

Targeting myeloid-derived suppressor cells in the treatment of hepatocellular carcinoma: current state and future perspectives

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Abstract: Systemic therapy for advanced hepatocellular carcinoma (HCC) has been focusing on overcoming tumor angiogenesis and immunosuppression. Myeloid-derived suppressor cells (MDSCs) promote both angiogenesis and immunosuppression in the tumor microenvironment (TME). Multiple clinical studies have demonstrated the prognostic implications of and suggested the translational significance of MDSCs in patients with HCC. In preclinical HCC models, targeting MDSCs has been shown to enhance antitumor efficacy of sorafenib or immune checkpoint inhibitors. Reversing the protumor effects of MDSCs could be achieved by depleting MDSCs, blocking MDSC trafficking and migration into TME, and inhibiting the immunosuppressive functions of MDSCs. To date, these strategies have not yet been validated to be clinically useful in patients with malignancy including HCC. Future studies should focus on identifying specific markers for human MDSCs and developing combination approaches incorporating MDSC-targeting therapy in the treatment of HCC.

Keywords: myeloid-derived suppressor cells, MDSCs; hepatocellular carcinoma, immunosuppression, angiogenesis, immunotherapy, immune checkpoint inhibitor

Introduction

Primary liver cancer, of which the vast majority of cases are hepatocellular carcinoma (HCC), has been the leading cause of cancer mortality worldwide for decades. The GLOBOCAN 2018 database estimated that 841,000 new cases of primary liver cancer and 782,000 deaths from it would occur annually, making it the sixth most commonly diagnosed and fourth most lethal malignancy worldwide.¹ The carcinogenesis of HCC is attributed to chronic inflammation of the liver, predominantly as a result of hepatitis B virus (HBV) and hepatitis C virus (HCV) virus infections, as well as liver cirrhosis. Owing to significant advances in the primary prevention of HBV infection through universal vaccinations as well as effective antiviral therapy for HBV and HCV infections, the incidence of HCC is expected to decline between 2035 and 2040.² Overall, HCC remains a significant malignant disease that will continue to be a major burden on global health for decades to come.

Treatment of HCC is commonly directed by disease stage.³ For patients with early-stage localized HCC, curative-intent treatment modalities include resection, ablation, and liver transplantation, whereas for patients with intermediate-stage localized HCC, image-guided transcatheter tumor therapies such as transarterial chemoembolization have provided survival benefits.

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Unfortunately, the majority of patients with HCC either progress to or develop de novo locally advanced or metastatic diseases and are indicated for systemic therapy.

Recent advances in systemic therapy for HCC

Sorafenib, a multikinase inhibitor with antiangiogenic properties, is the first systemic therapy approved for HCC owing to two positive randomized placebo-controlled phase III trials.^{4,5} Since 2016, four other antiangiogenic agents, including three multikinase inhibitors and one anti-vascular endothelial growth factor receptor monoclonal

antibody, have been demonstrated to provide survival benefits for patients with advanced HCC in phase III clinical trials (Table 1).^{6–9} As a result, the Food and Drug Administration of the United States (US-FDA) and the regulatory agencies of multiple countries approved lenvatinib as a first-line systemic therapy for HCC, and approved regorafenib and cabozantinib for patients with HCC who have been previously treated with sorafenib.

Moreover, immunotherapy with immune checkpoint inhibitors (ICIs) such as monoclonal antibodies that target programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated

Table 1 Systemic therapy approved or with positive results in phase III trials for advanced hepatocellular carcinoma

| Drug and study | Mechanism of action | Trial design | Treatment arms (patient numbers) | Key findings | US-FDA approval |
|--|------------------------|--|---|--|-----------------|
| Pivotal trials of sorafenib | | | | | |
| Sorafenib, SHARP trial ⁴ | Multikinase inhibitors | Phase III, 1st-line, 1:1 randomization | Sorafenib (299) vs placebo (303) | Median OS: 10.7 m (sorafenib) vs 7.9 m (placebo), HR=0.69 (95% CI, 0.55–0.87), $P<0.001$ | 2007 |
| Sorafenib, Asia-Pacific trial ⁵ | Multikinase inhibitors | Phase III, 1st-line, 2:1 randomization | Sorafenib (150) vs placebo (76) | Median OS: 6.5 m (sorafenib) vs 4.2 m (placebo), HR=0.68 (95% CI, 0.50–0.93), $P=0.014$ | 2007 |
| Antiangiogenic agents | | | | | |
| Lenvatinib, REFLECT trial ⁷ | Multikinase inhibitors | Phase III, 1st-line, 1:1 randomization (noninferiority) | Lenvatinib (478) vs sorafenib (476) | Median OS: 13.6 m (lenvatinib) vs 12.3 m (sorafenib), HR=0.92 (95% CI, 0.79–1.06) | 2018 |
| Regorafenib, RESORCE trial ⁶ | Multikinase inhibitors | Phase III, 2nd-line, 2:1 randomization | Regorafenib (379) vs placebo (194) | Median OS: 10.6 m (regorafenib) vs 7.8 m (placebo), HR=0.63 (95% CI, 0.50–0.79), $P<0.001$ | 2017 |
| Ramucirumab, REACH II trial ⁹ | Anti-VEGFR mAb | Phase III, 2nd-line, 2:1 randomization (AFP ≥ 400 ng/mL) | Ramucirumab (197) vs placebo (95) | Median OS: 8.5 m (ramucirumab) vs 7.3 m (placebo), HR=0.71 (95% CI, 0.53–0.95), $P=0.0199$ | Pending |
| Cabozantinib, CELESTIAL trial ⁸ | Multikinase inhibitors | Phase III, 2nd- or 3rd-line, 2:1 randomization | Cabozantinib (470) vs placebo (237) | Median OS: 10.2 m (cabozantinib) vs 8.0 m (placebo), HR=0.76 (95% CI, 0.63–0.92), $P=0.009$ | 2019 |
| Immune checkpoint inhibitors | | | | | |
| Nivolumab, CheckMate 040 trial ¹⁰ | Anti-PD-1 mAb | Phase I/II, multicohort, Both 1st- and 2nd-lines (70% previously treated with sorafenib) | Nivolumab: dose-escalation (48); dose-expansion (214) | ORR: 20% (95% CI, 15–26) in the dose-escalation and 15% (95% CI, 6–28) in the dose-expansion cohorts | 2017* |
| Pembrolizumab, Keynote-224 trial ¹¹ | Anti-PD-1 mAb | Phase II, 2nd-line | Pembrolizumab (104) | ORR: 17% (95% CI, 11–26) | 2018* |

Note: *Accelerated approval.

