CCR5 ANTAGONIST: A NEW ERA FOR TREATMENT OF AIDS

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Summary

The CCR5 antagonists are a welcome addition to the therapeutic armamentarium available for antiretroviral-experienced patients. The two primary means of CCR5 antagonism –small molecule inhibition & monoclonal antibody steric blockade are under the trials. CCR5 stands for chemokine (C-C motif) receptor 5. HIV uses it as a co-receptor to get into target cells: the CD4 T-cells or helper cells, the main coordinators of the immune system. When the co-receptor sees the HIV virus it signals to the main CD4 cell receptor to allow the HIV antigen into the target T-cell. By blocking the CCR5 co-receptor, CCR5 antagonists stop strains of HIV known as "R5-tropic", an HIV variant that is common in earlier infection. CCR5 antagonists stop the virus from getting into cells by blocking the main entry point common to most people who have the infection. Three pharmaceutical companies were racing for the first approved small molecule CCR5 antagonist; GlaxoSmithKline (GSK), with their compound aplaviroc, Schering-Plough with vicriviroc and Pfizer with maraviroc. The CCR5 antagonist maraviroc is FDA-approved for treatment-experienced patients with R5 virus. INCB9471 is currently in phase II development, where it has shown promising evidence of antiviral efficacy.

Keywords: CCR5 receptor, AIDS, gp120 protein, maraviroc, vicriviroc, aplaviroc

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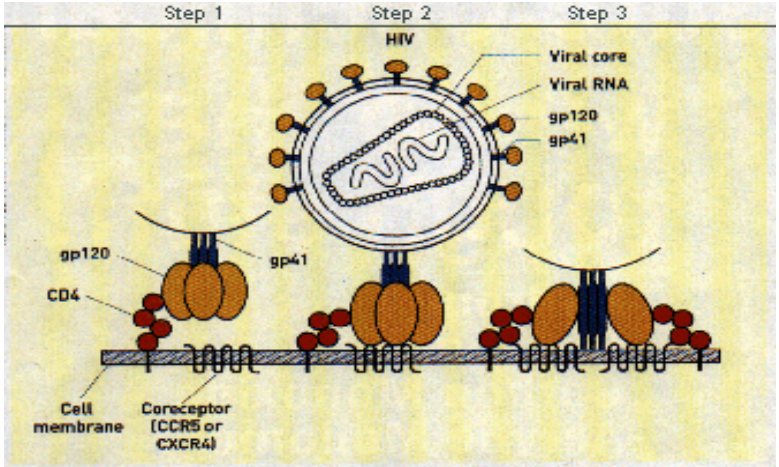
Introduction

Though potent anti-HIV therapy has spectacularly reduced the morbidity and mortality of human immunodeficiency virus (HIV)-1 infection in the advanced countries, it continues to be associated with substantial toxicity, drug-drug interactions, difficulties in adherence, and abnormal cost. As a result, better effective, safe antiretroviral drugs and treatment strategies keep on to be pursued. In this process, CCR5 (chemokine receptor 5) inhibitors are a new class of antiretroviral drug used in the treatment of HIV. The life cycle of the Human Immunodeficiency Virus (HIV) presents potential targets for drug therapy, one of them being the viral entry pathway. The C-C motif chemokine receptors CCR5 and CXCR4 are the main chemokine receptors involved in the HIV entry process. These receptors belong to the seven transmembrane G-protein-coupled receptor (GPCR) families and are predominantly expressed on human T-cells, dendritic cells and macrophages. They play an important role as co-receptors that HIV type 1 (HIV-1) uses to attach to cells before viral fusion and entry into host cells. HIV isolates can be divided into R5 and X4 strains. R5 strain is when the virus uses the co-receptor CCR5 and X4 strain is when it uses CXCR4. The location of CCR5 receptors at the cell surface, both large and small molecules has the potential to interfere with the CCR5-viral interaction and inhibit viral entry into human cells [1-2].

