Review

Cancer Research

Recent Advances Targeting CCR5 for Cancer and Its Role in Immuno-Oncology

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Abstract

Experiments of nature have revealed the peculiar importance of the G-protein–coupled receptor, C-C chemokine receptor type 5 (CCR5), in human disease since ancient times. The resurgence of interest in heterotypic signals in the onset and progression of tumorigenesis has led to the current focus on CCR5 as an exciting new therapeutic target for metastatic cancer with clinical trials now targeting breast and colon cancer. The eutopic expression of CCR5 activates calcium signaling and thereby augments regulatory T cell (Treg) differentiation and migration to sites of inflammation. The misexpression of CCR5 in epithelial cells, induced upon oncogenic transformation, hijacks this migratory phenotype. CCR5 reexpression augments resistance to DNA-damaging agents and is sufficient to induce cancer metastasis and "stemness". Recent studies suggest important cross-talk between CCR5 signaling and immune checkpoint function. Because CCR5 on Tregs serves as the coreceptor for human immunodeficiency virus (HIV) entry, CCR5-targeted therapeutics used in HIV, [small molecules (maraviroc and vicriviroc) and a humanized mAb (leronlimab)], are now being repositioned in clinical trials as cancer therapeutics. As CCR5 is expressed on a broad array of tumors, the opportunity for therapeutic repositioning and the rationale for combination therapy approaches are reviewed herein.

C-C Chemokine Receptor Type 5 and Signal Transduction

CCR5 (C-C chemokine receptor type 5) is a seven transmembrane G-protein–coupled receptor (GPCR), that binds multiple ligands, CCL3 (MIP1α), CCL3L1, CCL4 (MP-1β), CCL5 (RANTES), CCL8 (MCP2), CCL11 (Eotaxin), CCL13 (MCP-4), and CCL16 (HCC-4; Fig. 1; ref. 1). Homeostatic or inflammatory chemokines, of which there are 48 in total, are low molecular weight (8–14 kDa) proteins, which are divided into four families, based on the location of the two cysteine residues located at the amino terminus (CXC, CC, XC, and CX3C; ref. 2). A total of 19 unique GPCRs interact with the 48 distinct chemokines. Upon binding of ligand, the cognate GPCR undergoes a conformational change, thereby dissociating the Gαi and the Gβγ subunits, inducing downstream signaling. Gβγ subunits activate phospholipase Cγ, to PIP2 and IP3, and a rapid increase in cytosolic Ca^{+2} or to diacylglycerol, inositol-1–4,5-triphosphate, protein kinase C, and inflammatory gene expression. Gαi activates adenyl cyclase. CCR5 activation of Ca^{+2} signaling and cellular migration is preserved in immune cells (3) and cancer cells (4, 5). Additional pathways induced by CCR5, include the PI-3′K pathway and thereby PDK1 and the serine/threonine kinase protein kinase B (AKT), which in turn induces cell survival, glycolysis, cell proliferation, growth and proliferation of progenitor and stem cells, immune cell differentiation, and the release of eIF4E to promote cap-dependent translation (Fig. 1A).

CCR5 mediates physiologic functions of immune cells [T cells, macrophages, eosinophils, myeloid-derived suppressor cells (MDSC), microglia, and dendritic cells; Fig. 1B]. Pathologic expression of CCR5 upon cellular transformation occurs in many types of cancer (Fig. 1C). CCR5 expression induced by transformation imbues the cell with dramatic alteration in gene expression, motility, and homing behavior to metastatic sites. A naturally occurring homozygous 32 bp deletion of the *CCR5* coding region (*CCR5Δ32*) occurs in the normal population. Individuals who carry *CCR5Δ32* are healthy but have an altered immune function when exposed to pathogens, specifically with increased resistance to human immunodeficiency virus (HIV; ref. 6, 7), poxvirus (8), and the *Staphylococcus aureus* pore-forming leukotoxin ED (9). CCR5 is an essential coreceptor for HIV, and has been strongly implicated in cancer, in particular metastatic cancer, precancerous diseases [nonalcoholic steatohepatitis (NASH)], and cancer therapy–related disease (bone marrow transplant–related GvHD). Because *CCR5Δ32* individuals are physiologically normal, whereas cancer cells selectively overexpress CCR5, recent interest has focused on targeting CCR5 to restrain cancer metastasis.