Abbreviations: US-FDA, Food and Drug Administration of the United States; OS, overall survival; m, months; HR, hazard ratio; VEGFR, vascular endothelial growth factor receptor; mAb, monoclonal antibody; PD-1, programmed cell death protein 1; AFP, α -fetoprotein; ORR, objective response rate.

antigen 4 (CTLA-4) has become a new paradigm of treatment for multiple cancers, including HCC. Nivolumab, an anti-PD-1 monoclonal antibody, induced a considerable and durable objective tumor response in patients with advanced HCC in a phase I/II study.¹⁰ Pembrolizumab, another anti-PD-1 monoclonal antibody, exhibited a similar response rate (RR) to nivolumab in patients with HCC who had previously been treated with sorafenib in a phase II study.¹¹ Nivolumab and pembrolizumab were granted accelerated approval for the treatment of HCC in 2017 and 2018, respectively, by the US-FDA (Table 1).

Overall, two classes of drugs now exist to treat patients with advanced HCC: one targets tumor angiogenesis and the other targets immunosuppression. Tumor angiogenesis and immune evasion are two major “cancer hallmarks”.^{12,13} Efforts have been ongoing to develop strategies that combine antiangiogenic therapy with PD-1/PD-L1 inhibitors in patients with advanced HCC.^{14–16} However, new studies that elucidate the mechanisms underlying angiogenesis promotion and immunosuppression of HCC may help inspire the development of new therapeutic strategies in the future.

Myeloid-derived suppressor cells: dual tumor-supporting effects by promoting immunosuppression and angiogenesis

Myeloid-derived suppressor cells (MDSCs) play a critical role in the immune tumor microenvironment (TME). MDSCs represent a heterogeneous population of immature myeloid cells with various states of differentiation and are distributed in the bone marrow, spleen, peripheral blood, and tumor tissues. MDSCs have various functions that support tumor growth, including the suppression of T and NK cells and the promotion

of angiogenesis. Therefore, targeting MDSCs is a potential strategy for enhancing the current treatment of cancers.¹⁷

In both humans and mice, MDSCs have two major types: monocytic MDSCs (M-MDSCs) and granulocytic or polymorphonuclear MDSCs (PMN-MDSCs). M-MDSCs share morphological characteristics with monocytes, whereas PMN-MDSCs present morphological characteristics of neutrophils (Table 2). In most cancers, PMD-MDSCs are predominant, representing approximately three-fourths of all MDSCs. In mice, MDSCs are defined using surface markers CD11b and Gr1, and Gr1 has shared epitopes with Ly6C and Ly6G, which are expressed in monocytic cells and granulocytes, respectively. Therefore, M-MDSCs are defined as CD11b⁺Ly6G[−]Ly6C^{high} cells and PMN-MDSCs are defined as CD11b⁺Ly6G^{high}Ly6C^{low} cells in mice. In human, MDSCs generally lack HLA-DR expression, and M-MDSCs are defined as CD11b⁺CD14⁺CD15[−]HLA-DR^{low/−} cells, whereas PMN-MDSCs are defined as CD11b⁺CD14[−]CD15⁺HLA-DR[−] or CD11b⁺CD14[−]CD66b⁺ cells. Moreover, an alternative immature cell subset in humans that is defined by a lack of lineage markers, including CD3, CD14, CD15, CD19, CD56, and HLA-DR, and the expression of CD33 is named early-stage MDSCs.¹⁸ Recently, Condamine et al identified lectin-type oxidized LDL receptor-1 (LOX-1) as a new marker for PMN-MDSCs in humans, further facilitating the discrimination of human PMN-MDSCs from mature neutrophils.¹⁹

Immunosuppression is the primary feature of MDSCs. Although MDSCs suppress diverse immune cells, their main immunosuppressive mechanisms are the inhibition of T cells and NK cells and induction of regulatory T cells (Treg). The major factors involved in MDSC-mediated immunosuppression include arginase (ARG1), inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), TGF-β, IL-10, COX2, indoleamine 2,3-dioxygenase (IDO), and others.^{17,20} ARG1

Table 2 Two major types of myeloid-derived suppressor cells and their immunosuppressive functions

| Types | Markers in mice | Markers in human | Main factors mediating immunosuppression | Mechanisms of immunosuppression |
|-----------|---|---|---|--|
| M-MDSCs | CD11b ⁺ Ly6G [−] Ly6C ^{high} | CD11b ⁺ CD14 ⁺ CD15 [−] HLA-DR ^{low/−} | NO, ARG1, and cytokines such as TGF-β and IL-10 | Suppress T-cell responses both in antigen-specific and nonspecific manners; production of NO and cytokines |
| PMN-MDSCs | CD11b ⁺ Ly6G ^{high} Ly6C ^{low} | CD11b ⁺ CD14 [−] CD15 ⁺ HLA-DR [−] or CD11b ⁺ CD14 [−] CD66b ⁺ or LOX-1 ⁺ | ROS, ARG1 | Suppressing immune responses primarily in an antigen-specific manner; ROS production |

