: A Review. Vol. 12, Biomolecules. NLM (Medline); 2022.
59. Russell BL, Sooklal SA, Malindisa ST, Daka LJ, Ntwasa M. The Tumor Microenvironment Factors That Promote Resistance to Immune Checkpoint Blockade Therapy. Vol. 11, Frontiers in Oncology. Frontiers Media S.A.; 2021.
60. de Haas N, de Koning C, Spilgies L, de Vries IJM, Hato S v. Improving cancer immunotherapy by targeting the STATe of MDSCs. Vol. 5, OncoImmunology. Taylor and Francis Inc.; 2016.
61. Akbay EA, Koyama S, Liu Y, Dries R, Bufe LE, Silkes M, et al. Interleukin-17A promotes lung tumor progression through neutrophil attraction to tumor sites and mediating resistance to PD-1 blockade. Journal of Thoracic Oncology. 2017;12(8):1268–79.
62. Morales JK, Kmieciak M, Knutson KL, Bear HD, Manjili MH. GM-CSF is one of the main breast tumor-derived soluble factors involved in the differentiation of CD11b-Gr1-bone marrow progenitor cells into myeloid-derived suppressor cells. Breast Cancer Res Treat. 2010;123(1):39–49.
63. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Vol. 12, Nature Reviews Immunology. 2012. p. 253–68.
64. Mu X, Liu K, Li H, Wang FS, Xu R. Granulocyte-macrophage colony-stimulating factor: an immunotarget for sepsis and COVID-19. Vol. 18, Cellular and Molecular Immunology. Springer Nature; 2021. p. 2057–8.
65. Ostrand-Rosenberg S, Sinha P. Myeloid-Derived Suppressor Cells: Linking Inflammation and Cancer. The Journal of Immunology. 2009 Apr 15;182(8):4499–506.
66. Yang G, Shen W, Zhang Y, Liu M, Zhang L, Liu Q, et al. Oncotarget 38378 www.impactjournals.com/oncotarget Accumulation of myeloid-derived suppressor cells (MDSCs) induced by low levels of IL-6 correlates with poor prognosis in bladder cancer [Internet]. Vol. 8, Oncotarget. 2017. Available from: www.impactjournals.com/oncotarget/
67. Ma P, Beatty PL, McKolanis J, Brand R, Schoen RE, Finn OJ. Circulating myeloid derived suppressor cells (MDSC) that accumulate in premalignancy share phenotypic and functional characteristics with MDSC in cancer. Front Immunol. 2019;10(JUN).
68. Lappatand Madisoncawein EJ. A Study of the Leukemoid Response to Transplantable A-280 Tumor in Mice* [Internet]. Available from: http://aacrjournals.org/cancerres/article-pdf/24/2_Part_1/302/2379287/cr0242p10302.pdf
69. Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. Vol. 13, Nature Reviews Cancer. 2013. p. 739–52.
70. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of Myeloid-Derived Suppressor Cells in Tumor-Bearing Mice. The Journal of Immunology. 2008 Oct 15;181(8):5791–802.
71. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. Vol. 9, Frontiers in Immunology. Frontiers Media S.A.; 2018.
72. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Vol. 7, Nature Communications. Nature Publishing Group; 2016.
73. Magrini E, Minute L, Dambra M, Garlanda C. Complement activation in cancer: Effects on tumor-associated myeloid cells and immunosuppression. Seminars in Immunology. Academic Press; 2022.
74. Hart KM, Byrne KT, Molloy MJ, Usherwood EM, Berwin B. IL-10 immunomodulation of myeloid cells regulates a murine model of ovarian cancer. Front Immunol. 2011;2(JUL).
75. Ostrand-Rosenberg S, Sinha P, Beury DW, Clements VK. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. Vol. 22, Seminars in Cancer Biology. 2012. p. 275–81.
76. Chai E, Zhang L, Li C. LOX-1+ PMN-MDSC enhances immune suppression which promotes glioblastoma multiforme progression. Cancer Manag Res. 2019;11:7307–15.

77. Mazzoni A, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P, et al. Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. The Journal of Immunology. 2002;168(2):689–95.
78. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res. 2004;64(16):5839–49.
79. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res. 2010;70(1):68–77.
80. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res. 2010 Jan 1;70(1):68–77.
81. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I Production in the Tumor Microenvironment by Mature Myeloid Cells Inhibits T-Cell Receptor Expression and Antigen-Specific T-Cell Responses [Internet]. Vol. 64, CANCER RESEARCH. 2004. Available from: http://aacrjournals.org/cancerres/article-pdf/64/16/5839/2516899/zch01604005839.pdf?casa_token=lL51toiPBHQAAAAA:M0QHYGBO8ttxZy5zfnPbu1B9t0LEshho10-YYeg6zMndcsnQdUwvwSwwxO-SdFzlNYgHCu1O
82. Draghiciu O, Lubbers J, Nijman HW, Daemen T. Myeloid derived suppressor cells—An overview of combat strategies to increase immunotherapy efficacy. Oncoimmunology. 2015 Jan 2;4(1):954829.
83. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a novel mechanism of CD8 + T cell tolerance in cancer.
84. Chen X, Song M, Zhang B, Zhang Y. Reactive Oxygen Species Regulate T Cell Immune Response in the Tumor Microenvironment. Vol. 2016, Oxidative Medicine and Cellular Longevity. Hindawi Publishing Corporation; 2016.
85. Molon B, Ugel S, del Pozzo F, Soldani C, Zilio S, Avella D, et al. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. Journal of Experimental Medicine. 2011 Sep 26;208(10):1949–62.
86. Schlecker E, Stojanovic A, Eisen C, Quack C, Falk CS, Umansky V, et al. Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. The Journal of Immunology. 2012;189(12):5602–11.
87. Itakura J, Ishiwata T, Shen B, Kornmann M, Korc M. Concomitant over-expression of vascular endothelial growth factor and its receptors in pancreatic cancer. Int J Cancer. 2000;85(1):27–34.
