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REVIEW ARTICLE

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Potentials of C-C motif chemokine 2–C-C chemokine receptor type 2 blockers including propagermanium as anticancer agents

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Abstract

Inflammation plays an essential role in the development and progression of most cancers. Chemokine C-C motif chemokine 2 (CCL2) and its receptor C-C chemokine receptor type 2 (CCR2) constitute a key signaling axis in inflammation that has recently attracted much interest on the basis of evidence showing its association with cancer progression. Propagermanium (3-oxygermylpropionic acid polymer) is an organogermanium compound that is given for the treatment of hepatitis B in Japan and which inhibits the CCL2-CCR2 signaling pathway. Herein, we review the importance of the CCL2-CCR2 axis as a target in cancer treatment as shown by studies in mice and humans with pharmacological agents including propagermanium.

KEYWORDS
CCL2, CCR2, inflammation, metastasis, propagermanium

1 | INTRODUCTION

Inflammation is associated with the development of many diseases including cancer.[1,2] Under normal physiological conditions, the immune system eliminates pathogens and unwanted cells such as those that are damaged, senescent, or immature. However, cancer hijacks this system to ensure tumor survival and long-term growth. Cancer cells recruit immune cells such as macrophages, neutrophils, and MDSC to form a microenvironment known as a "niche."[3] Cancer cells and their niche cells produce various cytokines, chemokines, growth factors, ECM proteins, and proteases that promote tumor growth and metastasis.

C-C motif chemokine 2 (CCL2, also known as monocyte chemoattractant protein 1) and its receptor CCR2 have attracted much interest in recent years because of their relation to cancer progression.[4,5] Although CCL2 was first described as a cytokine with a physiological role in the regulation of inflammation,[6,7] more recent studies have shown a protumorigenic function of CCL2 in the promotion of cancer development and metastasis (Figure 1). Binding of CCL2 to CCR2, a G protein-coupled receptor, triggers intracellular signaling in cancer and other cell types. CCL2-CCR2 signaling promotes cancer progression by supporting cancer cell proliferation and survival, inducing cancer cell migration and invasion, and stimulating inflammation and angiogenesis.[8] CCL2 is secreted by many cell types including endothelial cells, fibroblasts, monocytes, and cancer cells, whereas CCR2 is expressed at a high level in inflammatory monocytes, dendritic cells, and natural killer cells as well as at a low level in neutrophils and T and B lymphocytes. In the early stages

Abbreviations: ALT, alanine aminotransferase; CCL2, C-C motif chemokine 2; CCR2, C-C chemokine receptor type 2; GeSP, germanium straight-chain polymer; HBV, hepatitis B virus; MDSC, myeloid-derived suppressor cell; Mo-MDSC, monocytic myeloid-derived suppressor cell; PDAC, pancreatic ductal adenocarcinoma; PGe, propagermanium; RGe, repagermanium; TAM, tumor-associated macrophage.