Entry inhibitor

Entry inhibitors (fusion inhibitors**)** are a class of antiretroviral drugs, used in combination therapy for the treatment of HIV infection. This class of drugs interferes with the binding, fusion and entry of an HIV virion to a human cell. By blocking this step in HIV's replication cycle, such agents slow the progression from HIV infection to AIDS. There are several key proteins involved in the HIV entry process like CD4 (a protein receptor found on the surface of helper T cells in the human immune system, also called CD4+ T cells), gp120 (a protein on HIV surface that binds to the CD4 receptor), CCR5 (a receptor found on the surface of CD4+ cells, called a chemokine co-receptor), CXCR4 (chemokine co-receptor found on CD4+ cells), gp41 (a HIV protein, closely associated with gp120, that penetrates the cell membrane) [3-6].



ATTACHMENT Binding of HIV's gp120 complex to CD4 and then a coreceptor—either CCR5 or CXCR4—causes gp41 to approach the cell surface and initiate the process of membrane fusion.

HIV entry into human cells requires the following steps in sequence:

- The binding of HIV surface protein gp120 to the CD4 receptor
- A conformational change in gp120, which both increases its affinity for a coreceptor and exposes gp41
- The binding of gp120 to a coreceptor either CCR5 or CXCR4
- The penetration of the cell membrane by gp41, which approximates the membrane of HIV and the T cell and promotes their fusion
- The entry of the viral core into the cell

Entry inhibitors work by interfering with one aspect of this process.

CCR5 antagonist

Almost a decade ago now, the chemokine receptor CCR5 was identified as the major co-receptor for HIV-1 entry, besides the cellular CD4 receptor [7-11]. CCR5 plays an integral role in the R5-tropic HIV-1 entry process by serving as a critical co-receptor for the viral envelope protein gp120 [12-13]. The natural ligands of CCR5 (RANTES, MIP-1α, MIP-1β) and their derivatives [14-16], as well as some specific monoclonal antibodies against certain epitopes of CCR5 possess anti-HIV-1 activity, and homozygous individuals with a 32-base pair deletion in the gene encoding CCR5 do not express the functional receptor and are ultimately resistant to R5-tropic HIV-1 infection [17-18].

These facts have made CCR5 an attractive novel target for the pharmaceutical industry in the HIV-1 therapeutic area. In the last decade, numerous small molecule CCR5 antagonists have been reported. The discovery and development of CCR5 antagonists have been systmatically reviewed by Palani. They include anilide-, oximino-piperidino-piperidine-, chiral piperazine-, tropane-, spirodiketopiperazine-, acyclic and cyclic scaffold-based compounds. These efforts have resulted recently in the FDA approval of the first small molecule CCR5 antagonist, maraviroc for the treatment of HIV- 1 infection. But there are still various challenges and unknowns associated with CCR5 antagonists such as drug resistance, viral tropism and possible long term adverse events, so development of second generation CCR5 antagonists with improved properties is still much needed [19-20].

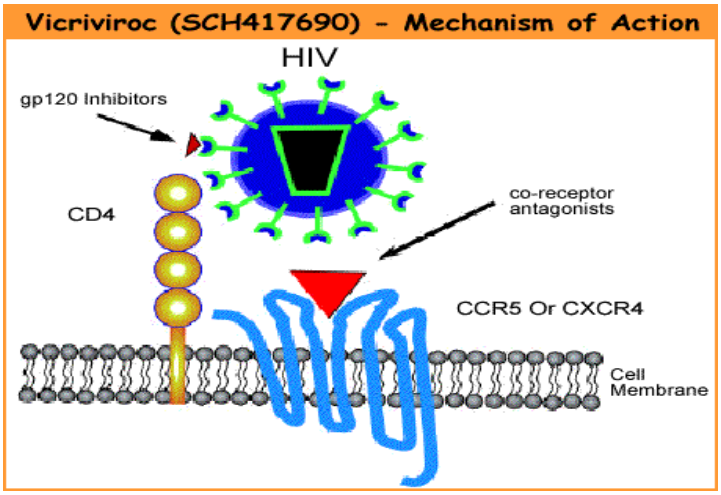
In August of 2007, the food & Drug Administration approved the first chemokine receptor 5 inhibitor maraviroc, for treatment experienced patients infected with R5 using virus. Chemokine receptor antagonists are the first antoretrovirals to bind a cellular protein of the host & as such, engender unique safety concerns. Here we will discuss the two primary means of CCR5 antagonism –small molecule inhibition & monoclonal antibody steric blockade. CCR5 inhibitors are a new class of antiretroviral drug used in the treatment of HIV. They are designed to prevent HIV infection of CD4 T-cells by blocking the CCR5 receptor. When the CCR5 receptor is unavailable, 'R5-tropic' HIV (the variant of the virus that is common in earlier HIV infection) cannot engage with a CD4 T-cell to infect the cell.