88. Koinis F, Vetsika EK, Aggouraki D, Skalidaki E, Koutoulaki A, Gkioulmpasani M, et al. Effect of first-line treatment on myeloid-derived suppressor cells' subpopulations in the peripheral blood of patients with non–small cell lung cancer. Journal of Thoracic Oncology. 2016;11(8):1263–72.
89. Vetsika EK, Koukos A, Kotsakis A. Myeloid-derived suppressor cells: Major figures that shape the immunosuppressive and angiogenic network in cancer. Vol. 8, Cells. MDPI; 2019.
90. Huang Y, Rajappa P, Hu W, Hoffman C, Cisse B, Kim JH, et al. A proangiogenic signaling axis in myeloid cells promotes malignant progression of glioma. J Clin Invest. 2017;127(5):1826–38.
91. Liu J, Deng W, Chen L, Li Y, Wu L, Ma S, et al. Inhibition of JAK2/STAT3 reduces tumor-induced angiogenesis and myeloid-derived suppressor cells in head and neck cancer. Mol Carcinog. 2018;57(3):429–39.
92. Liechtenstein T, Dufait I, Lanna A, Breckpot K, Escors D. Modulating co-stimulation during antigen presentation to enhance cancer immunotherapy. Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Immunology, Endocrine and Metabolic Agents). 2012;12(3):224–35.
93. Srivastava MK, Zhu L, Harris-White M, Kar U, Huang M, Johnson MF, et al. Myeloid suppressor cell depletion augments antitumor activity in lung cancer. PLoS One. 2012 Jul 16;7(7).
94. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, Leyvraz L, et al. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. Cancer Immunology, Immunotherapy. 2014;63(3):247–57.
95. Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. Cancer Res. 2004;64(17):6337–43.
96. Li W, Zhang X, Chen Y, Xie Y, Liu J, Feng Q, et al. G-CSF is a key modulator of MDSC and could be a potential therapeutic target in colitis-associated colorectal cancers. Protein Cell. 2016 Feb 1;7(2):130–40.
97. Sevko A, Michels T, Vrohlings M, Umansky L, Beckhove P, Kato M, et al. Antitumor Effect of Paclitaxel Is Mediated by Inhibition of Myeloid-Derived Suppressor Cells and Chronic Inflammation in the Spontaneous Melanoma Model. The Journal of Immunology. 2013 Mar 1;190(5):2464–71.
98. Song E, Tai WM, Messersmith WA, Bagby S, Purkey A, Quackenbush KS, et al. Potent antitumor activity of cabozantinib, ac-MET and VEGFR 2 inhibitor, in a colorectal cancer patient-derived tumor explant model. Int J Cancer. 2015;136(8):1967–75.
99. Rivera LB, Bergers G. Intertwined regulation of angiogenesis and immunity by myeloid cells. Trends Immunol. 2015;36(4):240–9.

100. Presta LG, Chen H, O'connor SJ, Chisholm V, Meng YG, Krummen L, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res. 1997;57(20):4593–9.
101. Koinis F, Vetsika EK, Aggouraki D, Skalidaki E, Koutoulaki A, Gkioulmpasani M, et al. Effect of first-line treatment on myeloid-derived suppressor cells' subpopulations in the peripheral blood of patients with non–small cell lung cancer. Journal of Thoracic Oncology. 2016;11(8):1263–72.
102. Peereboom DM, Alban TJ, Grabowski MM, Alvarado AG, Otvos B, Bayik D, et al. Metronomic capecitabine as an immune modulator in glioblastoma patients reduces myeloid-derived suppressor cells. JCI Insight. 2019;4(22).
103. Rodriguez PC, Ernstoff MS, Hernandez C, Atkins M, Zabaleta J, Sierra R, et al. Arginase I–producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res. 2009;69(4):1553–60.
104. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol. 2006;3(1):24–40.
105. Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. Vol. 9, Frontiers in Immunology. Frontiers Media S.A.; 2018.
106. Zhang J, Patel L, Pienta KJ. CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis. Cytokine Growth Factor Rev. 2010 Feb;21(1):41–8.
107. Steinberg SM, Shabaneh TB, Zhang P, Martyanov V, Li Z, Malik BT, et al. Myeloid Cells That Impair Immunotherapy Are Restored in Melanomas with Acquired Resistance to BRAF InhibitorsBRAF Inhibitor Resistance Restores Tumor Immune Suppression. Cancer Res. 2017;77(7):1599–610.
108. Liang H, Deng L, Hou Y, Meng X, Huang X, Rao E, et al. Host STING-dependent MDSC mobilization drives extrinsic radiation resistance. Nat Commun. 2017;8(1):1–10.
109. Blattner C, Fleming V, Weber R, Himmelhan B, Altevogt P, Gebhardt C, et al. CCR5+ myeloid-derived suppressor cells are enriched and activated in melanoma lesions. Cancer Res. 2018 Jan 1;78(1):157–67.
110. Tan MCB, Goedegebuure PS, Belt BA, Flaherty B, Sankpal N, Gillanders WE, et al. Disruption of CCR5-Dependent Homing of Regulatory T Cells Inhibits Tumor Growth in a Murine Model of Pancreatic Cancer. The Journal of Immunology. 2009 Feb 1;182(3):1746–55.
111. Zhang X, Haney KM, Richardson AC, Wilson E, Gewirtz DA, Ware JL, et al. Anibamine, a natural product CCR5 antagonist, as a novel lead for the development of anti-prostate cancer agents. Bioorg Med Chem Lett. 2010 Aug 1;20(15):4627–30.
112. Halama N, Zoernig I, Berthel A, Kahlert C, Klupp F, Suarez-Carmona M, et al. Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. Cancer Cell. 2016;29(4):587–601.
113. Marini O, Costa S, Bevilacqua D, Calzetti F, Tamassia N, Spina C, et al. Mature CD10+ and immature CD10− neutrophils present in G-CSF–treated donors display opposite effects on T cells. Blood, The Journal of the American Society of Hematology. 2017;129(10):1343–56.