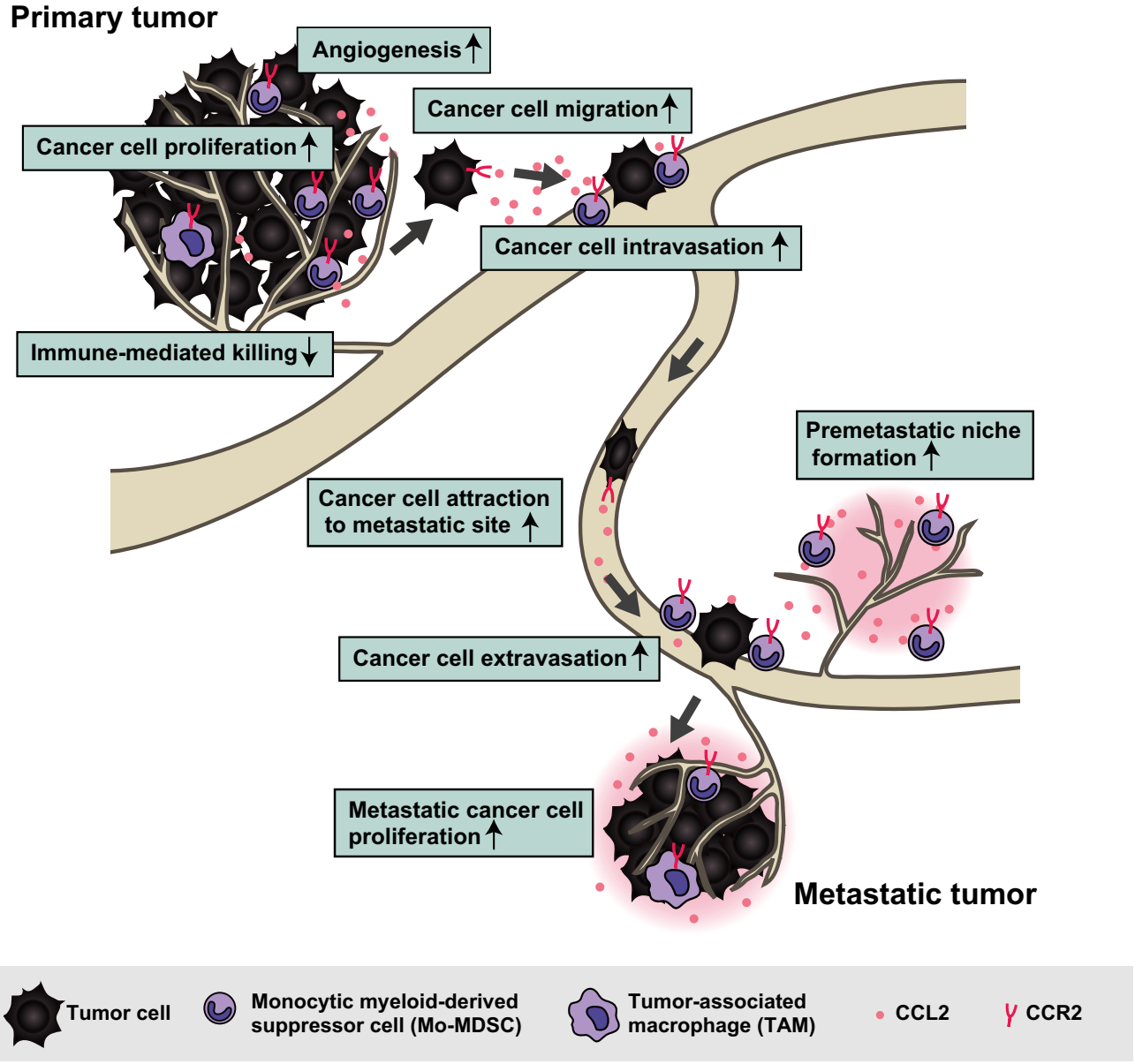


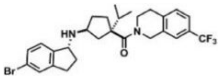
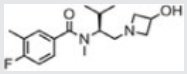
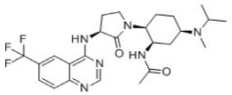
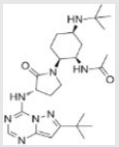
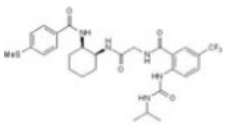
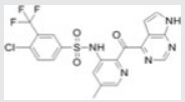
FIGURE 1 Role of chemokine C-C motif chemokine 2–C-C chemokine receptor type 2 (CCL2-CCR2) signaling in cancer progression. CCL2 is secreted by cancer cells and surrounding stromal cells. It induces tumor cell proliferation at the primary tumor site, and $CCR2^+$ myeloid cells attracted by CCL2 suppress immune-mediated killing of tumor cells. CCL2 also promotes tumor cell migration and invasion into the surrounding ECM followed by tumor cell intravasation into the circulation. The subsequent dissemination of cancer cells is directed by a chemotactic gradient of CCL2 toward potential sites of metastasis. CCL2 and $CCR2^+$ cells then promote tumor cell extravasation and colonization and growth at such metastatic sites

of metastasis, CCL2 guides tumor cell migration by interacting with CCR2 expressed on the surface of cancer cells.[9,10] CCL2 also induces expression of MMP2 and MMP9 in cancer cells, both of which facilitate cancer cell invasion,[11,12] and it promotes the intra- and extravasation of cancer cells by attracting TAM.[13] TAM and MDSC recruited by CCL2 trigger an angiogenic switch[14,15] and suppress immune-mediated attack of cancer cells.[16] In addition, CCL2 attracts cancer cells to future sites of metastasis and supports their proliferation and survival at such premetastatic niches.[17]

