Blockers Reveals Wide Target Compatibility and Synergic Cobinding to CCR5, ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, pp. 6215-6223; October (2014).

In vitro studies to investigate the effects of CCR5 receptor blockade by maraviroc on activated human T cells on potential immunological mechanisms have been conducted. It was found that blocking CCR5 by maraviroc not only can block CCR5 and CCR2 internalization processes induced by CCL5 and CCL2, but can also inhibit T cell chemotactic activities toward their cognate ligands, respectively. Further, blocking CCR5 with maraviroc at high doses tends to decrease production of TNF-α and IFN-γ. It was also noted that the effect of maraviroc on CCR5 was temporary and reversible. Yuan et al., *In Vitro Immunological Effects of Blocking CCR5 on T Cells*, INFLAMMATION, vol. 38, no. 2, (2015); *see* Arberas et al., *In vitro effects of the CCR5 inhibitor maraviroc on human T cell function*, J. ANTIMICROB. CHEMOTHER., 68: 577-586 (2013).

There exists a need, however, for improved competitive inhibitors to the CCR5 receptor and methods of use that can be used to inhibit, dampen, interrupt, block, alter, or modify the CCR5/CCL5 receptor/ligand axis for therapeutic purposes without triggering, or that reduce the impact of, unintended side effects. Further, there is a need for such competitive inhibitors to the CCR5 receptor and methods of use that cause fewer and less severe side effects, are longer-lasting, and facilitate improved patient compliance due to decreased dosing demands and improved patient experience (due to fewer undesirable side effects), including side effects caused by the competitive inhibitor itself.

Optimal therapeutic modalities using the CCL5/CCR5 axis as a therapeutic target will need to accommodate two opposing demands: the need to inhibit the detrimental involvement of CCL5 and CCR5 in specific malignant diseases while protecting their potentially beneficial activities in immunity.

SUMMARY

The present disclosure is directed to methods of use and treatment using competitive inhibitors to the CCR5 receptor that blunt, inhibit, dampen, reduce, or block the effects of CCL5 binding on the CCL5 receptor. The present disclosure comprises competitive inhibitors to the CCR5 receptor that blunt, inhibit, dampen, reduce, or block the effects of CCL5 binding to the CCR5 receptor to decrease cAMP levels. The present disclosure also comprises competitive inhibitors to the CCR5 receptor that blunt, inhibit, dampen, reduce, or block cell migration otherwise induced

by CCL5 binding to the CCR5 receptor. The competitive inhibitors to the CCR5 receptor of the present disclosure may or may not have CCL5 agonist activity upon binding to the CCR5 receptor. Preferred competitive inhibitors to the CCR5 receptor of the present disclosure do not have CCL5 agonist activity upon binding to the CCR5 receptor.

In one aspect, the present invention refers to use of a competitive inhibitor which comprises leronlimab, or a part, fragment, or conjugate of leronlimab in the manufacture of a medicament for treating inflammation associated with one of an autoimmune disorder, infectious agents other than human immunodeficiency virus-1 (HIV-1), chronic inflammation, and cancer in a subject in need of immunomodulatory intervention, wherein the competitive inhibitor to the CCR5 cell receptor does not itself have CCL5 agonist activity; and reduces CCL5 ligand and CCR5 receptor signaling in the subject.

In another aspect, the present invention refers to use of a CCR5 cell receptor competitive inhibitor comprising leronlimab, or a part, fragment, or conjugate of leronlimab, in the manufacture of a medicament for treating inflammation associated with one of autoimmune disorders, infectious agents other than human immunodeficiency virus-1 (HIV-1), chronic inflammation, and cancer such as breast cancer and prostate cancer in a subject wherein the competitive inhibitor inhibits CCL5 ligand bound to CCR5 receptor activity; and is measurably reducing the immunomodulatory downstream effects of the CCL5 ligand bound to CCR5 receptor activity.

In a preferred example, the competitive inhibitors to the CCR5 receptor of the present disclosure when bound to the CCR5 receptor do not have detectable CCL5 agonist activity (in terms of decreased cAMP measurements or induced cell migration) and at the same time stymie the effects of CCL5 ligands bound to CCR5 receptors. That is, in an example of the present disclosure, the competitive inhibitors to the CCR5 receptor do not have independent CCL5 agonist activity.