CCR5 Antagonists Retasked in Cancer

Several CCR5 antagonists developed for HIV treatment are being retasked for cancer and cancer-related diseases. The pyrimidine small-molecule CCR5 inhibitors, maraviroc and vicriviroc,

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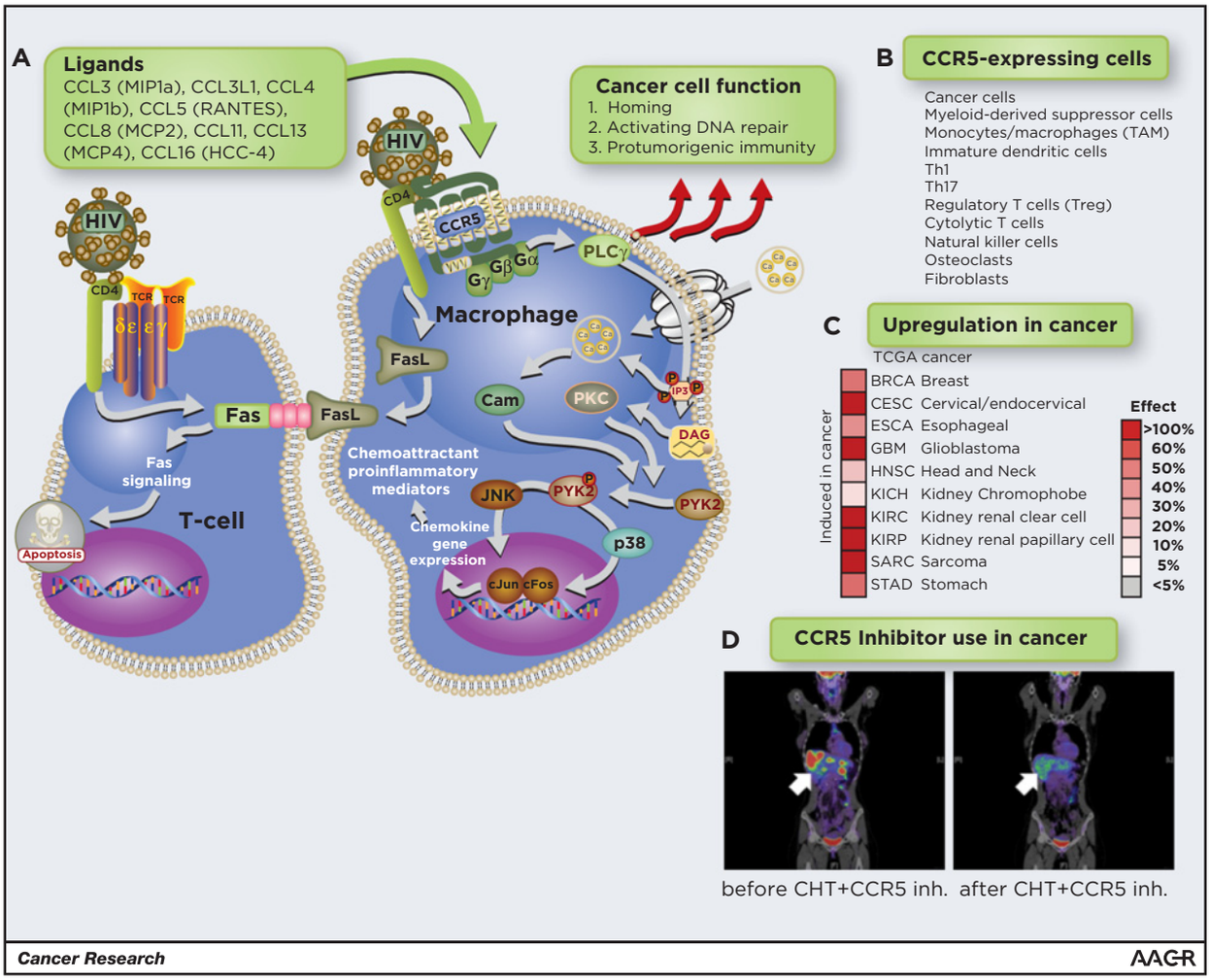

Figure 1.