Increased levels of CCL2 in tumor cells or serum have been detected in individuals with melanoma or with breast, prostate, colorectal, gastric, or ovarian cancer, and they are frequently associated with disease progression, tumor grade, or metastasis, suggestive of the clinical importance of the CCL2-CCR2 signaling pathway.[18-27] These observations also suggest that targeting of the CCL2-CCR2 axis might be an effective strategy for cancer treatment.

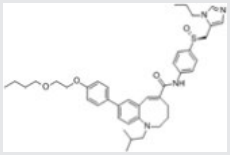
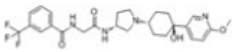
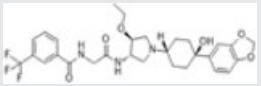
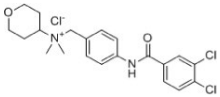
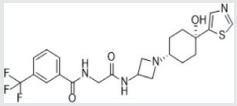
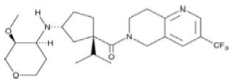
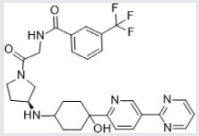
Herein, we review currently available agents that target the CCL2-CCR2 axis and studies of their efficacy for cancer treatment. Among such agents, we focus on an organogermanium compound, PGe (3-oxygermylpropionic acid polymer), that has been shown to act as a blocker of CCR2 signaling.

TABLE 1 Structures, IC_{50}, and clinical trials for C-C chemokine receptor type 2 blockers

Reagent	Structure	Originator	IC_{50} (in vitro binding)	IC_{50} (chemotaxis)	Clinical trial	ClinicalTrials.gov	Status
15a			50 nmol/L	–	–		
AZD2423		AstraZeneca	2.6 nmol/L	4.4 nmol/L	Chronic obstructive pulmonary disease (COPD)	NCT01153321	Completed (inhibition of)
					Chronic obstructive pulmonary disease (COPD)	NCT01215279	Completed (no effect)
					Post-traumatic neuralgia	NCT01200524	Completed (modest improvement)
					Painful diabetic polyneuropathy	NCT01201317	Completed (no effect)
BMS741672		Bristol-Myers Squibb	1.4 nmol/L	–	Type 2 diabetes mellitus	NCT00699790	Completed
					Neuropathic pain	NCT00683423	Completed
BMS813160		Bristol-Myers Squibb	6.2 nmol/L	0.8 nmol/L	Pancreatic ductal adenocarcinoma (PDAC)	NCT03496662	Recruiting
					Pancreatic ductal adenocarcinoma (PDAC)	NCT03767582	Ongoing
					Colorectal cancer and pancreatic cancer	NCT03184870	Recruiting
					Diabetic kidney disease	NCT01752985	Completed (no result published)
					Accelerated intimal hyperplasia	NCT01049165	Completed (no result published)
CAS 445479-97-0		Bristol-Myers Squibb	5.1 nmol/L	1 nmol/L	–		
CCX140-B		ChemoCentryx	17 nmol/L	8 nmol/L	Diabetic nephropathy	NCT01447147	Completed (modest improvement)
					Type 2 diabetes mellitus	NCT01440257	Completed (no result published)
					Type 2 diabetes mellitus	NCT01028963	Completed (no result published)
					Focal segmental glomerulosclerosis	NCT03536754	Recruiting

(Continues)

TABLE 1 (Continued)