Abbreviations: M-MDSCs, monocytic myeloid-derived suppressor cells; NO, nitric oxide; ARG1, arginase; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells.

depletes L-arginine and leads to cell cycle arrest in the G0-G1 phase of tumor-infiltrating T cells. Depleted L-arginine, increased NO production by iNOS, and increased ROS all result in the downregulation or desensitization of the T-cell receptor and induction of T-cell anergy. IDO degrades L-tryptophan and leads to the suppression of T and NK cells and activation of Treg.²⁰ Studies have also suggested that the immunosuppressive mechanisms of MDSCs may vary at different sites. In peripheral lymphoid structures, PMN-MDSCs have a high level of ROS production and suppress T cells function in an antigen-specific manner. By contrast, M-MDSCs suppress not only antigen-specific but also nonspecific T-cell responses by expressing various factors such as ARG1, NO, TGF- β , and IL-10. In TME, because of hypoxia, ROS levels in PMN-MDSCs are substantially reduced; however, the levels of ARG1 and other factors responsible for nonspecific T-cell suppression are increased.¹⁷

Additionally, MDSCs influence TME by inducing tumor angiogenesis through the production of several angiogenic factors and vascular-modulating enzymes.²¹ For example, Bv8 (bombina variegata peptide 8, a homolog of endocrine-gland-derived vascular endothelial growth factor), produced by MDSCs through granulocyte colony-stimulating factor (G-CSF)-dependent STAT3 signaling, was demonstrated to promote angiogenesis and hematopoietic cell mobilization.²² Actually, MDSC accumulation in TME was associated with tumor refractoriness to anti-VEGF treatment; anti-G-CSF therapy or anti-Bv8 therapy could enhance the responsiveness of anti-VEGF treatment.^{23,24} Moreover, matrix metalloproteinase-9 (MMP-9)-expressing CD11b⁺ myelomonocytic cells have been shown to be critical for the formation of tumor vasculature. Tumor growth could be inhibited in MMP-9 knockout mice or by the deletion of MMP-9 in CD11b⁺Gr1⁺ MDSCs.^{25,26} In addition, MDSCs could acquire endothelial cell properties in TME and directly incorporate into tumor endothelium.²⁶

Clinical significance of MDSCs in human HCC

Previous studies have demonstrated the role of MDSCs in various chronic liver diseases such as HBV or HCV-related hepatitis, and non-alcoholic fatty liver disease (NAFLD).^{27–30} MDSCs were shown to inhibit T cells and moderate HBV-related liver damage during viral replication through ARG1-dependent manner.²⁷ MDSCs may protect liver from detrimental necroinflammation, but also

contribute to persistence of HBV infection.³¹ HCV infection could induce MDSCs, which suppressed T cells and antiviral NK cell responses via ROS and ARG1.^{28,29} Moreover, MDSCs accumulated in the livers of NAFLD mice and had strong suppression effect on T cells, which was dependent on NO production by iNOS.³⁰

Clinical studies of MDSCs in HCC have mainly focused on analyzing M-MDSCs in the peripheral blood of patients with HCC, probably because the cryopreservation process may negatively affect PMN-MDSCs.³² Several groups have studied M-MDSCs, defined by CD14⁺HLA-DR^{low} cells, in the peripheral blood mononuclear cells (PBMCs) of patients with HCC. They found that these MDSCs increased in the PBMCs of patients with HCC compared with patients with only hepatitis or cirrhosis and healthy controls.^{33–35} Moreover, a high frequency of MDSCs in PBMCs has been associated with aggressive tumor features and poor clinical outcomes after hepatectomy, local ablation, or hepatic arterial infusion chemotherapy.^{34–36} Another report defined MDSCs as CD33⁺HLA-DR^{low/-}CD11b⁺CD14⁺ cells, and found PD-L1⁺ MDSCs to be increased in the PBMCs of patients with HCC. In addition, tumor-infiltrating leukocytes contained markedly higher percentages of PD-L1⁺ MDSCs than liver-infiltrating leukocytes and PBMCs.³⁷ Mechanistically, M-MDSCs isolated from the PBMCs of patients with HCC have been proven to be immunosuppressive, as well as shown to have high ARG1, suppress autologous T cell proliferation, inhibit autologous NK cell cytotoxicity, and induce Tregs when cultured ex vivo.^{33,38}

Other studies have utilized various markers to define MDSCs in circulation in patients with HCC. Kalathil et al measured multiple immunosuppressive factors in HCC, and found that the frequency of CD14⁺HLA-DR⁺CD11b⁺CD33⁺ MDSCs, Tregs, and PD-1⁺-exhausted T cells as well as immunosuppressive cytokines levels were increased in the peripheral blood of patients with HCC compared with healthy donors. Combined depletion of MDSCs, Tregs, and PD-1⁺-exhausted T cells in PBMCs isolated from patients with advanced HCC restored the production of granzyme B by CD8⁺ T cells in vitro.³⁹ Recently, Nan et al employed a novel marker, LOX-1, to analyze PMN-MDSCs in patients with HCC and determined that LOX-1⁺CD15⁺ cells were significantly increased in the PBMCs of patients with HCC compared with patients with hepatitis or cirrhosis and healthy controls. The levels of LOX-1⁺CD15⁺ PMN-MDSCs in circulation were associated with those

identified in HCC tissues. LOX-1⁺CD15⁺ PMN-MDSCs suppressed the proliferation and interferon (IFN)- γ production of T cells in vitro, whereas the LOX-1⁺CD15⁺ PMNs did not.⁴⁰

Table 3 summarizes the results of studies on MDSCs in patients with HCC, emphasizing the immunosuppressive activities and prognostic implications of MDSCs.