C-C chemokine receptor type 5 also known as CCR5 is a protein that in humans is encoded by the *CCR5* gene. chemokine abbreviation signify whether the first 2 cysteine residues of each protein are adjacent (cc) or separated by a variable amino acid (cxc), with R abbreviating receptor. The CCR5 protein has also recently been designated CD195: "Cluster of differentiation" 195 (for cell surface molecules present on White blood cells).

The CCR5 protein is a seven transmembrane protein which functions as a chemokine receptor in the CC chemokine group. The natural chemokine ligands that bind to this receptor are RANTES (Regulated upon activation normal T cell expressed & secreted) MIP-1α and MIP-1β. CCR5 is predominantly expressed on, macrophages, dendritic cells and microglia. The tyrosine sulfated N-terminus of CCR5 is the essential determinant of binding to gp120. The discovery that CCR5 receptors were critical for HIV's ability to enter the T cell taught scientists two things: 1)Blocking the CCR5 receptor might stop HIV 2)Since people born without CCR5 receptors are healthy, a medicine that blocks the CCR5 receptor probably wouldn't hurt anyone [19-22].

Mechanism of action of CCR5 antagonist

According to current models of HIV-1 entry, sequential binding of gp120 & CD4 & co-receptor leads to release of gp41 from its metastable conformation. Upon CD4 binding to gp 120, the V3 loop is exposed & extends towards the target cell membrane. This loop acts as molecular "hook" that interacts with either CCR5 or CXCR4. The V3 loop interacts with both the N terminus & 2nd extracellular loop of CCR5 leading to release of gp41 by unknown mechanism. gp41 rearranges & two trimeric coiled type structure termed HR1 & HR2 (hepted repeat 2) domains brings together to form a 6 helix bundle thats leads to fusion.



HIV enters host cells in the blood by attaching itself to receptors on the surface of the CD4+ cell. Viral entry to the CD4+ cell begins with attachment of the R5 HIV-1 glycoprotein 120 (gp120) to the CD4+ T-cell receptor. Although the virus binds to CD4+ on the cell surface, this interaction alone is not sufficient for the entry & productive infection. Expression of other cell surface molecules co-receptor present on T cell & monocyte is required for HIV-1 infection. The infection of co-receptor of a T cell is assisted by the T cell co-receptor CXCR4. An analogous receptor called CCR5 function for the monocytes & macrophages. Interaction of CD4+ & gp120 leads a conformational change in gp120 and allows it to bind to CCR5, thereby triggering glycoprotein 41 (gp41) mediated fusion of the viral envelope with the cell membrane and the nucleocapsid enters the host cell (Figure 1). CCR5 co-receptor antagonists prevent HIV-1 from entering and infecting immune cells by blocking CCR5 cell-surface receptor. Small molecule antagonists of CCR5 bind to a hydrophobic pocket formed transmembrane helices of the CCR5 receptor. They are thought to interact with the receptor in an allosteric manner locking the receptor in a conformation that prohibits its co-receptor function [23-27].

CCR5 Antagonist-binding

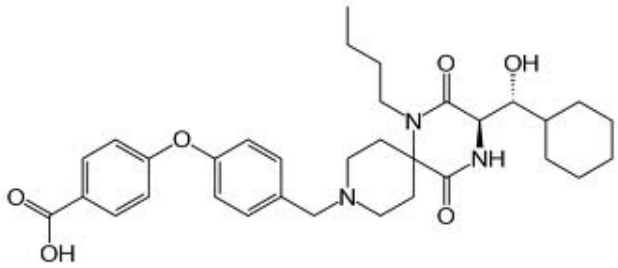
CCR5 is a member of G-protein-coupled, seven transmembrane segment receptors. The CCR5 antagonists are predicted to bind to a putative binding pocket which is buried inside the transmembrane domain, enclosed by the seven transmembrane helices. The binding pocket is very hydrophobic with multiple aromatic residues lining the pocket. The key residues are tryptophan 86 and 248 (Trp86, Trp248), tyrosine 108 and 251 (Tyr108, Tyr251), phenylalanine 109 (phe109), threonine 195 (Thr195), isoleucine 198 (Ile198), glutamic acid 283 (Glu283). CCR5 antagonists are very different in shape and electrostatic potential although they all share the same binding pocket. The interesting thing about the binding of these molecules is that significant difference in binding modes although they all establish an extensive interaction network with CCR5 [28-30].

