CCR5-001, a novel Drug Fc-Conjugate (DFC) targeting CCR5, demonstrates potent efficacy in a colorectal cancer mouse model

Simon Döhrmann*, Amanda Almaguer, Nicholas Dedeic, Doug Zuill, Elizabeth Abelovski, Joanne Fortier, Qiping Zhao, Maria Hernandez, Karin Amundson, Madison Moniz, Hongyuan Chen, Dhanya Panickar, Thanh Lam, Grayson Hough, Thomas P. Brady, Allen Borchardt, Jeffrey B. Locke, James Levin, Jason N. Cole, and Leslie W. Tari.
Cidara Therapeutics, Inc., 6310 Nancy Ridge Drive, Suite 101, San Diego, CA, USA

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BACKGROUND

The approval of immune checkpoint inhibitors (ICI) such as PD-(L)1 inhibitors has revolutionized cancer therapy and established a role for immune effector cells, most notably CD8$^+$ T cells, in tumor control and destruction. While anti-PD-(L)1 therapy has demonstrated durable responses, only a small subset of patients benefit and respond to therapy. Chemokine receptor 5 (CCR5) is expressed on multiple immune cells (e.g. T cells, NK cells and myeloid cells) and CCR5-dependent migration of these cells contribute to an immune-suppressive tumor microenvironment (TME) thereby limiting response to ICI therapy. In addition, expression of CCR5 on cancer cells has been linked to increased metastasis and resistance to ICI therapy. Upregulation of CCL5/CCR5 has been associated with poor outcomes in multiple solid tumor indications, including colorectal cancer (CRC). Therefore, targeting CCR5 has the potential to increase response rates to ICI therapy and improve outcomes. Herein, we describe a first-in-class CCR5 targeting DFC, CCR5-001, a multivalent conjugate of a potent, small molecule CCR5 antagonist conjugated to an immune-silent proprietary human IgG1 Fc (**Fig. 1**). This CCR5 targeting DFC has the potential to improve responses to ICI therapy by preventing pro-tumorigenic reprogramming of the TME induced by CCL5/CCR5 expressing tumor and myeloid cells.

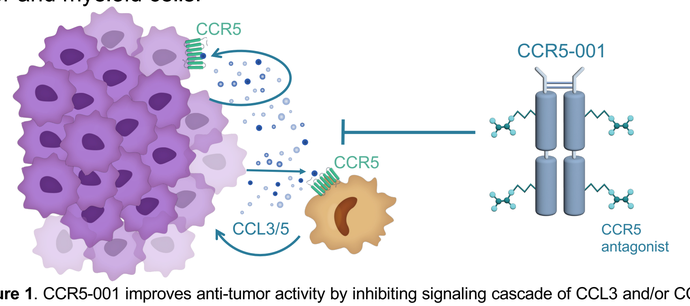

Figure 1. CCR5-001 improves anti-tumor activity by inhibiting signaling cascade of CCL3 and/or CCL5, the chemokine ligands of CCR5, immuno-suppressive CCR5$^+$ immune cell infiltration ('recruitment') and the autocrine effects of CCR5$^+$ cancer cells ('metastasis and resistance').

METHODS

Binding of CCR5-001 to recombinant, human CCR5 was measured by surface plasmon resonance (SPR), by ELISA or to CCR5$^+$ PathHunter cells (DiscoverX) by flow cytometry. Functional activity was determined in CCR5$^+$ PathHunter β-arrestin assay with CCL3 to induce CCR5 signaling. CCR5 expression on monocytes, competitive binding with labeled CCL5 to CCR5 and chemotaxis across 5 µm transwell was determined by flow cytometry. Efficacy was determined in a syngeneic MC38 model in C57BL/6 mice. CCR5-001 was dosed at 20 mg/kg twice per week for two weeks starting 5 days post cancer cell injection. Tumor volumes were measured and statistical analysis was conducted by two-way ANOVA in Prism (GraphPad) software.

RESULTS

CCR5 is a G protein-coupled receptor with two biological functions (a) chemokine ligand scavenging and (b) migration along a chemokine gradient[1]. Upon binding to the cognate chemokine ligands, CCL5 (RANTES) or CCL3 (MIP-1α), CCR5 undergoes a conformational change that triggers a downstream signaling cascade involving G protein coupled reactions and β-arrestin recruitment. The only approved CCR5 inhibitor is the small molecule antagonist, maraviroc, developed for the treatment of HIV.

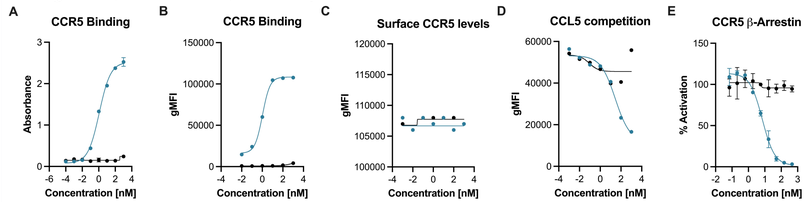

Figure 2. Binding to (**A**) recombinant, human CCR5 by ELISA or (**B**) engineered CCR5$^+$ cells as determined by flow cytometry. Effect of CCR5-001 (blue) or unconjugated Fc (black) on (**C**) CCR5 surface expression or (**D**) competition with 100 nM labeled CCL5 and (**E**) functional activity in a β-arrestin assay.