Reagent	Structure	Originator	IC_{50} (in vitro binding)	IC_{50} (chemotaxis)	Clinical trial	ClinicalTrials.gov	Status
CCX872-B	Undisclosed	ChemoCentryx	3 nmol/L	32 nmol/L	Pancreatic adenocarcinoma	NCT02345408	Ongoing
Cenicriviroc		National Institute for Health Research; Takeda	5.9 nmol/L	–	Nonalcoholic steatohepatitis (NASH)	NCT02217475	Completed (no result published)
					HIV-1 infection	NCT01092104	Completed (no result published)
					Nonalcoholic steatohepatitis (NASH)	NCT03028740	Recruiting
					Nonalcoholic fatty liver disease (NAFLD)	NCT02330549	Completed (no result published)
					Primary sclerosing cholangitis	NCT02653625	Completed (modest improvement)
INCB3284		Incyte Corporation	3.7 nmol/L	4.7 nmol/L	–		
INCB3344		Incyte Corporation	5.1 nmol/L	3.8 nmol/L	–		
JNJ-17166864		Johnson & Johnson	–	–	Allergic rhinitis	NCT00604123	Completed (no result published)
JNJ-41443532		Johnson & Johnson	–	–	Type 2 diabetes mellitus	NCT01230749	Completed (modest improvement)
MK0812		Merck & Co.	3.2 nmol/L	8 nmol/L	Rheumatoid arthritis	NCT00542022	Completed (no result published)
					Multiple sclerosis	NCT00239655	Terminated
PF04136309		Pfizer	5.2 nmol/L	3.9 nmol/L	Metastatic pancreatic ductal adenocarcinoma	NCT02732938	Terminated
					Pancreatic adenocarcinoma	NCT01413022	Completed (no result published)
					Osteoarthritic pain	NCT00689273	Completed (no result published)
					Chronic hepatitis C infection	NCT01226797	Terminated

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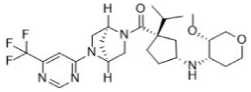
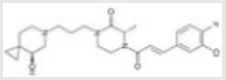
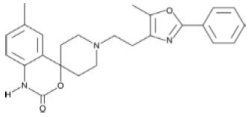
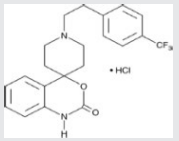
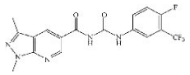
Reagent	Structure	Originator	IC_{50} (in vitro binding)	IC_{50} (chemotaxis)	Clinical trial	ClinicalTrials.gov	Status
PF04634817		Pfizer	3.68 nmol/L	–	Type 2 diabetes and overt nephropathy	NCT01712061	Completed (modest improvement)
					Renal insufficiency	NCT01791855	Completed (no result published)
					Diabetic macular edema	NCT01994291	Terminated
RO5234444		Roche	22 nmol/L	50.2 nmol/L	–		
RS504393		Roche	89 nmol/L	330 nmol/L	–		
RS102895		Roche	360 nmol/L	1700 nmol/L	–		
TLK19705		Telik, Inc.	–	700 nmol/L	–		

[a]'-' indicates that there is no data available.

2 | INHIBITORS OF THE CCL2-CCR2 AXIS

2.1 | Antibodies to CCL2 and CCR2

Experimental studies and clinical information have implicated the CCL2-CCR2 axis in tumor promotion and suggested that its inhibition might be of therapeutic value. One approach to inhibition of this axis is the administration of neutralizing antibodies to CCL2, which has been shown to suppress the growth of tumors formed by transplanted human prostate,[28,29] breast,[30] or pancreatic[31] cancer cells in mice as well as to attenuate macrophage infiltration in breast cancer. This strategy was also found to inhibit metastatic seeding in the lung and to prolong survival in mice with tumors formed by transplanted human breast cancer cells[32]or mouse melanoma cells.[33] Administration effects of antibodies to CCL2 were associated with inhibition of the recruitment to the tumors of $CCR2^+$ inflammatory monocytes.