CCR5-Drug development

As mentioned, the CCR5 receptor is a G-protein coupled receptor (GPRC) and before the discovery of CCR5's role in HIV infection many pharmaceutical companies had already built a substantial collection of compounds that target GPRCs. Three pharmaceutical companies were racing for the first approved small molecule CCR5 antagonist; GlaxoSmithKline (GSK), with their compound aplaviroc, Schering-Plough with vicriviroc and Pfizer with maraviroc All the compounds reached clinical trials in humans but only maraviroc has been approved by the U.S. Food and Drug Administration (FDA) [31].

Aplaviroc

Aplaviroc was a Glaxosmithkline compound that demonstrated antiviral activity with minimal toxicities during short term monotherapy studies. Aplaviroc is originated from a class of spirodiketopiperazine derivatives. The problem with this compound was not its CCR5 selectivity but the oral bioavailability. Unfortunately despite the promising preclinical and early clinical results some severe liver toxicity was observed in the treatment of naive and treatment-experienced patients that led to the discontinuation in further development of aplaviroc.

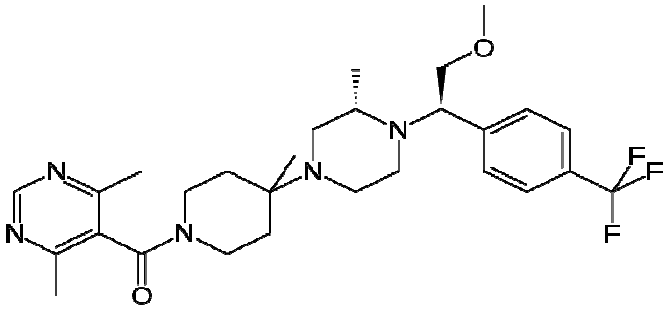


APLAVIROC

In phase 2 trials, however, 4 out of roughly 300 patients developed severe hepatotoxicity that, on liver biopsy, was found to be consistent with drug-induced hepatitis. This finding leads to the cessation of the trial. Similarly, 1 out of 26 patients participating in a phase 3 trial of aplaviroc demonstrated elevation of alanin aminotransferase of 24 times normal levels. This trial was also stopped & clinical development of aplaviroc was terminated [31-33].

Vicriviroc

Schering-Plough identified an active compound during screening. The lead compound contained a piperazine scaffold and was a potent muscarinic acetylcholine receptor (M2) antagonist with modest CCR5 activity. The changes that were made on the left hand side of the lead compound and the addition of a methyl group on the piperazine group ((S)-methylpiperazine) resulted in the intermediate compound that had good affinity for CCR5 receptors but very little affinity for muscarinic activity, however, the compound did show affinity for the hERG ion channel [34-35].



VICRIVIROC

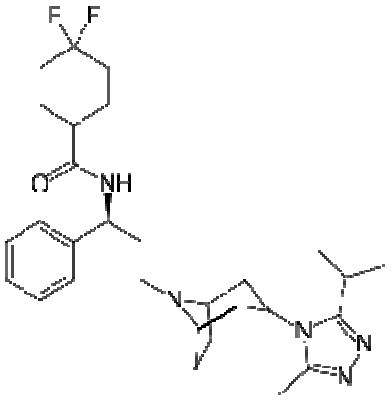
Further reconstruction led to the development of the final compound vicriviroc, when Schering discovered that the pyridyl N-oxide on the intermediate could be replaced by 4,6-dimethylpyrimidine carboxamide. Vicriviroc had an excellent selectivity for CCR5 receptor over muscarinic and hERG affinity was greatly reduced [36-37].