114. Condamine T, Dominguez GA, Youn JI, Kossenkov A v, Mony S, Alicea-Torres K, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol. 2016;1(2):aaf8943–aaf8943.
115. Chai E, Zhang L, Li C. LOX-1+ PMN-MDSC enhances immune suppression which promotes glioblastoma multiforme progression. Cancer Manag Res. 2019;11:7307.
116. Fultang L, Panetti S, Ng M, Collins P, Graef S, Rizkalla N, et al. MDSC targeting with Gemtuzumab ozogamicin restores T cell immunity and immunotherapy against cancers. EBioMedicine. 2019;47:235–46.
117. Fultang L, Panetti S, Ng M, Collins P, Graef S, Rizkalla N, et al. MDSC targeting with Gemtuzumab ozogamicin restores T cell immunity and immunotherapy against cancers. EBioMedicine. 2019;47:235–46.
118. Eksioglu EA, Chen X, Heider KH, Rueter B, McGraw KL, Basiorka AA, et al. Novel therapeutic approach to improve hematopoiesis in low risk MDS by targeting MDSCs with the Fc-engineered CD33 antibody BI 836858. Leukemia. 2017;31(10):2172–80.
119. Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1+/CD11b + myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. Clinical Cancer Research. 2005 Sep 15;11(18):6713–21.
120. Vincent J, Mignot G, Chalmin F, Ladoire S, Bruchard M, Chevriaux A, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res. 2010 Apr 15;70(8):3052–61.
121. Greenhough A, Smartt HJM, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE 2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009;30(3):377–86.
122. Chen Y, Hughes-Fulford M. Prostaglandin E2 and the protein kinase A pathway mediate arachidonic acid induction of c-fos in human prostate cancer cells. Br J Cancer. 2000;82(12).

123. Rodríguez-Ubreva J, Català-Moll F, Obermajer N, Álvarez-Errico D, Ramirez RN, Company C, et al. Prostaglandin E2 leads to the acquisition of DNMT3A-dependent tolerogenic functions in human myeloid-derived suppressor cells. Cell Rep. 2017;21(1):154–67.
124. Mao Y, Sarhan D, Steven A, Seliger B, Kiessling R, Lundqvist A. Inhibition of Tumor-Derived Prostaglandin-E2 Blocks the Induction of Myeloid-Derived Suppressor Cells and Recovers Natural Killer Cell ActivityRescue of NK Cells by Blocking the Induction of MDSCs. Clinical cancer research. 2014;20(15):4096–106.
125. Obermajer N, Muthuswamy R, Odunsi K, Edwards RP, Kalinski P. PGE2-Induced CXCL12 Production and CXCR4 Expression Controls the Accumulation of Human MDSCs in Ovarian Cancer EnvironmentPGE2 Controls CXCR4-Driven Accumulation of MDSCs. Cancer Res. 2011;71(24):7463–70.
126. Pruthi RS, Derksen E, Gaston K. Cyclooxygenase-2 as a potential target in the prevention and treatment of genitourinary tumors: a review. J Urol. 2003;169(6):2352–9.
127. Meyera C, Sevko A, Ramacher M, Bazhin A v., Falk CS, Osena W, et al. Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. Proc Natl Acad Sci U S A. 2011 Oct 11;108(41):17111–6.
128. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med. 2005;202(7):931–9.
129. Rong Y, Yuan CH, Qu Z, Zhou H, Guan Q, Yang N, et al. Doxorubicin resistant cancer cells activate myeloid-derived suppressor cells by releasing PGE2. Sci Rep. 2016;6(1):1–11.
130. Albu DI, Wang Z, Huang KC, Wu J, Twine N, Leacu S, et al. EP4 Antagonism by E7046 diminishes Myeloid immunosuppression and synergizes with Treg-reducing IL-2-Diphtheria toxin fusion protein in restoring anti-tumor immunity. Oncoimmunology. 2017 Aug 3;6(8).
131. Orillion A, Hashimoto A, Damayanti N, Shen L, Adelaiye-Ogala R, Arisa S, et al. Entinostat Neutralizes Myeloid-Derived Suppressor Cells and Enhances the Antitumor Effect of PD-1 Inhibition in Murine Models of Lung and Renal Cell CarcinomaHDAC and PD-1 Inhibition. Clinical cancer research. 2017;23(17):5187–201.
132. Christmas BJ, Rafie CI, Hopkins AC, Scott BA, Ma HS, Cruz KA, et al. Entinostat converts immune-resistant breast and pancreatic cancers into checkpoint-responsive tumors by reprogramming tumor-infiltrating MDSCs. Cancer Immunol Res. 2018 Dec 1;6(12):1561–77.
133. Inoue A, Fujimoto D. BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS ENZYMATIC DEACETYLATION OF HISTONE. Vol. 36. 1969.
134. Seto E, Yoshida M. Erasers of histone acetylation: The histone deacetylase enzymes. Cold Spring Harb Perspect Biol. 2014;6(4).
135. Cui Y, Cai J, Wang W, Wang S. Regulatory effects of histone deacetylase inhibitors on myeloid-derived suppressor cells. Front Immunol. 2021;12:690207.
136. Hashimoto A, Fukumoto T, Zhang R, Gabrilovich D. Selective targeting of different populations of myeloid-derived suppressor cells by histone deacetylase inhibitors. Cancer Immunology, Immunotherapy. 2020 Sep 1;69(9):1929–36.
137. Andersson K. PDE5 inhibitors–pharmacology and clinical applications 20 years after sildenafil discovery. Br J Pharmacol. 2018;175(13):2554–65.
138. Serafini P, Meckel K, Kelso M, Noonan K, Califano J, Koch W, et al. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. J Exp Med. 2006;203(12):2691–702.
139. Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. Nat Rev Drug Discov. 2002;1(9):674–82.
140. Rotella DP. Phosphodiesterase 5 inhibitors: current status and potential applications. Nat Rev Drug Discov. 2002;1(9):674–82.