CCR5 signaling in immune and cancer cells. **A,** Schematic representation of a T-cell–expressing CCR5 with the intracellular signaling cascade activated by cognate ligands. The diverse ligands for CCR5 are shown in green. The pathologic response induced by CCR5 on cancer cells is shown in the green box. **B,** The diverse types of cells expressing CCR5 are shown. **C,** CCR5 expression derived from The Cancer Genome Atlas (TCGA), with squares indicating upregulation in cancer versus normal tissue, shown as a calorimetric display of fold increase in expression as a HR. RNA-seq TCGA data (v2 RSEM values) were downloaded using Firebrowse and FPKM values were $\log_2$-scaled and quantile normalized (mean differences between cancer and normal tissues were calculated). **D,** Representative PET-MRI images from a patient receiving chemotherapy (CHT) after participation in the phase I pilot MARACON study, in which patients with advanced-stage mCRC who were refractory to standard chemotherapy were treated with maraviroc (26). White arrow, liver with metastatic lesions. Red spots, high glucose uptake typical for metastases; green, low background glucose uptake. Adapted from Cancer Cell, Vol. 29, 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, p. 587-601, 2016, with permission from Elsevier.

and the humanized monoclonal anti-CCR5 antibody, leronlimab, have been used in HIV. Maraviroc and leronlimab achieved their primary endpoints in phase III HIV clinical trials (10–12). Leronlimab has been used in more than 760 patients with HIV, without serious adverse events related to the agent and achieved its primary efficacy endpoints in a phase III (pivotal) study (11, 13). TAK-779 is a quaternary ammonium derivative that reduced regulatory T cell (Treg) infiltration and tumor growth in a pancreatic cancer mouse model (14). Anibamine is a natural product CCR5 antagonist that reduced prostate cancer cell growth, adhesion, and invasion (15). Met-CCL5 is a competitive chemokine receptor blocker that decreased breast tumor growth and infiltrating macrophages in murine cancer models (16). Aplaviroc, a 2,5-diketopiperazine CCR5 entry inhibitor, was discontinued because of hepatotoxicity (17). A saponin, DT-13, reduced CCR5 expression, and thereby reduced cancer cell migration (18). Other approaches to reduce CCR5 include siRNA (19) and a zinc finger nuclease (20). OTR4120 and OTR4131, GAG mimetics that inhibit CCL5 binding to GAG, reduced CCL5-induced migration and invasion of hepatocellular carcinoma cells (21). INCB9471 (Incyte Corporation) was discontinued after a phase II trial for HIV (22). Cinicriviroc (TBR-652; Takeda) is a dual CCR2-CCR5 inhibitor that completed a phase IIb clinical trial for HIV and is currently being tested in NASH. In addition, a soluble receptor–based fusion protein, mCCR5-Ig, inhibits CCR5 (23).

CCR5 is overexpressed in breast cancer (4, 5), gastric adenocarcinoma (24), prostate cancer (25), colorectal carcinoma (26, 27), melanoma (28), Hodgkin lymphoma (29), head and neck cancer (30), gastric cancer (31), esophageal cancer (32), pancreatic cancer (33), acute lymphocytic leukemia (33, 34), and other tumors (Fig. 1B). In analysis of >2,200 patients with breast cancer, >50% of patient's tumors were $CCR5^+$ and >95% of triple-negative breast cancer (TNBC) were $CCR5^+$ (4). Higher cytoplasmic CCR5 staining correlated with poor prognosis (5). CCR5 is induced by oncogenic transformation (Ha-Ras, c-Myc, ErbB2, and c-Src; ref. 4), DNA damage (5), and CCL5 stimulation. CCR5 receptor levels correlate with poor prognosis in breast cancer and gastric adenocarcinoma (5, 23, 24). Although CCR5 binds many ligands that are overexpressed in cancer, elevated levels of the ligand CCL5 indicate poor prognosis in breast cancer (35, 36), cervical cancer (36), prostate cancer (37), ovarian cancer (38), gastric cancer (23, 39), metastatic colorectal carcinoma response to regorafenib (40), and pancreatic cancer (33). Elevated level of CCL5 in tissues or plasma is indicative of unfavorable outcome in patients with melanoma, breast, cervical, prostate, gastric, or even pancreatic cancer (41–43).