Phase I clinical trial of vicriviroc gave promising results, so a phase II study in the treatment of naive patients was initiated. The phase II study was discontinued since there was a viral breakthrough in the vicriviroc group compared to the control group. These results suggested that vicriviroc was not effective in the treatment of treatment-naive patients compared to current treatments. Another phase II clinical study was performed in treatment-experienced patients. The results were that vicriviroc did have strong antiviral activity but five instances of cancer among the participants were reported, however, the study was continued since there was lack of causal association of the malignancies and vicriviroc. In late 2009 vicriviroc was assigned in phase III studies in treatment-experienced patients, and in phase II studies in treatment for naive patients [31].

Maraviroc

Pfizer turned to high-throughput screening in their search for a good starting point for a small molecule CCR5 antagonist. Maraviroc, originally designated UK-427857, was developed by the Pfizer drug company in Sandwich, UK labs. On April 24, 2007 the U.S. Food and Drug administration advisory panel reviewing maraviroc's new drug application unanimously recommended approval for the new drug and the drug received full FDA approval on August 6, 2007 for use in treatment experienced patients. On September 24, 2007, Pfizer announced that the European Commission approved maraviroc. Industry experts forecast annual maraviroc sales of 500 million dollars by 2011 [38].

Two randomized, placebo-controlled clinical trials, known as MOTIVATE 1 & 2, compared 209 patients receiving optimized therapy plus a placebo to 426 patients receiving optimized therapy plus 150 mg maraviroc once daily and 414 patients receiving optimized therapy plus 150 mg maraviroc twice daily. At 48 weeks, 55% of participants receiving maraviroc once daily and 60% of participants receiving the drug twice daily achieved a viral load of less than 400 copies/mL compared with 26% of those taking placebo; about 44% of the once-daily and 45% of the twice-daily maraviroc group had a viral load of less than 50 copies/mL compared with about 23% of those who received placebo. In addition, those who received the entry inhibitor had a mean increase in CD4 cells of 110 cells/μL in the once-daily group, 106 cells/μL in the twice daily group and 56 cells/μL in the placebo group [39-40].

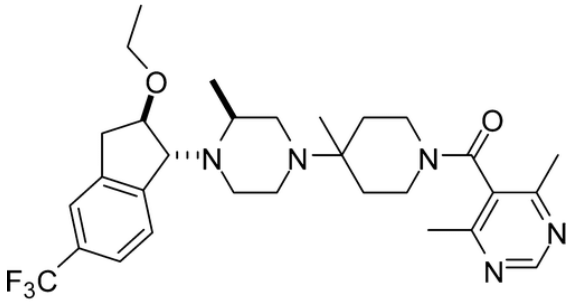


MARAVIROC

The strongest interaction is estimated to be between maraviroc and glutamic acid (Glu283) through a strong salt-bridge interaction. The interaction between tryptophan (Trp86) and maraviroc involves T-shaped π-π stacking while the interaction with phenylalanine (Phe109) is predicted to be hydrophopic. Tyrosine (Tyr108) is thought to interact with the phenyl group on maraviroc through a parallel displaced interaction. The interaction between maraviroc and isoleucine (Ile198) is predicted to be mostly hydrophobic in nature and the interaction between maraviroc and tyrosine (Tyr251) is very limited [29].

INCB9471

INCB9471 is currently in phase II development, where it has shown promising evidence of antiviral efficacy. INCB9471 in HIV patients suggest it possesses potent and prolonged antiviral effects against R5-tropic HIV in both treatment-naïve and treatment-experienced patients.



INCB9471

In this 14-day, 23-patient placebo-controlled study, administration of a 200mg once-daily dose of INCB9471 achieved rapid and prolonged reduction in viral load with a mean maximal decline of 1.81 log10 at day 16. Consistent with its long plasma half-life, viral load suppression continued well beyond the 14-day dosing period with a mean 0.81 log10 decline in viral load observed at day 28. INCB9471 appeared safe and well tolerated in this small study. Previous phase I safety studies in volunteers had shown that single doses of up to 300mg and multiple doses of up to 200mg for ten days were well tolerated; no dose-limiting toxicity was observed [41-42].