The competitive inhibitors to the CCR5 receptor of the present disclosure may cause therapeutic effect in a dose dependent manner. Accordingly, it is contemplated that the amount of active agent administered to a subject may be adjusted to meet their immunomodulatory needs, whether these are of minor, moderate, or severe nature.

In a particularly preferred example, the competitive inhibitor of the CCR5 receptor is PRO 140, or an isomer thereof, or a fragment, or derivative of PRO 140, any PRO 140 isomer, or fragment thereof. In this example, the competitive inhibitor has no, or no detectable, CCL5 agonist activity when bound to the CCR5 receptor and acts to down-regulate downstream effects caused by CCL5 ligand and CCR5 receptor binding. Specifically, such downstream effects may relate to one or both of CCL5 ligand induced decreases in cAMP levels or increases in cell migration.

Potential clinical applications of using the competitive inhibitor of the CCR5 receptor of the present disclosure to interfere with the CCR5 receptor and the CCL5 ligand axis are numerous. Such potential applications include diseases with an inflammatory component namely, respiratory tract infections (e.g., RSV, SARS), neuroinflammation (e.g., WNV, HSV, CMV), liver infections (e.g., HCV), asthma, autoimmunity (e.g., MS, Lupus, liver disease, psoriasis, Crohn's Disease, Inflammatory bowel disease, etc.), atherosclerosis, angiogenesis and cancer (e.g., prostrate, breast, melanoma, gastric, colon, ovarian, etc.), fibrosis and transplant rejection and GvHD. Methods and compounds of the presentdisclosure may also be used in connection with transplantation including GvHD, autoimmunity (e.g., MS, Lupus, Psoriasis, autoimmune liver disease, etc.), inflammation in respiratory viral diseases (e.g., RSV, SARS), other viral diseases (e.g., HCV, CMV, WNV), infectious agents, cancer applications for angiogenesis and unlocking Treg suppression of antitumor CTLs, and atherosclerosis and fibrosis. As indicated here, the interaction between the CCL5 ligand and the CCR5 receptor is implicated in several disease states.

Various CCR5 receptor binding agents are known. Competitive binding studies involving the CCR5 receptor and various anti-CCR5 binding agents including its natural ligand CCL5, and PRO 140 and maraviroc demonstrate that each of these components has a different binding capacity, and each binds to one or more distinct portions of the CCR5 receptor. PRO 140 binds to extracellular portions of the CCR5 receptor and, as exemplified below, effectively diminishes the downstream immunomodulatory effects of CCL5 binding on the CCL5 receptor. Also, unlike maraviroc, PRO 140 is shown to have no CCL5 receptor agonist activity when bound to CCR5 with respect to cAMP levels or cell migration. Accordingly, PRO 140 is shown to provide an advantageous contribution to the art and gives rise to new uses

for this CCR5 receptor competitive inhibitor to inhibit, interrupt, block, mitigate, dampen, slow the progress of, and/or therapeutically treat conditions resulting, in whole or in part, from the downstream immunomodulatory effects induced by CCL5 ligand binding on the CCL5 receptor. Particularly, here the inventors provide evidence supporting methods of using the CCR5 receptor competitive inhibitors of the present disclosure to stymie the activity of naturally occurring CCL5 without giving rise to unintended downstream immunomodulatory effects caused by the CCR5 receptor competitive inhibitor itself, at least with respect to cAMP levels or cell migration.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows cAMP accumulation in unstimulated cells (without addition of forskolin (FSK)) as measured in fmoles cAMP/0.5E6 cells in the presence of rolipram, i.e., basal cAMP for control cells in the presence of rolipram (control), cells in the presence of rolipram and CCL5, cells in the presence of rolipram and PRO 140, and cells in the presence of rolipram, PRO 140, and CCL5.