CCR5 Induces the Hallmarks of Cancer

CCR5 induces cancer cell homing to metastatic sites (4, 34), augments the proinflammatory prometastatic immune phenotype (26), and enhances DNA repair (5), providing aberrant cell survival and resistance to DNA-damaging agents (see review in refs. 2 and 44).

Activating invasion and metastasis

Distinct dissociable mechanisms govern tumor invasion and metastasis (45, 46). Ectopic CCR5 expression within cancer epithelial cells is sufficient to drive cancer cell metastasis (4). CCR5-specific small-molecule inhibitors blocked metastasis of isogenic oncogene-transformed breast cancer cells in NOD/SCID mice (4) and prostate cancer metastasis in immunocompetent mice (25). CCR5 induced metastasis in $p53^-$ breast cancer cells *in vivo* (5). In one study, CCR5 siRNA did not reduce the metastatic phenotype of MDA-MB-231 cells in the absence of additional MDSC (47), however it must be noted that endothelial cells produce CCL5, and were shown to augmented breast cancer metastasis in another study (48).

Avoiding immune destruction

The antitumor immune response. The ligands for CCR5 are induced in tumors, and CCR5 participates in promoting protumorigenic and prometastatic inflammation through mechanisms that are distinct from the canonical immune checkpoint (49, 50). Furthermore, there is plausible evidence for potential synergy between CCR5 inhibitors and the canonical immune checkpoint inhibitors, consistent with the current clinical trials of Pfizer and Merck, in which CCR5 inhibitors (maraviroc or vicriviroc) are combined with a checkpoint inhibitor (pembrolizumab; below). The recruitment of immune cells, including tumor-infiltrating lymphocytes (TIL), MDSCs, tumor-associated macrophages (TAM), innate lymphoid cells, Tregs (51), mesenchymal stem cells (MSC), and immature dendritic cells (DC), contributes to tumor-induced immunosuppression (52). Tumors evade immune destruction by actively inducing immune tolerance through the recruitment of $CD4^+CD25^+Foxp3^+$ Tregs.

Many of these cell types express CCR5 and/or produce ligands for CCR5 (Fig. 1). For example, MSCs produce CCL3, CCCL4, and CCL5 and when mixed with either breast (47), or colon cancer cells (53), they promoted tumor metastasis. Maraviroc can reduce MDSC-induced colon cancer metastasis (53). Furthermore, $CD4^+Foxp3^+$ Tregs preferentially express CCR5 when compared with $CD4^+Foxp3^-$ effector T cells, and inhibition (by TAK-779), reduced Treg migration to tumors, and reduced pancreatic tumor size (14).

Lack of CCR5 ligands is associated with reduced infiltration of antigen-specific T cells and associated metastasis (54). Tumor-derived CCL5 has also been shown to impede antitumor T-cell responses and heighten the progression of murine mammary carcinoma (55), possibly via TGFβ (56). CCR5 is part of a CCL3-CCR5/CCR1–mediated DC migration to lymph nodes and the tumor microenvironment (TME). When $CD4^+$ T cells interact with DCs, CCL3 and CCL4 are released, which can guide CCR5-positive naïve $CD8^+$ T cells into tissues for activation (57). CCR5 and its ligands promote the proliferation of $CCR5^+$ polymorphonuclear (PMN)-MDSCs in the bone marrow and, later, potentiate their tumor immunosuppressive activities at the tumor site in part by inducing arginase-1. CCR5 directs the mobilization of $CD11b^+Gr1^+Ly6C^{low}$ PMN myeloid cells from the bone marrow to promote tumor development (49). Both MDSCs subtypes ($CD11b^+Ly6G^-Ly6C^{hi}$ monocytic MDSCs and $CD11b^+Ly6G^+Ly6C^{low}$ PMN-MDSCs support tumor growth and suppress antitumor immunity; ref. 49). In mice CCR5 blockade with anti-CCR5 antibody inhibited B16 melanoma growth and MDSC accumulation in tumor tissues. CCL8, an endogenous ligand of CCR5, is produced by $F4/80^+$ macrophages in the lungs of mice with metastatic primary tumors (58). Migration of Tregs toward CCL8 *ex vivo* was reduced in the presence of the CCR5 inhibitor, maraviroc. Importantly, treatment of mice with maraviroc reduced the level of $CCR5^+$ Tregs and metastatic tumor burden in the lungs (58).