Table 3 Previous clinical studies on myeloid-derived suppressor cells in hepatocellular carcinoma

| Studies | Markers in humans | HCC patient no. | Key findings | Mechanistic insight or translational implication |
|--|---|-----------------|---|--|
| Hoechst et al <i>Gastroenterology</i> 2008 ³³ | CD14 ⁺ HLA-DR ^{-/low} | 111 | The frequency of MDSCs increased in PBMCs compared with healthy donors or cirrhosis patients. | MDSCs suppress T-cell proliferation and induce Treg. |
| Hoechst et al <i>Hepatology</i> 2009 ³⁸ | CD14 ⁺ HLA-DR ^{-/low} | 30 | MDSCs inhibited NK cell cytotoxicity and IFN- γ release in vitro. | Suppression of NK cells by MDSCs was dependent on cell contact but independent of ARG1 or iNOS function. MDSCs inhibited NK cell function via the NKp30 receptor on NK cells. |
| Arihara et al <i>Cancer Immunol Immunother</i> 2013 ³⁴ | CD14 ⁺ HLA-DR ^{-/low} | 123 | The frequency of MDSCs in CD14 ⁺ PBMCs was significantly increased in patients with HCC compared with that in non-HCC controls. | The frequency of MDSCs was significantly decreased after RFA (33 patients). Patients with high frequency of MDSCs after RFA had worse RFS than those with low frequency of MDSCs after RFA. |
| Mizukoshi et al <i>Cancer Immunol Immunother</i> 2016 ³⁶ | CD14 ⁺ HLA-DR ⁻ | 36 | High frequency of MDSCs in PBMCs was associated with aggressive tumor features such as advanced stage, large tumor size, main PVT, and distant metastasis. | A low frequency of MDSCs was associated with tumor response and longer OS in patients with advanced HCC receiving HAIC. |
| Gao et al <i>Hepatology Res</i> 2017 ³⁵ | CD14 ⁺ HLA-DR ^{-/low} | 183 | The frequency of MDSCs increased in PBMCs of HCC patients compared with those of chronic hepatitis and healthy donors. | High MDSCs were associated with early recurrence and poor OS after hepatectomy. |
| Iwata et al <i>Sci Rep</i> 2016 ³⁷ | CD33 ⁺ HLA-DR ^{low} / CD11b ⁺ CD14 ⁺ | 122 | PD-L1 ⁺ MDSCs were increased in PBMCs from patients with HCC. TILs contained remarkably higher percentages of PD-L1 ⁺ MDSCs than liver-infiltrating lymphocytes and PBMCs (14 patients with HCC). | The percentages of PD-L1 ⁺ MDSCs in PB were significantly reduced by curative treatment for HCC (12 patients with HCC). Patients with low PD-L1 ⁺ MDSCs in PB before curative treatment had significantly longer DFS than those with high PD-L1 ⁺ MDSCs (55 patients with HCC). |
| Kalathil et al <i>Cancer Res</i> 2013 ³⁹ | CD14 ⁺ HLA-DR ⁻ CD11b ⁺ CD33 ⁺ | 23 | The frequency and absolute number of circulating MDSCs was significantly elevated in patients with HCC. | Depleting Tregs, MDSCs, and PD-L1 ⁺ T cells of patients with advanced HCC restored production of granzyme B by CD8 ⁺ T cells in vitro. |
| Nan et al <i>Immunology</i> 2018 ⁴⁰ | LOX-1 ⁺ CD15 ⁺ | 127 | MDSCs in PBMCs were significantly elevated in patients with HCC compared with healthy controls. | MDSCs significantly reduced proliferation and IFN- γ production of T cells in vitro through the ROS/ARG1 pathway induced by ER stress. |

Abbreviations: HCC, hepatocellular carcinoma; MDSCs, myeloid-derived suppressor cells; PBMCs, peripheral blood mononuclear cells; Treg, regulatory T cells; NK cell, natural killer cell; IFN- γ , interferon- γ ; ARG1, arginase; iNOS, inducible nitric oxide synthase; RFA, radiofrequency ablation; RFS, recurrence-free survival; PVT, portal vein thrombosis; OS, overall survival; HAIC, hepatic arterial infusion chemotherapy; PD-L1, programmed death-ligand 1; TILs, tumor-infiltrating leukocytes; PB, peripheral blood; DFS, disease-free survival; PD-L1, programmed cell death protein 1; ER, endoplasmic reticulum.

Biological significance of MDSCs in experimental models of HCC

Multiple mouse HCC models have demonstrated that MDSCs are present at an increased level in tumor-bearing mice and accumulate in HCCs. In orthotopic and subcutaneous tumors derived from the mouse HCC cell line RIL-175, MDSCs rapidly expanded in the liver, spleen, and blood, whereas in slow-growing diethylnitrosamine-induced HCC and MYC-expressing spontaneous HCC models, MDSCs were increased in more advanced stages.⁴¹ Some studies using transplantable HCC models have demonstrated that MDSCs not only inhibit T and NK cells but also suppress the T cell stimulating activity of dendritic cells and alter Kupffer cell function.^{42,43} Collectively, these results illustrate the roles of MDSCs playing in the development and progression of mouse HCCs.