141. Califano JA, Khan Z, Noonan KA, Rudraraju L, Zhang Z, Wang H, et al. Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. Clinical Cancer Research. 2015;21(1):30–8.
142. Weed DT, Vella JL, Reis IM, de La Fuente AC, Gomez C, Sargi Z, et al. Tadalafil reduces myeloid-derived suppressor cells and regulatory t cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma. Clinical Cancer Research. 2015 Jan 1;21(1):39–48.
143. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. J Clin Invest. 2010;120(2):457–71.
144. Poschke I, Mougiakakos D, Hansson J, Masucci G v, Kiessling R. Immature Immunosuppressive CD14+ HLA-DR−/low Cells in Melanoma Patients Are Stat3hi and Overexpress CD80, CD83, and DC-SignCharacterization of Melanoma CD14+ HLA-DR−/low MDSC. Cancer Res. 2010;70(11):4335–45.
145. O'Shea JJ, Schwartz DM, Villarino A v, Gadina M, McInnes IB, Laurence A. The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med. 2015;66:311–28.

146. Vasquez-Dunddel D, Pan F, Zeng Q, Gorbounov M, Albesiano E, Fu J, et al. STAT3 regulates arginase-I in myeloid-derived suppressor cells from cancer patients. J Clin Invest. 2013;123(4):1580–9.
147. Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, Pilon-Thomas S, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med. 2005;11(12):1314–21.
148. Hong D, Kurzrock R, Kim Y, Woessner R, Younes A, Nemunaitis J, et al. AZD9150, a next-generation antisense oligonucleotide inhibitor of STAT3 with early evidence of clinical activity in lymphoma and lung cancer [Internet]. Available from: https://www.science.org
149. Karin N. The development and homing of myeloid-derived suppressor cells: from a two-stage model to a multistep narrative. Front Immunol. 2020;11:557586.
150. Daurkin I, Eruslanov E, Vieweg J, Kusmartsev S. Generation of antigen-presenting cells from tumor-infiltrated CD11b myeloid cells with DNA demethylating agent 5-aza-2′-deoxycytidine. Cancer Immunology, Immunotherapy. 2010 May;59(5):697–706.
151. Nefedova Y, Fishman M, Sherman S, Wang X, Beg AA, Gabrilovich DI. Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells. Cancer Res. 2007 Nov 15;67(22):11021–8.
152. Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, Djeu JY. A novel chemoimmunomodulating property of docetaxel: Suppression of myeloid-derived suppressor cells in tumor bearers. Clinical Cancer Research. 2010 Sep 15;16(18):4583–94.
153. Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon Receptor Signaling Pathways Regulating PD-L1 and PD-L2 Expression. Cell Rep. 2017 May 9;19(6):1189–201.
154. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation. New England Journal of Medicine. 2011 Jun 30;364(26):2507–16.
155. Saw PE, Xu X, Kim S, Jon S. Biomedical Applications of a Novel Class of High-Affinity Peptides. Acc Chem Res. 2021 Sep 21;54(18):3576–92.
156. Kim D, Lee IH, Kim S, Choi M, Kim H, Ahn S, et al. A specific STAT3-binding peptide exerts antiproliferative effects and antitumor activity by inhibiting STAT3 phosphorylation and signaling. Cancer Res. 2014 Apr 15;74(8):2144–51.
157. Kim TW, Kim Y, Keum H, Jung W, Kang M, Jon S. Combination of a STAT3 inhibitor with anti-PD-1 immunotherapy is an effective treatment regimen for a vemurafenib-resistant melanoma. Mol Ther Oncolytics. 2022 Sep 15;26:1–14.
158. Ye Y, Fang Y, Xu W, Wang Q, Zhou J, Lu R. 3,3′-Diindolylmethane induces anti-human gastric cancer cells by the miR-30e-ATG5 modulating autophagy. Biochem Pharmacol. 2016 Sep 1;115:77–84.
159. Sun Q, Xiao L, Cui Z, Yang Y, Ma J, Huang Z, et al. 3,3′-Diindolylmethane improves antitumor immune responses of PD-1 blockade via inhibiting myeloid-derived suppressor cells. Chinese Medicine (United Kingdom). 2022 Dec 1;17(1).
160. Seo JS, Cho NY, Kim HR, Tsurumi T, Jang YS, Lee WK, et al. Cell cycle arrest and lytic induction of EBV-transformed B lymphoblastoid cells by a histone deacetylase inhibitor, Trichostatin A. Oncol Rep. 2008;19(1):93–8.
161. Hong EH, Heo EY, Song JH, Kwon BE, Lee JY, Park Y, et al. Trans-scirpusin A showed antitumor effects via autophagy activation and apoptosis induction of colorectal cancer cells. Oncotarget. 2017;8(25):41401.
162. Xie Z, Ago Y, Okada N, Tachibana M. Valproic acid attenuates immunosuppressive function of myeloid-derived suppressor cells. J Pharmacol Sci. 2018;137(4):359–65.
163. Youn JI, Kumar V, Collazo M, Nefedova Y, Condamine T, Cheng P, et al. Epigenetic silencing of retinoblastoma gene regulates pathologic differentiation of myeloid cells in cancer. Nat Immunol. 2013;14(3):211–20.
164. Briere D, Sudhakar N, Woods DM, Hallin J, Engstrom LD, Aranda R, et al. The class I/IV HDAC inhibitor mocetinostat increases tumor antigen presentation, decreases immune suppressive cell types and augments checkpoint inhibitor therapy. Cancer Immunology, Immunotherapy. 2018;67(3):381–92.
165. Orillion A, Hashimoto A, Damayanti N, Shen L, Adelaiye-Ogala R, Arisa S, et al. Entinostat Neutralizes Myeloid-Derived Suppressor Cells and Enhances the Antitumor Effect of PD-1 Inhibition in Murine Models of Lung and Renal Cell CarcinomaHDAC and PD-1 Inhibition. Clinical cancer research. 2017;23(17):5187–201.