PRO140, HGS101 and HGS004

CCR5 is the chemokine receptor for the ligands macrophage inflammatory protein-1 (MIP-1) α and β, RANTES (regulated on activation normal T cell expressed and secreted), and monocyte chemotactic protein-2. CCR5 is also the primary coreceptor for HIV-1 transmission and replication, from the early stages of disease through progression to AIDS. In all stages of HIV-1 infection, R5 and dual-tropic (R5/X4) viruses comprise the majority of viral strains detected, while X4-exclusive viruses constitute a minority of cases. Naturally occurring host defects in CCR5 expression have demonstrated the importance of this receptor in HIV-1 infection, as it has been observed that individuals with a homozygous deletion (CCR5Δ32) show resistance to the virus. In addition, CCR5 is a potentially safe target in HIV-1 treatment, as people lacking CCR5 expression appear healthy. Because MIP-1β and RANTES are proinflammatory, another potentially interesting role for this class of agents is their use in altering of the immune activation that has been shown to promote disease progression in HIV-1 [43-44].

Several monoclonal antibodies including PRO140(progenics) & HGS004(human genome sciences) are developed in addition to small molecules CCR5 antagonist.CCR5 monoclonal antibodies could have a distinct mechanism of action , presumably sterically hindering gp120- CCR5 binding through the much larger size of the antibodies structure. Compelling clinical data are not available for this class of entry inhibitor.

Further research has subsequently identified HGS101, an alternative anti-CCR5 MAb candidate. *In vitro* data suggest that HGS101 is 5.5-fold more potent against the clinical isolates from the Phase I trials. Other attributes of HGS101 are similar to those of HGS004, including favorable pharmacokinetics, strong *in vitro* evidence of anti-viral activity that is additive or synergistic in combination with approved therapeutic agents, and a low likelihood of causing development of resistance based on long-term *in vitro* culture [43-47].

Small molecule or antibody approaches require the constant presence of antagonist in high enough concentrations to block therapeutically relevant numbers of the CCR5 protein, of which there are approximately 10,000 copies on the surface of each T-cell. In contrast, brief exposure of T-cells to Sangamo's ZFNs(ZINK FINGER NUCLEASE) has been shown to result in permanent modification of the CCR5 gene and consequent alteration of the CCR5 protein. In its anti-HIV preclinical research program, Sangamo has designed ZFNs that can be used to disrupt the CCR5 gene, a receptor required for HIV entry into immune cells. The researchers found that ZFN-modified cells were resistant to HIV infection [43-46].

Attachment and fusion inhibitors in development

Fusion and attachment inhibitors in human trials include AK602, AMD070, BMS-378806, HGS004, INCB9471, Maraviroc, PRO 140, SCH532706, SP01A, TAK-652, TNX-355 and Vicriviroc (SCH 417690).

AK602 is a CCR5 blocker being developed by Kumamoto University in Japan. It is in early human trials. AMD070 by AnorMed blocks the CXCR4 receptor on CD4 T-cells to inhibit HIV fusion. Development is on hold because of liver problems in animal studies. BMS-378806 is an attachment inhibitor that attaches to gp120, a part of the virus, not the target cell. It is in Phase I trials. GSK 706769 by ViiV Healthcare is a new CCR5 antagonist in Phase I trials. HGS004 by Human Genome Sciences, a monoclonal antibody CCR5 blocker, successfully completed a Phase II trial. Ibalizumab (TNX-355) by TaiMed Biologics blocks the CD4 receptor. It is a genetically engineered drug, a "monoclonal antibody." It is being studied as an intravenous infusion every two or four weeks. It is administered along with antiretroviral medications. No significant side effects have shown up yet. It is in Phase II trials.

PF-232798 by Pfizer is a CCR5 blocker. It is in Phase II trials. PRO 140 by Progenics is now in Phase II trials. It blocks fusion by binding to a receptor protein on the surface of CD4 cells. PRO 140 has been granted fast-track status by the FDA. It is being studied as an intravenous infusion and by subcutaneous injections. SCH532706 by Schering is in Phase I studies. It is best used as part of a regimen that includes ritonavir where it can be administered once daily. SP01A by Samaritan Pharmaceuticals is an HIV entry inhibitor in a Phase III trial. TBR-652 by Tobira Therapeutics (formerly TAK-652 by Takeda) is a CCR5 blocker. It is in Phase II studies. VCH-286 by ViroChem Pharma is a CCR5 antagonist. A Phase II trial has received regulatory approval [43-48].