Other preclinical studies have explored the significance of MDSCs in TME of mouse HCCs while investigating the therapeutic effect of sorafenib. Chen et al demonstrated that sorafenib-induced tumor hypoxia and stroma-derived factor 1 alpha (SDF1- α) expression, which subsequently induced CD11b⁺Gr1⁺ MDSC-infiltration in an HCA-1 orthotopic mouse liver cancer model. Furthermore, CD11b⁺Gr1⁺ MDSCs mediated the resistance of sorafenib in liver tumors by promoting hepatic stellate cell differentiation and survival and inducing tumor fibrosis. Inhibiting C-X-C receptor type 4 (CXCR4), the receptor of SDF1- α , or targeting Gr-1 improved the therapeutic effect of sorafenib by reducing the growth of mouse HCCs.⁴⁴ Our group studied another orthotopic HCC model using BNL mouse liver cancer cells and found that tumor-infiltrating Ly6G⁺ PMN-MDSCs increased in mouse HCCs treated with sorafenib. Ly6G⁺ MDSCs suppressed T cell proliferation, induced IL-10 or TGF- β -expressing CD4⁺ T cells, and downregulated the cytotoxic activity of CD8⁺ T cells. Multiple proinflammatory and proangiogenic factors, including G-CSF, stroma-derived factor (SDF), TGF- β , tumor necrosis factor (TNF)- α , VEGF, IL-1 β , and IL-6 were found to be increased in sorafenib-treated mouse orthotopic liver tumors. Targeting MDSCs with anti-Ly6G or anti-IL-6 antibody significantly reduced the frequency of Ly6G⁺ MDSCs in orthotopic liver tumors, enhanced T cell proliferation, and improved the therapeutic effect of sorafenib.⁴⁵

Recent studies have investigated the roles of MDSCs in the efficacy of ICIs in mouse HCC models. Chiu et al studied multiple orthotopic mouse HCC models and found

tumor hypoxia induced the ectoenzyme, ectonucleoside triphosphate diphosphohydrolase 2 (ENTPD2), by stabilizing HIF-1 in cancer cells. ENTDP2 supported the maintenance of MDSCs, and targeting ENTDP2 inhibited tumor growth and enhanced the efficacy of PD-1/CTLA-4 blockade.⁴⁶ Zhou et al demonstrated that the overexpression of cell cycle-related kinase (CCRK), a cyclin-dependent kinase family member, increased MDSC accumulation and T cell suppression in liver-specific CCRK-inducible transgenic mice. Targeting CCRK or downstream IL-6 signaling reduced tumor-infiltrating MDSCs and increased intratumoral IFN- γ ⁺TNF- α ⁺CD8⁺ T cells. Furthermore, the inhibition of CCRK enhanced the anti-tumor effect of anti-PD-L1 therapy.⁴⁷

Overall, preclinical studies using mouse liver cancer models not only confirmed the roles of MDSCs in tumor formation and progression but also indicated the effects of MDSCs in the treatment efficacies of sorafenib and ICIs against HCCs (Table 4). Most studies have indicated that targeting MDSCs would improve the efficacy of sorafenib or ICIs—the currently approved therapeutic agents—in HCC.

Targeting MDSCs in the treatment of human HCC: clinical evidence to date

Numerous preclinical studies have investigated targeting MDSCs as a therapeutic strategy to improve tumor control in experimental animal models. Reversing the protumor effects of MDSCs could be achieved by depleting MDSCs, blocking MDSC trafficking and migration into TME, and inhibiting the immunosuppressive function of MDSCs (Figure 1). The scientific rationales and potential approaches of targeting MDSCs as cancer treatment have been previously reviewed by several groups.^{17,48–50} Herein, we discuss the clinical data concerning HCC by focusing on agents or approaches that have been directly or indirectly implicated in targeting MDSCs in preclinical studies.

Depletion of MDSCs

The number of MDSCs of cancer-bearing hosts could be reduced by inhibiting the myelopoiesis of bone marrow and inducing apoptosis of MDSCs; both these effects are commonly induced by chemotherapeutic agents. Indeed, several chemotherapeutic agents, including gemcitabine,

Table 4 Recent preclinical studies of myeloid-derived suppressor cells in experimental hepatocellular carcinoma models

| Studies | Preclinical models | Key findings | Mechanistic insight or translational implication |
|---|--|---|---|
| Inducing immunosuppression | | | |
| Hu et al <i>Scand J Gastroenterol</i> 2011 ⁴² | HepaI-6 subcutaneous mouse liver cancer models | Increased frequency of MDSCs in tumor development was detected in spleen, PB, LN, and tumor; and IL-10 levels were higher in MDSCs derived from tumor-bearing mice than in control. | MDSCs inhibited TLR-ligand-induced IL-12 production of DC through IL-10 production and suppressed T cell stimulatory activity of DC. |
| Lacotte et al <i>Oncoimmunology</i> 2016 ⁴³ | RIL-175 orthotopic mouse liver cancer models | Kupffer cells expressed less costimulatory CD86 and MHCII and more coinhibitory CD274 molecules in HCC-bearing livers than in control livers, indicating decreased antigen-presenting activity. | MDSC subsets (Ly6G ^{high} cells, Gr1 ^{high} cells, and Ly6C ^{low} cells) were identified and sorted from HCC-bearing mice. Primary isolated Kupffer cells in co-cultured with the three MDSC subsets showed a decrease in CCL2 and IL-18 secretion, increase in IL-10 and IL-1b secretion, and increased expression of CD86, CD274, and MHCII. |
| Engaging drug efficacy | | | |
| Chen et al <i>Hepatology</i> 2014 ⁴⁴ | HCA-I orthotopic mouse liver cancer models | Sorafenib induced tumor-infiltration of CD11b ⁺ Gr1 ⁺ MDSCs through SDF1- α /CXCR4 signaling. | CD11b ⁺ Gr1 ⁺ MDSCs mediated the resistance of sorafenib in liver tumors by promoting hepatic stellate cell differentiation and survival and inducing tumor fibrosis. Inhibition of CXCR4 or Gr-1 in combination with sorafenib inhibited HCC growth compared with sorafenib alone. |
| Chang et al <i>Int J Cancer</i> 2018 ⁴⁵ | BNL orthotopic mouse liver cancer models | MDSCs increased in orthotopic liver tumors after sorafenib treatment. | Targeting MDSCs with anti-Ly6G or anti-IL-6 antibodies improved antitumor efficacy of sorafenib. |
| Chiu et al <i>Nat Commun</i> 2017 ⁴⁶ | MHCC97L cells and HepaI-6 orthotopic mouse liver cancer models | Hypoxia, through stabilization of HIF-1, induced ENTPD2/CD39L1 expression in cancer cells. | Overexpression of ENTPD2 was a poor prognostic factor for patients with HCC. In mouse models, ENTPD2 promoted the maintenance of MDSCs by preventing their differentiation. ENTPD2 inhibition was able to mitigate cancer growth and enhance the efficacy of immune checkpoint inhibitors. |
| Zhou et al <i>Gut</i> 2018 ⁴⁷ | Liver-specific CCRK-inducible transgenic mice and HepaI-6 orthotopic mouse liver cancer models | Ccrk-IL-6 signaling drove liver tumorigenicity through MDSC immunosuppression. | Targeting tumorous CCRK signaling diminished MDSC-mediated immunosuppression and inhibited tumorigenicity of HCC. Tumorous CCRK depletion upregulated PD-L1 expression and increased intratumoral CD8 ⁺ T cells, thereby enhancing PD-L1 blockade efficacy to eradicate HCC. |