166. Xie Z, Ikegami T, Ago Y, Okada N, Tachibana M. Valproic acid attenuates CCR2-dependent tumor infiltration of monocytic myeloid-derived suppressor cells, limiting tumor progression. Oncoimmunology. 2020;9(1):1734268.
167. Weber JS, Levinson BA, Laino AS, Pavlick AC, Woods DM. Clinical and immune correlate results from a phase 1b study of the histone deacetylase inhibitor mocetinostat with ipilimumab and nivolumab in unresectable stage III/IV melanoma. Melanoma Res [Internet]. 2022;32(5). Available from: https://journals.lww.com/melanomaresearch/Fulltext/2022/10000/Clinical_and_immune_correlate_results_from_a_phase.4.aspx
168. Roussos Torres ET, Rafie C, Wang C, Lim D, Brufsky A, LoRusso P, et al. Phase I study of entinostat and nivolumab with or without ipilimumab in advanced solid tumors (ETCTN-9844). Clinical Cancer Research. 2021 Nov 15;27(21):5828–37.
169. Gray JE, Saltos A, Tanvetyanon T, Haura EB, Creelan B, Antonia SJ, et al. Phase I/Ib study of pembrolizumab plus vorinostat in advanced/metastatic Non–small cell lung cancer. Clinical Cancer Research. 2019;25(22):6623–32.

170. Saltos AN, Tanvetyanon T, Creelan BC, Shafique MR, Antonia SJ, Haura EB, et al. Phase II randomized trial of first-line pembrolizumab and vorinostat in patients with metastatic NSCLC (mNSCLC). American Society of Clinical Oncology; 2020.
171. Honda F, Kano H, Kanegane H, Nonoyama S, Kim ES, Lee SK, et al. The kinase Btk negatively regulates the production of reactive oxygen species and stimulation-induced apoptosis in human neutrophils. Nat Immunol. 2012 Apr;13(4):369–78.
172. Mueller H, Stadtmann A, Aken H van, Hirsch E, Wang D, Ley K, et al. Tyrosine kinase Btk regulates E-selectin-mediated integrin activation and neutrophil recruitment by controlling phospholipase C (PLC) 2 and PI3K pathways. 2010; Available from: http://ashpublications.org/blood/article-pdf/115/15/3118/1325791/zh801510003118.pdf
173. Collett G, Wood A, Alexander MY, Varnum BC, Boot-Handford RP, Ohanian V, et al. Receptor tyrosine kinase Axl modulates the osteogenic differentiation of pericytes. Circ Res. 2003 May 30;92(10):1123–9.
174. Wu X, Ma W, Zhou Q, Yan H, Lim ZF, Huang M, et al. AXL–GAS6 expression can predict for adverse prognosis in non-small cell lung cancer with brain metastases. J Cancer Res Clin Oncol. 2017 Oct 1;143(10):1947–57.
175. Holtzhausen A, Harris W, Ubil E, Hunter DM, Zhao J, Zhang Y, et al. TAM Family Receptor Kinase Inhibition Reverses MDSC-Mediated Suppression and Augments Anti–PD-1 Therapy in MelanomaTAM RTK Inhibition Reverses MDSC Suppression and Augments PD-1. Cancer Immunol Res. 2019;7(10):1672–86.
176. Synn CB, Kim SE, Lee HK, Kim MH, Kim JH, Lee JM, et al. SKI-G-801, an AXL kinase inhibitor, blocks metastasis through inducing anti-tumor immune responses and potentiates anti-PD-1 therapy in mouse cancer models. Clin Transl Immunology. 2022;11(1).
177. Patnaik A, Swanson KD, Csizmadia E, Solanki A, Landon-Brace N, Gehring MP, et al. Cabozantinib Eradicates Advanced Murine Prostate Cancer by Activating Antitumor Innate ImmunityActivation of Antitumor Innate Immunity in Prostate Cancer. Cancer Discov. 2017;7(7):750–65.
178. Lu X, Horner JW, Paul E, Shang X, Troncoso P, Deng P, et al. Effective combinatorial immunotherapy for castration-resistant prostate cancer. Nature. 2017;543(7647):728–32.
179. Kwilas AR, Ardiani A, Donahue RN, Aftab DT, Hodge JW. Dual effects of a targeted small-molecule inhibitor (cabozantinib) on immune-mediated killing of tumor cells and immune tumor microenvironment permissiveness when combined with a cancer vaccine. J Transl Med. 2014;12(1):1–15.
180. Nadal RM, Mortazavi A, Stein M, Pal SK, Davarpanah NN, Parnes HL, et al. Results of phase I plus expansion cohorts of cabozantinib (Cabo) plus nivolumab (Nivo) and CaboNivo plus ipilimumab (Ipi) in patients (pts) with with metastatic urothelial carcinoma (mUC) and other genitourinary (GU) malignancies. American Society of Clinical Oncology; 2018.
181. Administration USF and D. FDA approves nivolumab plus cabozantinib for advanced renal cell carcinoma. 2021.
182. Kudo M, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. The Lancet. 2018;391(10126):1163–73.
183. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25–34.
184. Sor afenib in Advanced Hepatocellular Carcinoma [Internet]. 2008. Available from: www.nejm.org
185. Kudo M, Ikeda M, Motomura K, Okusaka T, Kato N, Dutcus CE, et al. A phase Ib study of lenvatinib (LEN) plus nivolumab (NIV) in patients (pts) with unresectable hepatocellular carcinoma (uHCC): Study 117. Journal of Clinical Oncology [Internet]. 2020 Feb 1;38(4_suppl):513. Available from: https://doi.org/10.1200/JCO.2020.38.4_suppl.513
186. Finn RS, Ikeda M, Zhu AX, Sung MW, Baron AD, Kudo M, et al. Phase Ib Study of Lenvatinib Plus Pembrolizumab in Patients With Unresectable Hepatocellular Carcinoma [Internet]. Vol. 38, J Clin Oncol. 2020. Available from: https://doi.org/10.