FIGURE 2 shows cAMP accumulation in stimulated cells (with addition of forskolin (FSK)) as measured in fmoles cAMP/0.5E6 cells in the presence of rolipram and FSK (control), cells in the presence of rolipram, CCL5, and FSK, cells in the

CLAIMS

1. Use of a competitive inhibitor which comprises leronlimab, or a part, fragment, or conjugate of leronlimab in the manufacture of a medicament for treating inflammation associated with one of an autoimmune disorder, infectious agents other than human immunodeficiency virus-1 (HIV-1), chronic inflammation, and cancer in a subject in need of immunomodulatory intervention, wherein the

competitive inhibitor to the CCR5 cell receptor does not itself have CCL5 agonist activity; and reduces CCL5 ligand and CCR5 receptor signaling in the subject.

2. The use of claim 1, wherein the competitive inhibitor results in increased cAMP levels in the presence of CCL5 ligand in the subject.

3. The use of claim 1, wherein the competitive inhibitor reduces cell migration in the presence of CCL5 ligand in the subject.

4. The use of claim 1, further comprising that the subject is to be tested to measure the immunomodulation triggered by the use of the competitive inhibitor.

5. The use of claim 1, further comprising that the test subject is to be monitored to assess the therapeutic effectiveness of the competitive inhibitor.

6. The use of claim 1, further comprising that a dose amount of the competitive inhibitor is to be adjusted to achieve down-regulation of cell migration in the presence of CCL5 ligand in the subject.

7. The use of claim 1, further comprising that a dose amount of the competitive inhibitor is to be adjusted to achieve increased cAMP levels in the presence of CCL5 ligand in the subject.

8. The use of claim 1, further comprising that a second competitive inhibitor to the CCR5 cell receptor is to be administered.

9. The use of claim 1, further comprising that an immunomodulatory intervention with maraviroc is to be replaced with leronlimab, or a part, fragment, or conjugate of leronlimab.

10. The use of claim 1, further comprising that an immunomodulatory intervention with maraviroc is to be supplemented with leronlimab, or a part, fragment, or conjugate of leronlimab.

11. The use of claim 1, wherein said autoimmune disorder is one of Multiple Sclerosis, Lupus, psoriasis, liver disease, Crohn's Disease, and inflammatory bowel disease or said cancer is one of breast cancer and prostate cancer.

12. Use of a CCR5 cell receptor competitive inhibitor comprising leronlimab, or a part, fragment, or conjugate of leronlimab, in the manufacture of a medicament for treating inflammation associated with one of autoimmune disorders, infectious agents other than human immunodeficiency virus-1 (HIV-1), chronic inflammation, and cancer such as breast cancer and prostate cancer in a subject

wherein the competitive inhibitor inhibits CCL5 ligand bound to CCR5 receptor activity; and is measurably reducing the immunomodulatory downstream effects of the CCL5 ligand bound to CCR5 receptor activity.

13. The use of claim 12, wherein the competitive inhibitor is leronlimab, or a part, fragment, or conjugate of leronlimab.

14. The use of claim 12, wherein the competitive inhibitor results in increased cAMP levels in the presence of CCL5 ligand in the subject.

15. The use of claim 15, wherein the competitive inhibitor reduces cell migration in the presence of CCL5 ligand in the subject.

16. The use of claim 12, further comprising that the test subject is to be monitored to assess the therapeutic effectiveness of the competitive inhibitor.

17. The use of claim 12, further comprising that a dose amount of the competitive inhibitor is to be monitored to achieve down-regulation of cell migration in the presence of CCL5 ligand in the subject.

18. The use of claim 12, further comprising that a dose amount of the competitive inhibitor is to be adjusted to achieve increased cAMP levels in the presence of CCL5 ligand in the subject.

19. The use of claim 12, further comprising that an immunomodulatory intervention with maraviroc is to be replaced with leronlimab, or a part, or conjugate of leronlimab.

20. The use of claim 12, further comprising that an immunomodulatory intervention with maraviroc is to be supplemented with leronlimab, or a part, or conjugate of leronlimab.

21. The use of claim 12, further comprising that a second competitive inhibitor to the CCR5 cell receptor is to be administered.

22. The use of claim 12, for treating inflammation associated with autoimmune disorders such as Multiple Sclerosis, Lupus, psoriasis, liver disease, Crohn's Disease and inflammatory bowel disease.