Abbreviations: MDSCs, myeloid-derived suppressor cells; PB, peripheral blood; LN, lymph node; HCC, hepatocellular carcinoma; TLR, Toll-like receptor; DC, dendritic cell; MHC, major histocompatibility complex; HIF-1, hypoxia-inducible factor-1; ENTPD2, ectonucleoside triphosphate diphosphohydrolase 2; CCRK, cell cycle-related kinase; PD-L1, programmed death-ligand 1.

doxorubicin, paclitaxel, and 5-fluorouracil (5-FU), have been investigated in preclinical studies and demonstrated to reduce the number of MDSCs in circulation and in TME.^{51–54} Clinical trials conducted a decade or two ago,

most of which were small-scale single-arm phase II trials, demonstrated objective tumor RRs ranging from 0% to 33% for the aforementioned agents in patients with advanced HCC.^{55–62} However, the successful use of

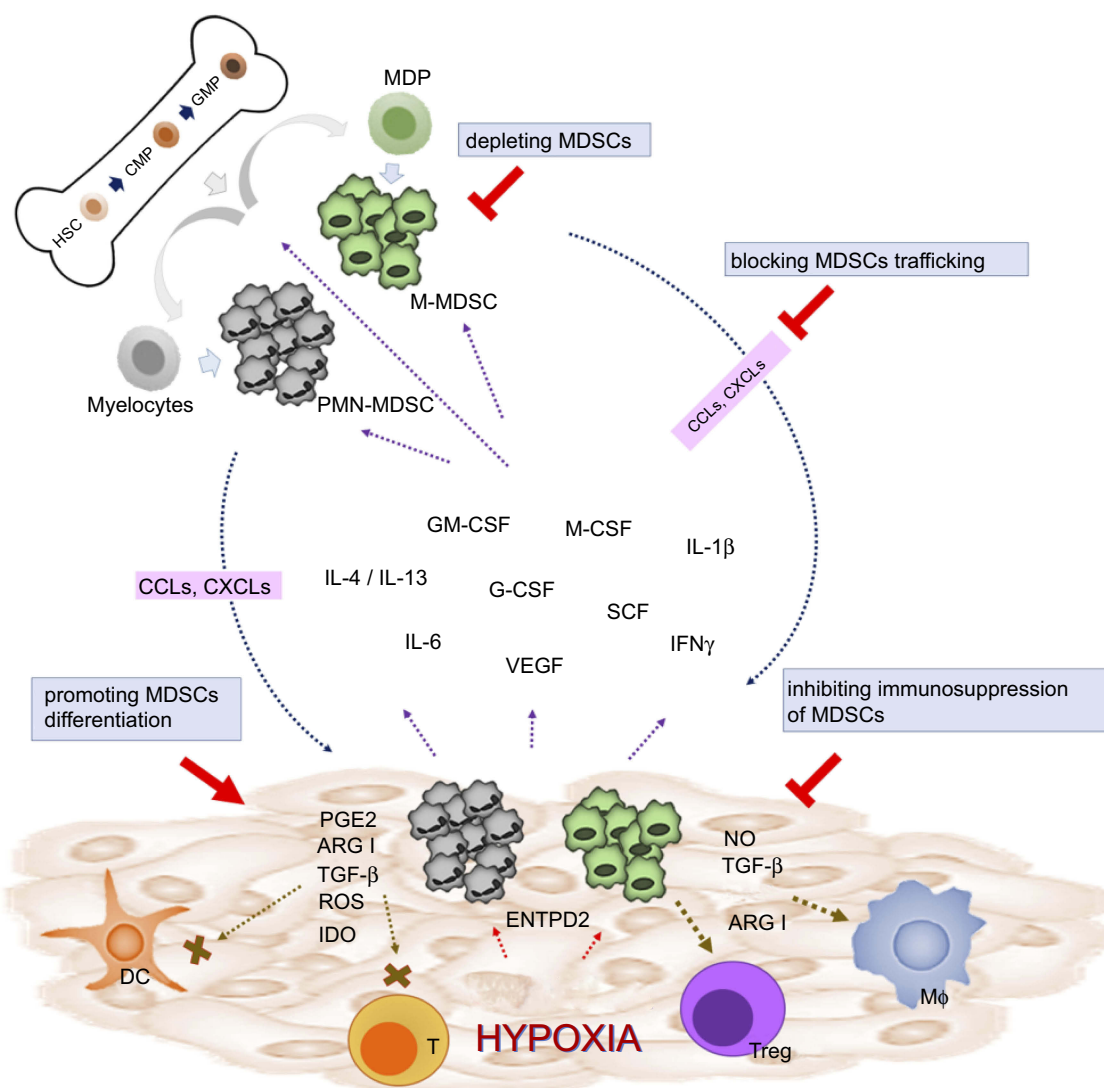


Figure 1 Strategies of targeting MDSCs in cancers. In physiological condition, HSCs differentiate into CMPs and GMPs, which subsequently become mature granulocytes or monocytes. In pathological condition such as malignancy, multiple tumor-derived factors affect the differentiation of myeloid cells, leading to the generation M-MDSCs and PMN-MDSCs. Both types of MDSCs migrate to the tumor site through the interaction of chemokine receptors and ligands (CCLs or CXCLs). In TME, MDSCs are activated and can support tumor growth by suppressing antitumor response of T cells through various mechanisms such as ARG1, iNOS, ROS, TGF- β , IL-10, and IDO. MDSCs can also promote macrophage polarization and induce Tregs and tolerogenic DCs. Reversing the protumor effects of MDSCs could be achieved by depleting MDSCs, promoting MDSC differentiation, blocking MDSC trafficking and migration into TME, and inhibiting the immunosuppressive function of MDSCs.