Antibodies to human CCL2 have been evaluated for safety, pharmacokinetic-pharmacodynamic profile, and antitumor activity in cancer patients. Phase 1 clinical trials (NCT00537368 and NCT01204996) and a phase 2 study (NCT00992186) for a mAb to human CCL2 (CNTO888, carlumab)[34-36] showed that it induced a transient decrease in the concentration of CCL2 in serum, but that this decrease was followed by an increase to levels higher than pretreatment baseline values. Pharmacodynamics analysis indicated that, in contrast to its high affinity for CCL2 (dissociation constant of 15 pmol/L) in vitro, the affinity of CNTO888 for CCL2 was much higher (dissociation constant of 2.4 nmol/L) in patients, suggestive of a reduced capacity to inhibit CCL2 in vivo.[34]

Another phase 2 clinical trial (NCT01015560) with a humanized mAb to CCR2 (MLN1202, plozalizumab) was conducted with regard to treatment of bone metastasis of unspecified tumors.[37] MLN1202 was given to 44 patients with bone metastasis to evaluate its effects on tumor cell proliferation, monocyte-macrophage trafficking, and osteoclast maturation. Urinary concentration of N-telopeptide, a biomarker of bone turnover, was decreased in 14% of the treated patients, and serious adverse events developed in 7% of the patients. A phase 1 clinical trial (NCT02723006) of MLN1202 in combination with the immune-checkpoint inhibitor nivolumab for patients with advanced melanoma was terminated as a result of the emergence of serious adverse events. Together, these results suggest that antibodies to CCL2 and CCR2 have limited potential for cancer therapy.

2.2 | Small-molecule compounds

As an alternative to neutralizing antibodies, chemical agents have been shown to restrain cancer progression by inhibiting the CCL2-CCR2 axis. Treatment with one such small-molecule antagonist of CCR2 (PF-04136309) alone or in combination with gemcitabine slowed tumor progression and reduced the number of infiltrating TAM in mice subjected to orthotopic transplantation of established PDAC tumors.[38] Similar results were obtained when PF-04136309 was combined with FOLFIRINOX chemotherapy in mice.[39] PF-04136309 in combination with gemcitabine also completely inhibited metastasis of pancreatic cancer cells injected into mice through the tail vein.[40] In addition, PF-04136309 treatment significantly attenuated lung metastasis of lung squamous carcinomas cells injected i.v. in mice.[41] Giving CCX872-B, another small-molecule antagonist of CCR2, prolonged overall survival in a mouse model of breast cancer, although it neither extended tumor-free survival nor suppressed tumor growth.[42]

On the basis of the preclinical findings for the potential of PF-04136309 for treatment of pancreatic cancer, a phase 1 clinical trial (NCT01413022) was conducted for this agent in combination with FOLFIRINOX in patients with nonmetastatic PDAC.[43] Incidence of adverse events of grade 3 or higher in patients treated with FOLFIRINOX plus PF-04136309 was similar to that in those who received FOLFIRINOX alone. Treatment with PF-04136309 prevented the PDAC-mediated mobilization of bone marrow-derived $CCR2^+$ monocytes into the peripheral circulation, resulting in a decrease in the number of TAM. Approximately half of the patients treated with FOLFIRINOX plus PF-04136309 achieved an objective tumor response, suggesting that such therapy is both effective and tolerable. A phase 1b/2 clinical study of PF-04136309 in combination with nab-paclitaxel and gemcitabine in patients with metastatic PDAC (NCT02732938) was terminated as a business-related decision by Pfizer. Clinical trials of small-molecule CCR2 blockers for patients with other inflammatory diseases such as insulin resistance, multiple sclerosis, nonalcoholic steatohepatitis, and rheumatoid arthritis have also been conducted (Table 1).

3 | CHEMICAL AND PHARMACOLOGICAL PROPERTIES OF PROPAGERMANIUM

Organogermanium compounds manifest various biological actions including antibacterial and antioxidant effects, effects on the blood circulation system, and anticancer activities. PGe, with the formula $(C_3H_5GeO_{3.5})_n$, is the only approved medicine among such compounds. It was discovered in 1979 together with other organic germanium polymers, and it was approved for the treatment of chronic hepatitis B in Japan in 1994, having now been given to such patients for 25 years. PGe has the same essential formula as RGe (or Ge-132), poly-*trans*-[(2-carboxyethyl) germasesquioxane] or $(C_{18}H_{30}Ge_6O_{21})_n$, although the physicochemical properties of the two compounds are distinct.[44] PGe is thus more susceptible to hydrolysis by water than is RGe. At low concentrations, PGe and RGe are hydrolyzed to the same monomer species. At high concentrations, however, whereas RGe is hydrolyzed to the same monomer, PGe is hydrolyzed to GeSP (Figure 2).