187. Lee SW, Yang SS, Lien HC, Peng YC, Tung CF, Lee TY. The Combining of Tyrosine Kinase Inhibitors and Immune Checkpoint Inhibitors as First-Line Treatment for Advanced Stage Hepatocellular Carcinoma. J Clin Med. 2022 Aug 1;11(16).
188. Yang J, Nie J, Ma X, Wei Y, Peng Y, Wei X. Targeting PI3K in cancer: Mechanisms and advances in clinical trials. Vol. 18, Molecular Cancer. BioMed Central Ltd.; 2019.
189. Lindemans CA, Coffer PJ. Regulation of granulocyte apoptosis by phosphatidylinositol 3-kinase. Biochem Soc Trans. 2004;32(3):480–4.
190. Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, et al. Central role for G protein-coupled phosphoinositide 3-kinase γ in inflammation. Science (1979). 2000;287(5455):1049–53.
191. Central role for G-protein coupled PI3K in Inflammation.
192. Enioutina EY, Bareyan D, Daynes RA. A Role for Immature Myeloid Cells in Immune Senescence. The Journal of Immunology. 2011 Jan 15;186(2):697–707.

193. de Henau O, Rausch M, Winkler D, Campesato LF, Liu C, Cymerman DH, et al. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kγ in myeloid cells. Nature. 2016;539(7629):443–7.
194. Kaneda MM, Messer KS, Ralainirina N, Li H, Leem CJ, Gorjestani S, et al. PI3Kγ 3 is a molecular switch that controls immune suppression. Nature. 2016;539(7629):437–42.
195. Nam W, Tak J, Ryu JK, Jung M, Yook JI, Kim HJ, et al. Effects of artemisinin and its derivatives on growth inhibition and apoptosis of oral cancer cells. Head Neck. 2007 Apr;29(4):335–40.
196. Paik IH, Xie S, Shapiro TA, Labonte T, Narducci Sarjeant AA, Baege AC, et al. Second generation, orally active, antimalarial, artemisinin-derived trioxane dimers with high stability, efficacy, and anticancer activity. J Med Chem. 2006 May 4;49(9):2731–4.
197. Cao Y, Feng YH, Gao LW, Li XY, Jin QX, Wang YY, et al. Artemisinin enhances the anti-tumor immune response in 4T1 breast cancer cells in vitro and in vivo. Int Immunopharmacol. 2019 May 1;70:110–6.
198. Zhang M, Wang L, Liu W, Wang T, de Sanctis F, Zhu L, et al. Targeting Inhibition of Accumulation and Function of Myeloid-Derived Suppressor Cells by Artemisinin via PI3K/AKT, mTOR, and MAPK Pathways Enhances Anti-PD-L1 Immunotherapy in Melanoma and Liver Tumors. J Immunol Res. 2022;2022:2253436.
199. Wang H, He X, Fang D, Wang X, Guan J, Shi ZW, et al. Gemcitabine-facilitated modulation of the tumor microenvironment and PD-1/PD-L1 blockade generate a synergistic antitumor effect in a murine hepatocellular carcinoma model. Clin Res Hepatol Gastroenterol. 2022 Apr 1;46(4).
200. Peng S, Hu P, Xiao YT, Lu W, Guo D, Hu S, et al. Single-Cell Analysis Reveals EP4 as a Target for Restoring T-Cell Infiltration and Sensitizing Prostate Cancer to Immunotherapy. Clinical Cancer Research. 2022 Feb 1;28(3):552–67.
201. Take Y, Koizumi S, Nagahisa A. Prostaglandin E Receptor 4 Antagonist in Cancer Immunotherapy: Mechanisms of Action. Vol. 11, Frontiers in Immunology. Frontiers Media S.A.; 2020.
202. Grauers Wiktorin H, Nilsson MS, Kiffin R, Sander FE, Lenox B, Rydström A, et al. Histamine targets myeloid-derived suppressor cells and improves the anti-tumor efficacy of PD-1/PD-L1 checkpoint blockade. Cancer Immunology, Immunotherapy. 2019 Feb 13;68(2):163–74.
203. Liang Y, Wang W, Zhu X, Yu M, Zhou C. Inhibition of myeloid-derived suppressive cell function with all-trans retinoic acid enhanced anti-PD-L1 efficacy in cervical cancer. Sci Rep. 2022 Dec 1;12(1).
204. Lesterhuis WJ, Rinaldi C, Jones A, Rozali EN, Dick IM, Khong A, et al. Network analysis of immunotherapy-induced regressing tumours identifies novel synergistic drug combinations. Sci Rep. 2015 Jul 21;5.
205. Prasad M, Zorea J, Jagadeeshan S, Shnerb AB, Mathukkada S, Bouaoud J, et al. MEK1/2 inhibition transiently alters the tumor immune microenvironment to enhance immunotherapy efficacy against head and neck cancer. J Immunother Cancer. 2022 Mar 15;10(3).
206. Highfill SL, Cui Y, Giles AJ, Smith JP, Zhang H, Morse E, et al. Disruption of CXCR2-Mediated MDSC Tumor Trafficking Enhances Anti-PD1 Efficacy [Internet]. Available from: https://www.science.org
207. Benguigui M, Vorontsova A, Timaner M, Levin S, Haj-Shomaly J, Deo A, et al. Bv8 Blockade Sensitizes Anti-PD1 Therapy Resistant Tumors. Front Immunol. 2022 Jul 7;13.
208. Obermajer N, Muthuswamy R, Lesnock J, Edwards RP, Kalinski P. Positive feedback between PGE2 and COX2 redirects the differentiation of human dendritic cells toward stable myeloid-derived suppressor cells. Blood. 2011 Nov 17;118(20):5498–505.
209. Rostom A, Dubé C, Lewin G, Tsertsvadze A, Barrowman N, Code C, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the US Preventive Services Task Force. Ann Intern Med. 2007;146(5):376–89.