TAMs express CCR5 and are comprised of an M1 to M2 spectrum of macrophages expressing variable levels of arginase, IL4, IL10, and IL13. $F4/80^+$ macrophages are well known participants in the onset and progression of mammary tumors in murine models and strongly implicated in human cancer progression (59). Ligands from the tissue microenvironment including RANTES, recruit TAMs to the TME (60). CCL3, which binds CCR5 and CCR1, promotes tumorigenesis through recruitment of protumor macrophages into the TME (61). Genetic deletion of *Ccl3* in macrophages reduced the number of lung metastasis, whereas adoptive transfer of wild-type inflammatory monocytes increased the number of lung metastasis in *Ccl3*-deficient mice (61). Additional ligands, including EGF, CSF1, HGF, CCL2, CXCR4/CXCl12, and Tie2, also participate in the local TME to recruit inflammatory cells, which collectively contribute to the diversity of inflammatory subtypes seen within the TME necessary for tumor progression (62). Importantly in this regard, single-cell sequencing assessing the expansion of immune cell phenotypes and diversity of cell states within the TME evidenced CCR5 as one of the top genes correlated with activation of this variance in the breast cancer TME (63).

In addition to augmenting the noncanonical tumor-promoting immune responses, several lines of evidence suggest CCR5 and its ligands appear to participate in the canonical immune checkpoint response. The programmed cell death protein 1 (also known as CD279 and PD-1) and its ligand PD-1 ligand (PD-L1) signaling

pathway is a critical immune checkpoint. PD-1 signaling is an important mechanism by which tumors escape antitumor immune responses. TILs are an important biomarker for predicting responses to PD-L1 blockade therapy. Analysis of responses to CTLA-4 and PD-1 antagonists revealed that tumors responsive to these immunotherapies tend to be infiltrated with T cells, referred to as a "T-cell–inflamed" TME (64–66). CCL5 was upregulated in PD-L1–positive melanoma tumors along with IFNγ and several IFNγ-regulated genes (67, 68). Tumor mutational burden and a T-cell–inflamed gene expression profile were independently predictive of response to the PD-1 antibody, pembrolizumab (69), and high levels of the CCR5 ligands, CCL3 and CCL4, in pretreatment tumor specimens were associated with worse patient overall survival after anti-CTLA4 and carboplatin/paclitaxel treatment in melanoma (70).

In addition, a role for MDSC has been described. $CCR5^{high}$ MDSCs have a higher immunosuppressive activity than $CCR5^{low}$ MDSCs (71), and disruption of MDSC trafficking enhances anti-PD1 therapy (72). As noted above, CCL5 promotes influx of $CD8^+$ T cells (54) and PD-L1 expression is often associated with increased TILs. In this regard the Keynote-028 study showed that the patients with tumors with high PD-L1 expression, high expression of T-cell–inflamed genes, and high tumor mutational burden were associated with high benefit from pembrolizumab treatment across several different tumor types (73). Collectively these studies suggest that the CCR5 noncanonical immune checkpoint may intersect the canonical immune checkpoint pathway.

Induction of proliferative signaling, angiogenesis, and resistance to cell death. CCR5 participates in angiogenesis (74, 75) and resistance to cell death (5). The requirement for CCR5 in oncogene-induced cellular proliferation was supported by elegant transgenic studies, in which MMTV-PyMT (mouse mammary tumor virus polyomavirus middle T-antigen)-induced mammary tumors were reduced in $ccr5^{-/-}$ mice (MMTV-PyMT; ref. 76). CCL5 exerts proangiogenic effects by promoting endothelial cell migration, spreading, neovessel formation, and VEGF secretion. Moreover, tumor cells, upon CCL5 stimulation, produce VEGF and by secreting CCL5 recruit CCR5-expressing TAMs (16, 77). CCR5 inhibitors also reduced lymphangiogenesis (78, 79).