Virus resistance to CCR5 antagonist

In the case of CCR5 inhibitors, the drug target is a host cell protein that will not undergo mutation in response to CCR5 antagonist therapy. Viral adaptation to CCR5 inhibitors could, however, involve changes in the viral envelop protein that alters dependence on CCR5. True resistance appears to be mediated by changes in HIV-1 gp 120 that allow binding to the complex of the CCr5 receptor & bound drug. Maraviroc resistance HIV-1 variants have been generated by serial passage in vitro. Resistance to CCR5 inhibitors is caused by sequence changes in the fusion peptide of HIV-1 gp41. Studies with engineered Env-chimeric and point-substituted viruses confirmed that these 3 FP residues were substantially responsible for VVC (vicriviroc) resistance without altering coreceptor usage, as assessed in both peripheral blood mononuclear cells and the TZM-bl cell line. Resistance to CCR5 antagonist emerges as viruses acquire the ability to use the inhibitor bound form of CCR5 for viral entry [49-52].

Resistance to CCR5 antagonists emerges as viruses acquire the ability to use the inhibitor-bound form of CCR5 for viral entry. Decreases in the percent maximal suppression seen with the emergence of CCR5 antagonist resistance is a function of the allosteric, noncompetitive nature of small-molecule CCR5 antagonism and reflects the ability of the virus to use either inhibitor-bound CCR5 or unbound CCR5 for entry. As the virus becomes more efficient at using antagonist-bound CCR5 for entry, the height of the plateau will decrease. Eventually, plateau height will decrease below 50%, making it impossible to calculate an IC_{50} for the resistant virus [50-51].

Consequences of CCR5 antagonism

CCR5 antagonists are the first antiretroviral drugs that target host proteins. The apparent absence of significant immunologic deficits amongst individuals with naturally occurring mutations (ie, *CCR5-Δ32* homozygotes) that result in a lack of functional CCR5 provides some reassurance that pharmacologic blockade of CCR5 will be relatively benign. Presumably, redundancy in the chemokine network allows other chemokine receptors to subsume the function of CCR5. However, pharmacologic blockade of a receptor in mature individuals may have different consequences than congenital absence of the receptor. Thus, the long-term safety of CCR5 blockade remains to be proven [53].

Several studies of CCR5 knockouts in mice as well as epidemiologic data from human *CCR5-Δ32* homozygotes suggest this deletion may have previously unrecognized consequences. For example, although no overt pathologic changes were noted in a mouse CCR5 knockout, alterations of macrophage function and increased susceptibility to cryptococcal infections of the brain have been reported. Moreover, increased mortality from West Nile virus encephalitis was seen in CCR5–/– mice and was linked to decreased leukocyte trafficking into the brain. Mice that are CCR5 deficient also have an abnormal immune response to ocular infection with herpes simplex virus type 1 [54-57].

In a mouse model of T cell –mediated hepatitis, CCR5 deficiency increased mortality and liver injury. The lack of signaling due to the absence of CCR5 was thought to prevent downregulation of the natural killer T-cell response, resulting in fulminant hepatitis. The relevance of this finding to the cases of aplaviroc-induced hepatotoxicity is unclear. *CCR5Δ32* heterozygotes have a six-fold increased risk for severe morbidity from West Nile virus infection and a five-fold increased risk of mortality [58-59].

Conclusion

CCR5, as the major co-receptor for HIV-1 entry, is an attractive novel target for the pharmaceutical industry in the HIV-1 therapeutic area. The CCR5 antagonists are a welcome addition to the therapeutic armamentarium available for antiretroviral-experienced patients. Currently, their use in antiretroviral-naive patients should be restricted to enrollment in ongoing or planned clinical trials. The CCR5 antagonist maraviroc is FDA-approved for treatment-experienced patients with R5 virus (only), and no patient should receive maraviroc without first undergoing a tropism assay. Although the cost of this additional test is substantial, the possibility of combining maraviroc with at least 2 other active drugs to achieve undetectable viral loads may justify the added expense. Ongoing clinical monitoring will provide important follow-up data to address the theoretical safety concerns surrounding CCR5 inhibition. The approval of drugs from several new drug classes onto the market in 2007 through 2008 will herald a major step forward in the treatment of antiretroviral-experienced patients with multidrug-resistant HIV-1.

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