Abbreviations: HSC, hematopoietic stem cells; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MDP, macrophage/dendritic cell progenitor; MDSCs, myeloid-derived suppressor cells; M-MDSCs, monocytic myeloid-derived suppressor cells; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; G-CSF, granulocyte colony-stimulating factor; SCF, stem cell factor; IFN- γ , interferon- γ ; PGE2, prostaglandin E2; ARG1, arginase; IDO, indoleamine 2,3-dioxygenase; NO, nitric oxide; DC, dendritic cell; T, T cell; Treg, regulatory T cell; M ϕ , macrophage; ENTPD2, ectonucleoside triphosphate diphosphohydrolase 2; CCLs, CC chemokine ligands; CXCLs, C-X-C chemokine ligands; TME, tumor microenvironment; iNOS, inducible nitric oxide synthase.

systemic chemotherapy in the treatment of HCC has been hampered by the inevitable toxicities associated with maximum tolerated dose-type chemotherapy and the poor tolerance by patients with HCC because of impaired organ function and decreased bone marrow reserves.

Administration of chemotherapeutic agents in a low-dose and uninterrupted manner is referred to as metronomic chemotherapy. Metronomic chemotherapy was originally described as an antiangiogenic chemotherapy⁶³ and

has recently been demonstrated to modulate TME, including through an effect on the immune system.^{64,65} Notably, Servo et al demonstrated in a mouse melanoma model that an ultralow and nontoxic dose of paclitaxel could reduce MDSC numbers, improve immunosuppressive functions, and prolong the survival of tumor-bearing mice.⁵³ Clinical studies of metronomic chemotherapy have been conducted in patients with advanced HCC, mainly using oral 5-FU preparations either alone or in combination of

antiangiogenic agents.^{66–69} Although the treatments were well tolerated by HCC patients, their RRs were only modest. These trials did not investigate whether metronomic chemotherapy affects MDSCs in circulation or TME.

Previous studies have shown that treatment with sunitinib, a multikinase inhibitor with antiangiogenic activity, decreased the number of circulating MDSCs in patients with cancer.^{70,71} Multiple preclinical studies have demonstrated that sunitinib was able to deplete the number of MDSCs in circulation as well as in tumors.^{72,73} Another preclinical study demonstrated that cabozantinib reduced intratumoral PMN-MDSCs and enhanced the therapeutic effect of ICIs in a prostate cancer model.⁷⁴ With regard to the clinical efficacy in advanced HCC, sunitinib failed to provide similar clinical efficacy as sorafenib as a first-line therapy for advanced HCC in a phase III trial,⁷⁵ whereas cabozantinib demonstrated significant survival benefits compared with a placebo in patients with HCC who had been previously treated with sorafenib and became an approved agent for advanced HCC.⁸

A recent preclinical study demonstrated that MDSCs could be selectively targeted by TRAIL receptor 2 (TRAIL-R2/DR5) agonist.⁷⁶ A phase I trial testing the agonistic TRAIL-R2 antibody DS-8273a in patients with advanced cancer, including HCC, found that DS-8273a eliminated MDSCs without affecting mature myeloid or lymphoid cells, and the decrease in MDSCs was associated with progression-free survival (PFS).⁷⁷ Another randomized phase II study evaluated tigatuzumab, a humanized monoclonal antibody directed against TRAIL-R2, in patients with advanced HCC.⁷⁸ Although patients treated with tigatuzumab plus sorafenib had numerically longer median PFS and overall survival than those treated with sorafenib alone, the differences did not reach statistical significance. The combination of tigatuzumab with sorafenib was well tolerated in patients with HCC; however, the effect on MDSCs was not investigated.

Another strategy to reduce the number of MDSCs in TME is to facilitate MDSCs differentiating into dendritic cells and macrophages. This MDSC differentiation strategy can be achieved through the inhibition of retinoic acid signaling using all-trans retinoid acid (ATRA).⁷⁹ ATRA has been determined in clinical trials to downregulate MDSCs, and a significant reduction of MDSCs was observed in patients with renal cell carcinoma and small-cell lung cancer.^{80,81} A case report by Hungarian

investigators detailed how a patient received ATRA treatment for hematological malignancy and experienced significant tumor remission in liver tumors, which were clinically diagnosed as HCC because of moderately elevated alpha-fetoprotein and the presence of portal vein thrombosis.⁸² In addition, polyprenoid acid, a synthetic retinoid derivative, has been demonstrated to prevent second primary HCCs in patients who underwent surgical resection for HCC.^{83,84} Polyprenoid acid may work through multiple mechanisms to achieve its chemopreventive effect on HCC.^{85,86} However, whether it would affect anticancer immunity or MDSCs is unclear.