210. Karlsson A, Dahlgren C. Assembly and activation of the neutrophil NADPH oxidase in granule membranes. Antioxid Redox Signal. 2002;4(1):49–60.
211. Tacke RS, Lee H, Goh C, Courtney J, Polyak SJ, Rosen HR, et al. Myeloid suppressor cells induced by hepatitis C virus suppress T-cell responses through the production of reactive oxygen species. Hepatology. 2012;55(2):343–53.
212. Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, et al. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. The Journal of Immunology. 2009;182(9):5693–701.
213. Martner A, Wiktorin HG, Lenox B, Ewald Sander F, Aydin E, Aurelius J, et al. Histamine Promotes the Development of Monocyte-Derived Dendritic Cells and Reduces Tumor Growth by Targeting the Myeloid NADPH Oxidase. The Journal of Immunology. 2015 May 15;194(10):5014–21.
214. Horowitz NA, Rowe JM. Histamine dihydrochloride for maintaining remission in acute myeloid leukemia. Int J Hematol Oncol. 2014;3(2):137–43.
215. Huang Meng-er B, Yu-chen Y, Shu-rong Chai Jin-ren C, Jia-Xiang L, Lin Z, Long-jun G, et al. Use of All-Trans Retinoic Acid in the Treatment of Acute Promyelocytic Leukemia. Vol. 72, Blood. 1988.
216. Barault L, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008 May 15;122(10):2255–9.

217. Dudley DT, Pang L, Decker SJ, Bridgest AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade (protein phosphorylation/growth factor/cell proliferation) [Internet]. Vol. 92, Biochemistry. 1995. Available from: https://www.pnas.org
218. Pages G, Lenormand P, L'allemain G, Chambard JC, Meloche S, Pouyssagurt J, et al. Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation (protein kinase cascade/kinase antiense/dominant neptve mutants/growth control/H-killing selection) [Internet]. Vol. 90, Proc. Natl. Acad. Sci. USA. 1993. Available from: https://www.pnas.org
219. Sebolt-Leopold JS, Herrera R. Targeting the mitogen-activated protein kinase cascade to treat cancer. Vol. 4, Nature Reviews Cancer. 2004. p. 937–47.
220. Franklin DA, Sharick JT, Ericsson-Gonzalez PI, Sanchez V, Dean PT, Opalenik SR, et al. MEK activation modulates glycolysis and supports suppressive myeloid cells in TNBC. JCI Insight. 2020;5(15).
221. Ebert PJR, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity. 2016;44(3):609–21.
222. Kuske M, Westphal D, Wehner R, Schmitz M, Beissert S, Praetorius C, et al. Immunomodulatory effects of BRAF and MEK inhibitors: Implications for Melanoma therapy. Pharmacol Res. 2018;136:151–9.
223. Verma V, Jafarzadeh N, Boi S, Kundu S, Jiang Z, Fan Y, et al. MEK inhibition reprograms CD8+ T lymphocytes into memory stem cells with potent antitumor effects. Nat Immunol. 2021;22(1):53–66.
224. Gilmartin AG, Bleam MR, Groy A, Moss KG, Minthorn EA, Kulkarni SG, et al. GSK1120212 (JTP-74057) is an inhibitor of MEK activity and activation with favorable pharmacokinetic properties for sustained in vivo pathway inhibition. Clinical Cancer Research. 2011 Mar 1;17(5):989–1000.
225. Uppaluri R, Winkler AE, Lin T, Law JH, Haughey BH, Nussenbaum B, et al. Biomarker and tumor responses of oral cavity squamous cell carcinoma to trametinib: A phase II neoadjuvant window-of-opportunity clinical trial. Clinical Cancer Research. 2017 May 1;23(9):2186–94.
226. Kang SH, Keam B, Ahn YO, Park HR, Kim M, Kim TM, et al. Inhibition of MEK with trametinib enhances the efficacy of anti-PD-L1 inhibitor by regulating anti-tumor immunity in head and neck squamous cell carcinoma. Oncoimmunology. 2019 Jan 2;8(1).
227. Liu L, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, et al. The BRAF and MEK inhibitors dabrafenib and trametinib: Effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. Clinical Cancer Research. 2015 Apr 1;21(7):1639–51.
228. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res. 2005;65(3):671–80.
229. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, et al. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. New England Journal of Medicine. 2020 May 14;382(20):1894–905.
230. Ng KYY, Wong LWJ, Ang AJS, Lee AWX, Tay DSH, Tan JJE, et al. Immune checkpoint inhibitors and tyrosine kinase inhibitors in patients with advanced hepatocellular carcinoma: Does the sequence matter? Asia Pac J Clin Oncol [Internet]. 2022 Aug 10;n/a(n/a). Available from: https://doi.org/10.1111/ajco.13837
231. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I Production in the Tumor Microenvironment by Mature Myeloid Cells Inhibits T-Cell Receptor Expression and Antigen-Specific T-Cell Responses [Internet]. Vol. 64, CANCER RESEARCH. 2004. Available from: http://aacrjournals.org/cancerres/article-pdf/64/16/5839/2516899/zch01604005839.pdf?casa_token=uL8c8tbTHEIAAAAA:XxAKF8mPocCi8i6EEBqpQ-4SFsreaHsK2kTgF2AkuQKb0-x6JCiYibwEVXFgqoxIwxCldXuZ
232. Chang AL, Miska J, Wainwright DA, Dey M, Rivetta C v., Yu D, et al. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory t cells and myeloid-derived suppressor cells. Cancer Res. 2016 Oct 1;76(19):5671–82.
233. Chun E, Lavoie S, Michaud M, Gallini CA, Kim J, Soucy G, et al. CCL2 Promotes Colorectal Carcinogenesis by Enhancing Polymorphonuclear Myeloid-Derived Suppressor Cell Population and Function. Cell Rep. 2015 Jul 14;12(2):244–57.