PGe exerts immunomodulatory effects through interaction with glycosylphosphatidylinositol-anchored proteins associated with CCR2. It thus interrupts the CCL2-CCR2 signaling pathway and thereby suppresses the chemotaxis of monocytes-macrophages without disrupting the receptor-ligand interaction.[45] Inhibition of such signaling by treatment with PGe promotes retention of all leukocyte subsets—in particular, inflammatory monocytes—in bone

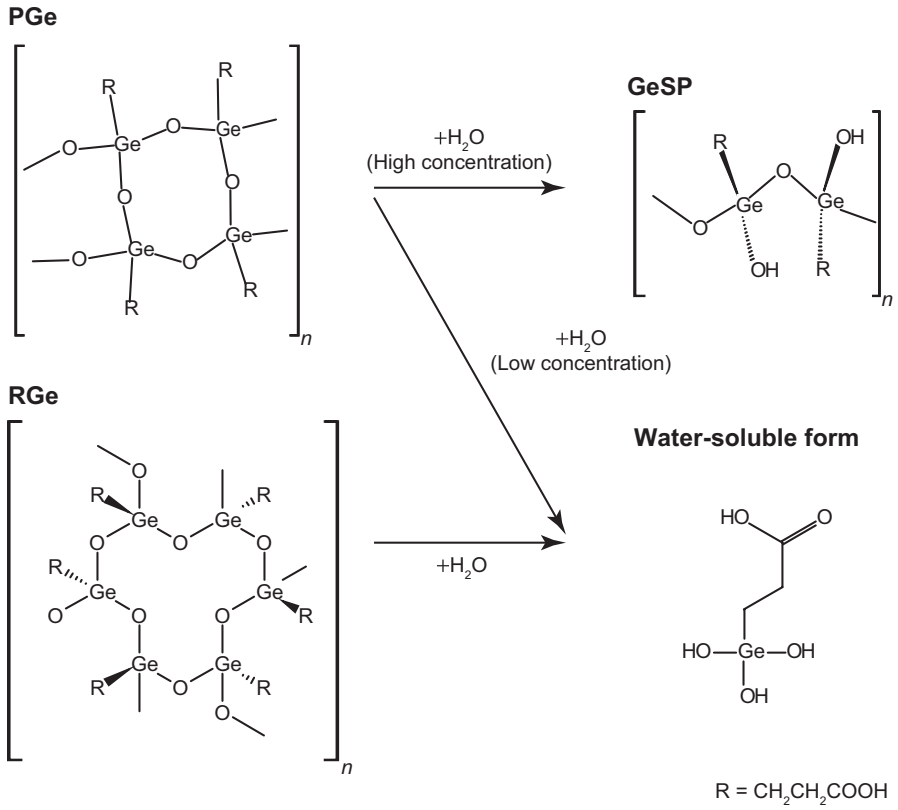


FIGURE 2 Solid and water-soluble forms of propagermanium (PGe) and repagermanium (RGe). GeSP, germanium straight-chain polymer

marrow, resulting in a reciprocal reduction in the number of these cells in peripheral blood and consistent with the suppressive effect of PGe on a range of chronic inflammatory conditions mediated primarily by inflammatory monocytes and macrophages.