Deregulated cellular energetics and cancer stem cells. Tumor cells require higher rates of glucose and catabolite uptake, transfer, and utilization (80) and CCR5-induced Akt phosphorylation, stimulating glucose uptake, glycolysis, the pentose phosphate pathway, fatty acid synthesis, and glutamine metabolism (2, 81). Single-cell analysis of breast cancer cells revealed that CCR5 governs dramatic (>1,000-fold) activation of RNA abundance for PI3K/Akt, ribosomal biogenesis, and cell survival signaling pathways (5). $CCR5^+$ breast cancer epithelial cells showed features of cancer stem cells forming mammospheres and initiated tumors with >60-fold greater efficiency in mice (5).

Preclinical Studies of CCR5 Inhibitors in Metastatic Cancer

The CCR5 antagonists maraviroc and vicriviroc and leronlimab blocked metastasis of human breast cancer xenografts (MDA-MB-231 cells) in immunodeficient mice via the inhibition of homing, and enhanced cell killing by DNA-damaging chemotherapeutic agents (4, 5, 82). Targeting CCL5 in the bone marrow via nanoparticle-delivered expression silencing, in combination with maraviroc, augmented antitumor immunity (83). Maraviroc and vicriviroc reduced prostate cancer cell metastasis to the bones, brain, and viscera in immunocompetent mice (25). Maraviroc reduced the growth of orthotopically injected colon cancer cells in part via limiting cancer-associated fibroblast accumulation (84). In mice, an anti-CCR5 antibody inhibited B16 melanoma growth and MDSC accumulation in tumor tissues (85). TAK-779 reduced pancreatic cancer cell growth and metastasis with reduced migration of Tregs into the tumors (14). Chemokines participate in the development of NASH, which in turn may progress to hepatocellular cancer (86). Maraviroc reduced lipogenesis, insulin resistance, and β-oxidation in NASH, reduced steatosis and improved the NASH score in high-fat diet–induced NASH (87), and ameliorated the development of hepatocellular carcinoma in a murine model (88).

Growth of acute lymphoblastic leukemia cells and lymphoma was reduced by maraviroc (34). Deadly hematologic malignancies may also be treated by bone marrow transplantation. Chronic GvHD (cGvHD), which is often preceded by acute GvHD (aGvHD), continues to be a significant cause of morbidity and mortality, affecting an estimated 50% of allogeneic hematopoietic stem cell transplantation (HSCT) patients (89). $CCR5^+$ CD146-expressing CD4 T cells (both conventional Tcon and Treg subsets) are increased in patients with cGvHD, express greater levels of T-bet and IFNγ, and contribute to cGvHD (90). Leronlimab, reduced aGvHD in a dose-response fashion in a xenogeneic mouse model of aGvHD (NOD-scid $IL2Ry^{null}$ mice transplanted with human bone marrow stem cells) without significantly altering engraftment (91).

Clinical Studies

In the phase I pilot MARACON study, patients with advanced stage metastatic colorectal cancer who were refractory to standard chemotherapy (26), were treated with maraviroc. All tumor samples showed reduced proliferation by Ki-67. CCR5 inhibition correlated with an anti-tumoral macrophage-polarized M1 morphology. T cells at the invasive margins of human colorectal cancer liver metastases produced CCL5, which reprogrammed immunosuppressive TAMs toward a protumorigenic phenotype. An inverse correlation was found between "immune CCR5" levels and the maturation status of tumor-infiltrating neutrophils as well as 5-year survival rates (83). From the 11 patients of the core cohort, 5 were reexposed to chemotherapy, and 3 of the 5 patients had objective partial responses comparing favorably with the historic objective response rates in patients with mCRC, on or after the third-line of chemotherapy, of around 5%–10%. A representative PET-MRI image, from a patient with advanced-stage mCRC who was refractory to standard chemotherapy, clearly showed tumor shrinkage after maraviroc treatment (Fig. 1D; ref. 26).