234. Li BH, Garstka MA, Li ZF. Chemokines and their receptors promoting the recruitment of myeloid-derived suppressor cells into the tumor. Mol Immunol [Internet]. 2020;117:201–15. Available from: https://www.sciencedirect.com/science/article/pii/S0161589019304304
235. Raman D, Baugher PJ, Thu YM, Richmond A. Role of chemokines in tumor growth. Vol. 256, Cancer Letters. Elsevier Ireland Ltd; 2007. p. 137–65.
236. Wang D, Sun H, Wei J, Cen B, DuBois RN. CXCL1 is critical for premetastatic niche formation and metastasis in colorectal cancer. Cancer Res. 2017 Jul 1;77(13):3655–65.
237. Curtis VF, Wang H, Yang P, McLendon RE, Li X, Zhou QY, et al. A PK2/Bv8/PROK2 Antagonist Suppresses Tumorigenic Processes by Inhibiting Angiogenesis in Glioma and Blocking Myeloid Cell Infiltration in Pancreatic Cancer. PLoS One. 2013 Jan 29;8(1).
238. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, et al. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. Nature. 2007 Dec 6;450(7171):825–31.

239. Toor SM, Syed Khaja AS, el Salhat H, Faour I, Kanbar J, Quadri AA, et al. Myeloid cells in circulation and tumor microenvironment of breast cancer patients. Cancer Immunology, Immunotherapy. 2017;66(6):753–64.
240. Speigl L, Burow H, Bailur JK, Janssen N, Walter CB, Pawelec G, et al. CD14+ HLA-DR−/low MDSCs are elevated in the periphery of early-stage breast cancer patients and suppress autologous T cell proliferation. Breast Cancer Res Treat. 2018;168(2):401–11.
241. Blixt NC, Faulkner BK, Astleford K, Lelich R, Schering J, Spencer E, et al. Class II and IV HDACs function as inhibitors of osteoclast differentiation. PLoS One. 2017;12(9):e0185441.
242. Danilin S, Merkel AR, Johnson JR, Johnson RW, Edwards JR, Sterling JA. Myeloid-derived suppressor cells expand during breast cancer progression and promote tumor-induced bone destruction. Oncoimmunology. 2012;1(9):1484–94.
243. Shou D, Wen L, Song Z, Yin J, Sun Q, Gong W. Suppressive role of myeloid-derived suppressor cells (MDSCs) in the microenvironment of breast cancer and targeted immunotherapies. Oncotarget. 2016;7(39):64505.
244. Chun E, Lavoie S, Michaud M, Gallini CA, Kim J, Soucy G, et al. CCL2 promotes colorectal carcinogenesis by enhancing polymorphonuclear myeloid-derived suppressor cell population and function. Cell Rep. 2015;12(2):244–57.
245. Katoh H, Wang D, Daikoku T, Sun H, Dey SK, DuBois RN. CXCR2-expressing myeloid-derived suppressor cells are essential to promote colitis-associated tumorigenesis. Cancer Cell. 2013;24(5):631–44.
246. Wu P, Wu D, Ni C, Ye J, Chen W, Hu G, et al. γδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. Immunity. 2014;40(5):785–800.
247. de Cicco P, Sanders T, Cirino G, Maloy KJ, Ianaro A. Hydrogen sulfide reduces myeloid-derived suppressor cell-mediated inflammatory response in a model of Helicobacter hepaticus-induced colitis. Front Immunol. 2018;9:499.
248. Zhang Y, Wang J, Wang W, Tian J, Yin K, Tang X, et al. IL-17A produced by peritoneal macrophages promote the accumulation and function of granulocytic myeloid-derived suppressor cells in the development of colitis-associated cancer. Tumor Biology. 2016;37(12):15883–91.
249. Yan J, Huang J. Innate γδT17 cells convert cancer-elicited inflammation into immunosuppression through myeloid-derived suppressor cells. Oncoimmunology. 2014;3(8):e953423.
250. Stanojevic I, Miller K, Kandolf-Sekulovic L, Mijuskovic Z, Zolotarevski L, Jovic M, et al. A subpopulation that may correspond to granulocytic myeloid-derived suppressor cells reflects the clinical stage and progression of cutaneous melanoma. Int Immunol. 2016;28(2):87–97.
251. Weide B, Martens A, Zelba H, Stutz C, Derhovanessian E, di Giacomo AM, et al. Myeloid-Derived Suppressor Cells Predict Survival of Patients with Advanced Melanoma: Comparison with Regulatory T Cells and NY-ESO-1-or Melan-A–Specific T CellsPrognostic Impact of MDSCs and Tregs in Stage IV Melanoma. Clinical Cancer Research. 2014;20(6):1601–9.
252. Filipazzi P, Huber V, Rivoltini L. Phenotype, function and clinical implications of myeloid-derived suppressor cells in cancer patients. Cancer Immunology, Immunotherapy. 2012;61(2):255–63.
253. Stiff A, Trikha P, Mundy-Bosse B, McMichael E, Mace TA, Benner B, et al. Nitric Oxide Production by Myeloid-Derived Suppressor Cells Plays a Role in Impairing Fc Receptor–Mediated Natural Killer Cell FunctionMDSC Inhibit Antibody Therapy via Nitric Oxide Production. Clinical Cancer Research. 2018;24(8):1891–904.
254. Xiu B, Lin Y, Grote DM, Ziesmer SC, Gustafson MP, Maas ML, et al. IL-10 induces the development of immunosuppressive CD14+ HLA-DRlow/− monocytes in B-cell non-Hodgkin lymphoma. Blood Cancer J. 2015;5(7):e328–e328.
255. Sinistro A, Ciaprini C, Natoli S, Sussarello E, Carducci FC, Almerighi C, et al. Lipopolysaccharide desensitizes monocytes–macrophages to CD40 ligand stimulation. Immunology. 2007;122(3):362–70.
256. Mancino A, Schioppa T, Larghi P, Pasqualini F, Nebuloni M, Chen IH, et al. Divergent effects of hypoxia on dendritic cell functions. Blood, the Journal of the American Society of Hematology. 2008;112(9):3723–34.