A double-blind controlled trial of PGe was conducted with HBV antigen-positive chronic hepatitis in the late 1980s.[46] Titers of antibodies to HBV were significantly increased after treatment in the PGe group, whereas HBV antigen titers were significantly lower in the PGe group than in the placebo group. Serum HBV antigen levels and serum (ALT levels were significantly lower in the PGe group than in the control group at 12 and 16 weeks after treatment onset. PGe is also effective for the treatment of other types of liver injury in mice. Giving oral PGe thus inhibited the development of liver injury induced by injection of concanavalin A or lipopolysaccharide injection, *Corynebacterium parvum* infection or genetic deletion of fumarylacetoacetate hydrolase.[47-49] PGe also has therapeutic effects in other inflammatory diseases such as atherosclerosis,[50,51] fibrosis,[52-54] and obesity induced by a high-fat diet.[55-57]

4 | PROPAGERMANIUM FOR TREATMENT OF CANCER

Recent studies have shown that interference with key mediators of metastatic development is a promising alternative strategy for cancer treatment. The CCL2-CCR2 signaling pathway is an attractive therapeutic target in such an approach, given its key functions in metastasis described above. We previously showed that Fbxw7 (also known as Fbw7, Sel-10, hCdc4, or hAgo), a receptor protein of SCF (Skp1-Cul1-F-box protein)-type ubiquitin ligases, suppresses cancer metastasis by inhibition of CCL2-dependent inflammation.[58] A low level of Fbxw7 in tumor cells and peripheral blood is associated with poor prognosis in cancer patients. Moreover, mice in which *Fbxw7* is specifically ablated in bone marrow (*Fbxw7*$^{bm\Delta/\Delta}$ mice) manifest enhanced metastasis of melanoma, lung carcinoma, and breast adenocarcinoma. Serum level of CCL2 was also increased in *Fbxw7*$^{bm\Delta/\Delta}$ mice compared with control mice both before and after orthotopic transplantation of breast cancer cells. In addition, the numbers of Ly6C$^+$ Mo-MDSC and F4/80$^+$ macrophages were increased in peripheral blood and at sites of metastasis in *Fbxw7*$^{bm\Delta/\Delta}$ mice (Figure 3).

Giving PGe significantly attenuated metastasis of melanoma cells and breast cancer cells in *Fbxw7*$^{bm\Delta/\Delta}$ mice, with the size of metastatic nodules of breast adenocarcinoma in the lungs being reduced.[58] Such treatment also reduced the number of Ly6C$^+$ Mo-MDSC found in the lungs of *Fbxw7*$^{bm\Delta/\Delta}$ mice after transplantation of breast cancer cells (Figure 3).

Treatment with PGe in a mouse model of colon carcinogenesis reduced the number and size of tumors as well as the number of TAM, and it attenuated adenocarcinomatous changes in the colon tumors.[59] A phase 2 clinical trial (UMIN000017123) is underway to assess the efficacy of PGe in 15 patients with untreatable advanced or metastatic gastric cancer. Another study found that PGe treatment in 10 multiple myeloma patients resulted in complete remission in two patients, partial remission in two patients, stable disease in four patients, and progressive disease in two patients.[60] After discontinuation of PGe, the multiple myeloma progressed in two patients