Three additional studies targeting CCR5 for metastatic cancer have been approved by the FDA. Each study combines a drug and a biologic for $CCR5^+$ metastatic cancer. The first is a phase I study of pembrolizumab with maraviroc in patients with refractory microsatellite stable (MSS)-colorectal cancer. The second, a phase II study is assessing safety and efficacy of vicriviroc in combination

Table 1. Active clinical trials using CCR5 inhibitors

CCR5 antagonist	Mechanism	Administration	Reference
Maraviroc UK-427857 (Pfizer) approved for HIV 2007		Maraviroc (days 1–21 of each cycle) + pembrolizumab (day 1, day 22)	(i) Phase I study of pembrolizumab with maraviroc in patients with refractory MSS colorectal cancer. NCT03274804. (ii) GVHD, phase I NCT00948753. Phase II NCT02167451.
Vicriviroc CH 417690 (Merck)	Pyrimidine CCR5 inhibitor	Vicriviroc + pembrolizumab	(i) Phase II, vicriviroc in combination with pembrolizumab (MK-3475) in patients with advanced metastatic MSS colorectal cancer. NCT03631407.
Leronlimab (pro-140; CytoDyn)	Humanized mAb	Weekly self-injection	(i) Phase II study for $CCR5^+$ TNBC using carboplatin and leronlimab NCT03838367. (ii) Phase II GVHD NCT02737306.

with pembrolizumab (MK-3475) in patients with advanced metastatic MSS-colorectal cancer. The third is a phase Ib/II study for $CCR5^+$ metastatic TNBC using carboplatin and leronlimab. The study is evaluating the impact on progression-free survival with secondary objectives to assess the overall response rate, the number of circulating tumor cells, and assess benefit based on time to new metastasis.

In studies of hepatocellular cancer prevention targeting NASH, liver fibrosis improved after 1 year of therapy with cenicriviroc, leading to the implementation of a phase III trial (AURORA; ref. 92). Tropifexor (LJN452) and cenicriviroc are being assessed for safety, tolerability, and efficacy in patients with NASH and liver fibrosis (TANDEM; NCT03517540).

Valuable progress has been made with CCR5 inhibitors in treatment of bone marrow transplant–related GvHD. In a trial of reduced intensity allo-HSCT with standard GvHD prophylaxis plus maraviroc compared with a contemporary control cohort receiving standard GvHD prophylaxis alone (NCT01785810), maraviroc treatment was associated with a lower incidence of aGvHD without increased risk of disease relapse (93), extending earlier studies with maraviroc suggesting a role for CCR5 in aGvHD. Maraviroc is also being assessed for GvHD prophylaxis in pediatric and adult stem cell transplant recipients (NCT02167451). Leronlimab has been deployed in a clinical trial of GvHD because of the dramatic reduction of aGvHD in a murine model (91). An open-label, single-arm, phase II multicenter study of the safety and efficacy of leronlimab (Pro-140) for prophylaxis of aGvHD in patients undergoing reduced intensity conditioning allogeneic stem cell transplantation was initiated (NCT02737306).

The retasking of CCR5 inhibitors for cancer prevention and treatment of metastatic cancer leverages the substantial prior clinical experience with these compounds and their known safety profiles in patients with HIV. Several additional agents, when combined with CCR5 inhibitors have shown promise in preclinical studies including an anti-IL6 receptor antibody for TNBC and PD-L1 inhibitors in gastric and colon cancer. The finding that CCR5 inhibitors enhance cancer cell killing mediated by radiation and DNA-damaging chemotherapeutic agents (5) suggests the potential for combining these biological agents with chemotherapeutics to potentially reduce the dose-dependent side effects of chemotherapy.

Disclosure of Potential Conflicts of Interest

X. Jiao is the principal investigator and reports receiving a commercial research grant from CytoDyn. N. Halama reports receiving a commercial research grant from Bristol-Myers Squibb, Ono Pharma, and NOXXON Pharma, has ownership interest (including stock, patents, etc.) in CCR5 Inhibitor IP owned by University Clinic Heidelberg, and is a consultant/advisory board member for Merck Serono. D. Jaeger reports having ownership of relevant IP for CCR5 Inhibition of University of Heidelberg. R.G. Pestell is chief medical officer at, reports receiving commercial research grants from, has ownership interest (including stock, patents, etc.) in, and is a consultant/advisory board member for CytoDyn. No potential conflicts of interest were disclosed by the other authors.

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