CCR5-001 is a proof-of-concept DFC molecule developed for targeting CCR5 in cancer. CCR5-001 displayed a K_D of 4.2 nM to immobilized human CCR5 by SPR. Similarly, CCR5-001 demonstrated high affinity to plate-bound human CCR5 with an IC_{50} of 1.16 nM by ELISA (**Fig. 2A**) and to engineered CCR5$^+$ expressing cells with an IC_{50} of 0.63-0.98 nM as determined by flow cytometry (**Fig. 2B**). CCR5-001 did not impact the CCR5 surface expression on engineered cells (**Fig. 2C**), but outcompeted binding of 100 nM CCL5 to CCR5 after 1 h incubation at 37ºC (**Fig. 2D**). This high affinity binding translated to potent functional inhibition of CCL3-induced, CCR5-dependent β-arrestin recruitment with an IC_{50} of 7.04 nM (**Fig. 2E**). CCR5 is expressed on CD14$^+$ monocytes and CD4$^+$ and CD8$^+$ T cells within human PBMCs as visualized by tSNE analysis (**Fig. 3A, B**). In agreement with the CCR5 receptor expression on PBMCs, labeled CCL5 bound predominantly to CD14$^+$ monocytes and to a lesser extent CD4$^+$ and CD8$^+$ T cells (**Fig. 3C)**. Functionally, CCR5-001 outcompeted binding of labeled CCL5 to CCR5 (**Fig. 3D**). CCR5-001 also prevented the CCL5-dependent internalization of surface CCR5 expression by 100 nM CCL5 as compared to the no CCL5 control (**Fig. 3E**). CCR5-001 inhibited chemotaxis of human monocytes induced at 3 h time point (**Fig. 3F**).

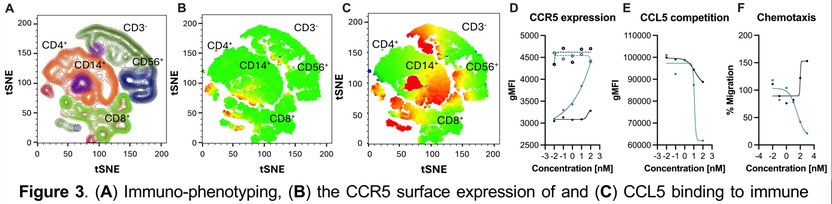

Figure 3. (**A**) Immuno-phenotyping, (**B**) the CCR5 surface expression of and (**C**) CCL5 binding to immune cell subsets within human PBMCs by tSNE analysis. The effect of CCR5-001 (blue) or unconjugated Fc (black) on (**D**) surface CCR5 expression in the absence (open circle) or presence of 100 nM CCL5 (filled circles), (**E**) binding of 100 nM labeled CCL5 or (**F**) chemotaxis to 10 nM CCL5 of human CD14$^+$ monocytes.

RESULTS

To translate the *in vitro* findings, a CCL5-driven syngeneic tumor mouse model with the CRC cell line, MC38, was selected[2]. CCR5-001 was dosed at 20 mg/kg twice a week for two weeks starting 5 days after subcutaneous injection of MC38 cancer cells. CCR5-001 demonstrated potent, statistically significant ($p = 0.0052$) tumor volume reduction in this syngeneic mouse model. CCR5-001 demonstrated a time-dependent increase in tumor growth inhibition (TGI) starting at 9.5% on day 6 post-injection to maximal TGI of 76% by day 13 as compared to the vehicle control arm. The study was terminated on day 13 because mice in the vehicle grouped reached terminal tumor volume of 2000 mm^3.

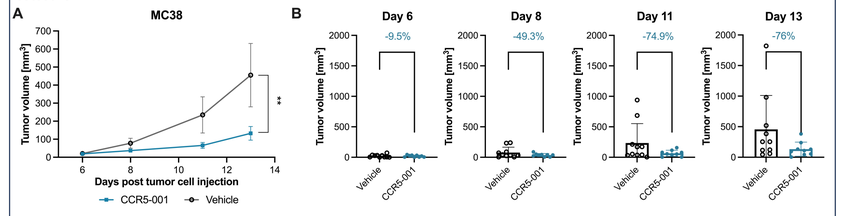

Figure 6. (**A**) CCR5-001 (blue) vs. vehicle (black) demonstrated potent tumor volume reduction in the mouse syngeneic CRC tumor model reaching statistical significance ($p = 0.0052$) and (**B**) TGI.

SUMMARY

CCR5-001 is a proof-of-concept DFC molecule that is being developed for the treatment of solid cancers.

CCR5-001 has demonstrated potent CCR5 binding, outcompeted the chemokine ligand CCL5 for binding to the CCR5 receptor and demonstrated functional inhibition of CCR5 signaling in a cell-based β-arrestin assays. This *in vitro* activity translated to robust efficacy as a monotherapy in a syngeneic mouse model.

Based on these results and other emerging data, CCR5-001 represents an exciting new avenue for targeting CCR5 with DFCs in solid cancers where the pathology is driven by the CCR5/CCL5 axis on two levels (1) cancer-extrinsic by prevention of tumor infiltration of immuno-suppressive immune cells and (2) cancer-intrinsic by prevention of autocrine CCR5 signaling (e.g. metastasis).

DISCLOSURE & REFERENCES

All authors are shareholders & employees of Cidara Therapeutics.
*corresponding author: *sdoehrmann@cidara.com*

[1]Zhang et al., 2021 PMID: 34230484
[2]Zhang et al., 2018 PMID: 29991744