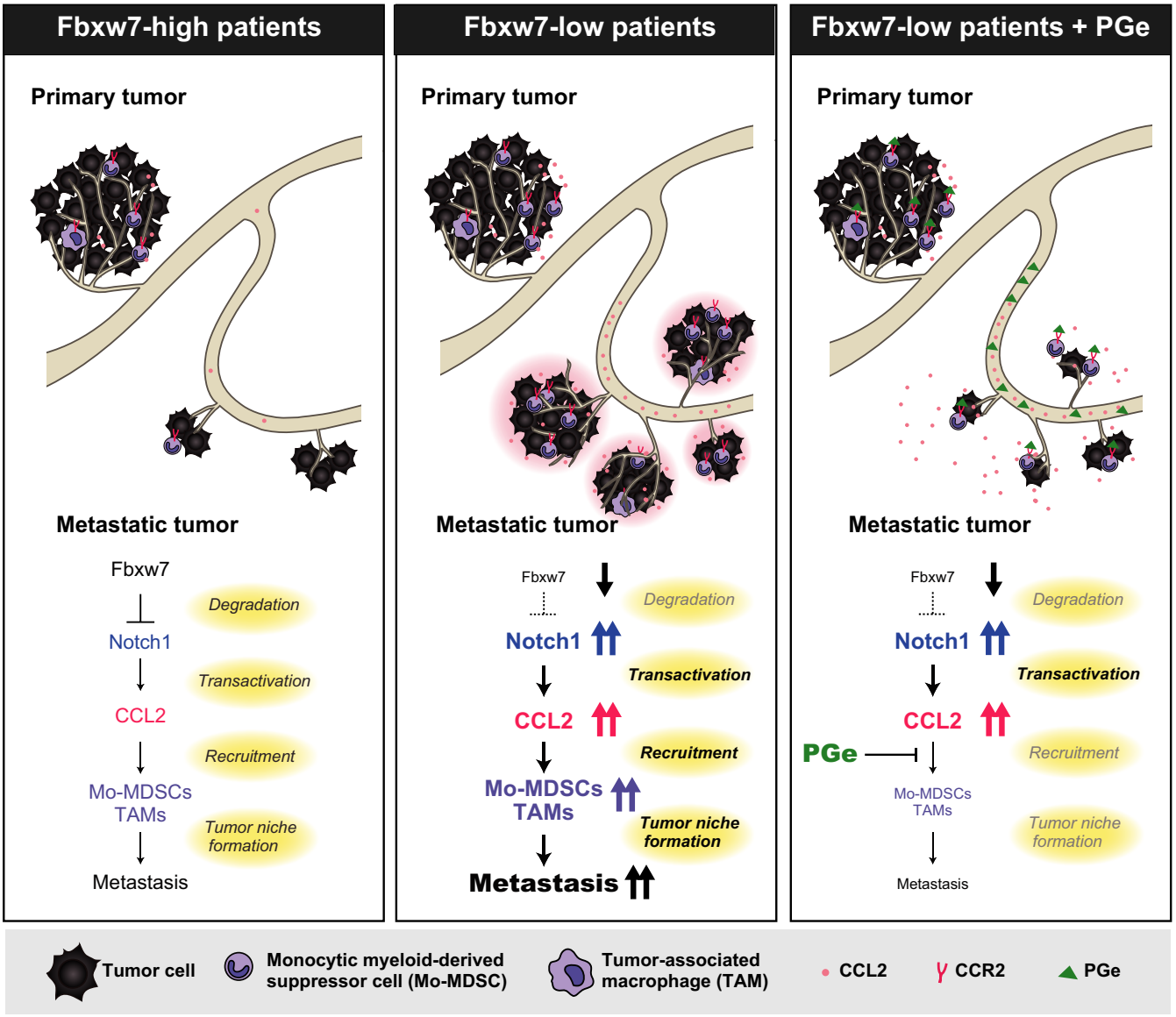


FIGURE 3 Model for the promotion of cancer metastasis by loss of Fbxw7 in the host environment and its suppression by treatment with propagermanium (PGe). Excessive signaling by Notch1 due to the impairment of its degradation as a result of Fbxw7 ablation gives rise to increased production of chemokine C-C motif chemokine 2 (CCL2). Consequent recruitment of monocytic myeloid-derived suppressor cells (Mo-MDSC) and tumor-associated macrophages (TAM) facilitates metastatic tumor growth. PGe suppresses CCL2-dependent recruitment of Mo-MDSC and TAM and thereby attenuates cancer metastasis. CCR2, C-C chemokine receptor type 2

who had achieved stable disease and in the two patients who had achieved partial remission. Phase 1 studies are also ongoing to evaluate the safety and effectiveness of PGe for patients with breast cancer (UMIN000022494), pancreatic cancer (UMIN000017715), and colorectal cancer (UMIN000022129).

5 | CONCLUSIONS

The CCL2-CCR2 signaling pathway plays a central role in inflammatory diseases including cancer metastasis. Treatment targeted to CCR2 such as that with PGe alleviates pathological phenotypes associated with these diseases. PGe has been approved for the treatment of hepatitis B in Japan, and its bioavailability and safety have been established. Drug repositioning, which aims to identify new indications for existing drugs, has been gaining popularity as an approach to drug discovery. PGe is thus a candidate for drug repositioning with regard to its suppressive effect on cancer metastasis. Further studies will determine whether this agent is truly effective for the treatment of cancer.

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DISCLOSURE

Authors declare no conflicts of interest for this article.

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