Blockade of MDSC trafficking

Entry of MDSCs into TME is critical for their main immunosuppressive function to be manifested. Therefore, inhibiting chemokine receptors may reduce the number of MDSCs in TME. Chemokine receptor CCR2 and the interaction of its ligand CCL2 are required not only for the recruitment of M-MDSCs and tumor-associated macrophages but also for their suppressive function.^{87,88} A CCR2 inhibitor, PF-04136309, has been tested in combination with FOLFIRINOX chemotherapy in a phase Ib clinical trial of patients with advanced pancreatic cancer. Compared with patients who received FOLFIRINOX alone, those who received chemotherapy plus PF-04136309 had a significantly lower ratio of blood to bone marrow CCR2-positive monocytes, demonstrating the effect of CCR2 blockade on the inhibition of the mobilization of bone marrow-derived monocytes into circulation.⁸⁹

CCR5 is another chemokine receptor that is expressed in many immune cells, and the CCR5–CCR5 ligand axis was found to be critical for the mobilization of PMN-MDSCs.⁹⁰ Targeting CCR5⁺ MDSCs has been demonstrated to prevent MDSC migration and suppress tumor growth in preclinical studies.^{91,92} Recently, a phase Ib/2 clinical trial testing a small-molecule CCR2/5 dual antagonist, BMS-813160, as monotherapy or in combination with chemotherapy or nivolumab in patients with advanced pancreatic or colorectal cancer began to recruit patients.⁹³ CXCR2 is another chemokine receptor expressed on PMN-MDSCs and tumor-associated neutrophils. Blocking CXCR2 has limited the recruitment of PMN-MDSCs and enhanced the efficacy of anti-PD-1 therapy or chemotherapy in preclinical studies.^{94,95} However, there has been no clinical development of inhibitors of these chemokine receptors in HCC.

Inhibition of the immunosuppressive function of MDSCs

STAT3 is a critical transcription factor for immunosuppressive activity and proliferation of MDSCs. A STAT3 oligonucleotide inhibitor, danvatirsen (AZD9150), was tested in a phase I clinical trial of patients with advanced HCC (NCT01839604); 39 patients with HCC actually received the study agent in the escalation or expansion cohort, and only one patient in the escalation cohort had a partial response. The most common adverse events were transaminase elevation and thrombocytopenia.⁹⁶ In a recent phase Ib/2 study testing danvatirsen with or without durvalumab, an anti-PD-L1 antibody, in patients with head and neck squamous cell carcinoma (HNSCC), no responses were reported to monotherapy with danvatirsen; however, a relatively high RR of 23% was reported in the danvatirsen plus durvalumab combination arm.⁹⁷

Histone deacetylase (HDAC) inhibitors may suppress MDSC function by reducing ARG1, iNOS, and COX-2 levels.⁹⁸ Entinostat, a class I HDAC inhibitor, was demonstrated to inhibit the immunosuppressive function of both PMN-MDSCs and M-MDSCs in lung cancer and renal cancer mouse models. The antitumor effect of PD-1 blockade was also enhanced by adding entinostat *in vivo*.⁹⁸ Several clinical trials have tested various HDAC inhibitors in HCC.^{99,100} Although these agents were generally tolerated, their activities as single-agent appeared to be low because of low RRs and short PFS. Further, the effect of such therapy on MDSCs was not evaluated.

Phosphodiesterase-5 (PDE-5) inhibition downregulated ARG1 and iNOS activities in several preclinical models.^{101–103} Tadalafil, the FDA-approved PDE-5 inhibitor, has been tested in HCC mouse models; MDSC suppressor function was reversed and the antitumor effect of cytokine-induced killer-cell therapy was enhanced by the addition of tadalafil.¹⁰³ Tadalafil has been tested in clinical trials of patients with HNSCC and melanoma, but not in patient with HCC. The treatment was well tolerated, MDSCs were suppressed in circulation and tumor tissues, and T cell immunity was determined to be elevated.^{104–106}

Targeting MDSCs in the treatment of HCC: future perspectives

Ensuring that the targeting MDSCs is a clinically useful therapy is challenging because of the following reasons. First, MDSCs are a heterogeneous group of immature myeloid cells that require multiple markers to define and differentiate

their subtypes. In humans, no single-specific marker exists for defining MDSCs or their subtypes. This limitation makes direct demonstration of MDSCs in human HCC tumors and tracking their dynamic changes in humans cumbersome. Lack of specific makers also renders the development of “targeted therapy” for specifically targeting MDSCs difficult in humans. Second, although multiple therapeutic strategies focusing on depleting, inhibiting the trafficking, and down-regulating the immunosuppressive function of MDSCs have been proposed in preclinical models, most agents have exhibited multiple biological functions, thereby making the true contribution of targeting MDSCs to therapeutic effects less convincing. Third, the clinical data of potential MDSCs-targeting agents, revealed by preclinical studies, suggest that these strategies when administered alone are of limited efficacy against HCC.

Therefore, future studies should focus on identifying specific markers of and developing reliable assays for detecting human MDSCs and their subtypes. Specific markers will be invaluable for helping to develop more specific approaches for targeting MDSCs. Assays that could reliably detect MDSCs in both circulation and tissues, and in freshly prepared and archival samples, are of great importance for confirming the significance of MDSCs in patients with HCC who undergo therapeutic approaches. Furthermore, future studies should focus on developing combined approaches for treating HCC, especially those that incorporate MDSC-targeting therapy with ICIs or antiangiogenic agents, the two approved therapeutic strategies for treating HCC.

In conclusion, MDSCs play critical roles in promoting immunosuppression and angiogenesis, two major “cancer hallmarks” and two crucial therapeutic targets for HCC. The prognostic significance of MDSCs has been demonstrated in multiple clinical studies of patients with HCC. Thus, targeting MDSCs may be a potential therapeutic strategy for treating HCC. Although multiple preclinical studies have demonstrated the promising therapeutic efficacy of targeting MDSCs, additional well-designed clinical studies incorporating strong immunological, molecular, and biochemical research are warranted for the successful development of targeting MDSCs in the treatment of HCC.

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Disclosure

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