



Progress in HIV-1 Prevention, Control and Treatment: Genetic Manipulation or Pharmacological Blockade of Chemokine Receptor 5?

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Authors' contributions

This article was written in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

For Human immunodeficiency virus type 1 (HIV-1) to invade the host cells it requires human cluster of differentiation (CD4) receptor and a chemokine receptor, principally chemokine receptor 5 (CCR5). Although the viral particles interact with several receptors on cell surface, a key receptor, CD4 and a co-receptor act in succession to facilitate the fusion of the viral glycoprotein with cellular membranes allowing the entry of the virus into cells. The CCR5 is the predominant co-receptor for HIV-1. HIV-1 is the most common pathogenic strain and its genetic hyper-variability makes the virus resistant to antiretroviral drug therapy. Current approaches focus on the CCR5 as the emerging target for HIV-1 control. Here, we highlight the current trend in HIV-1 control, prevention and treatment, compare the two promising approaches: Genetic manipulation of CCR5 gene and the pharmacological blockade of CCR5 using chemokine receptor antagonists.

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1. INTRODUCTION

The initial phase of Human Immunodeficiency Virus (HIV-1) infection to host cells occurs by binding of viral envelope (Env) surface glycoprotein to specific receptors on plasma cell membrane. CD4 receptor is one of these receptors found in T-helper lymphocytes, macrophages and dendritic cells. HIV-1 infection shows a lot of tropism. The T cell line-tropic (T-tropic) and macrophage-tropic (M-tropic) HIV-1 isolates mediate their infection to human cluster of differentiation 4 (CD4+) cells using different but similar viral envelope proteins 120 (gp120) and glycoprotein 41 (gp41) respectively [1]. T-tropic HIV-1 adsorbs to target cell membrane upon binding of gp120 to CD4 receptor and initiates conformational changes in gp120 enabling it to bind to a co-receptor (Fig. 1). Among 19 chemokine receptors considered *in vitro* as co-receptors for HIV, only CCR5 and C-X-C chemokine receptor type 4 (CXCR4) (members of the G protein-coupled receptor superfamily) have been identified as the principal co-receptors for T-tropic and M-tropic HIV-1 isolates, respectively and shown to be the most relevant in the pathogenesis of HIV infection [2].

The chemokine receptor CCR5 is the predominant co-receptor for HIV-1. Some variants evolve in some infected individuals that switch to use CXCR4 and perhaps other co-receptors. Other cell surface receptors, mannose binding protein found on macrophages and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) on dendritic cells also interact with gp120 of virus but the interactions do not lead to fusion and virus entry [3].

HIV-1 is the most common pathogenic strain and its genetic hyper-variability makes the virus resistant to antiretroviral drug therapy. Hence the need for new approaches that can complement or even replace the existing therapies for long-term control of the disease with minimum resources. Among the various HIV control strategies CCR5 genetic modification and pharmacological blockage are showing promises in effective HIV-1 control. These two approaches consider the fact that individuals who lack CCR5 expression due to the homozygous $\Delta 32$ deletion in the CCR5 gene are resistant to HIV infection and appear to be normal [4,5]. However, heterozygous individuals with a reduced CCR5

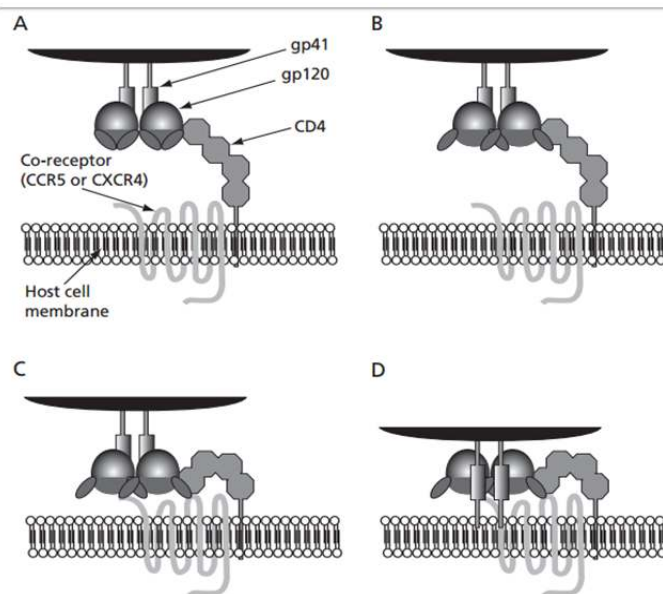


Fig. 1. Sequence of molecular interaction events leading to the entry of virus into host cell. HIV gp120 binds to CD4 (A). This induces conformational changes in gp120 and exposure of the co-receptor binding site (B). Exposure of the co-receptor binding site permits binding of gp120 to the co-receptor (C). Co-receptor binding induces conformational changes in gp41 and insertion of a 'fusion peptide' into the host cell membrane (D), resulting in fusion of viral and cell membranes [14]

surface expression have lower plasma viral load and delayed progression of the disease [6]. Current approaches focus on the CCR5 as the emerging target for HIV-1 therapy. In this review we highlight the current trend in the genetic manipulation of CCR5 gene and the pharmacological blockade of CCR5 using chemokine receptor antagonists and compare the success reported and challenges faced by each approach.

2. HIV-1 TROPISM, INTERACTION WITH RECEPTOR AND CO-RECEPTOR

Based on the co-receptor use, HIV-1 strains are classified according to their tropism, into CCR5 tropic (R5); CXCR4 (X4) and dual/mixed tropic (R5/X4). The different conformations taken by co-receptors on cell surfaces and on different cell types influence their ability to allow HIV infection [7]. They take such conformations by forming dimers [8] or by association with other cell surface molecules as shown for CCR5 and CD4 [9]. There are two sites on co-receptors centered on the N-terminus and E2, involved in HIV entry. N-terminal domain of CCR5 was shown, by mutagenesis studies, to be important for co-receptor activity for CCR5-using HIV-1s [10]. R5 strains differ considerably in their use of CCR5 due to a wide variation in their capacity of infecting cells that express different chimeric human/mouse CCR5s [11]. Other chemokine receptors also take part in the infection in certain strains. Choe and colleagues reported "CCR3 facilitated infection by restricted strain of the virus, however, binding of the CCR3 ligand, eotaxin, blocked infection by these isolates, suggesting that utilization of CCR3 and CCR5 on the target cell depended on the sequence of the third variable (V3) region of the HIV-1 gp120 exterior envelope glycoprotein" [12]. Similarly, a study by He J and his team showed that CCR3 and CCR5 promote HIV-1 infection of the Central Nervous System (CNS) [13].

Regarding the chemistry of the interaction, Cormier et al. [15] and others have indicated that acidic residues, including tyrosines located within the CCR5 amino-terminal domain, are essential for CCR5-mediated fusion and entry of R5 and R5X4 HIV-1 strains [16-19]. A study by Farzan and his team uncovered that tyrosine residues in the CCR5 Nt are sulfated [20]. The electrostatic interactions involved are thought to enhance gp120/co-receptor interactions. The N-terminal region of CCR5 and related family members are negatively charged due to 3 acidic amino acids and 4 (potentially) sulfated tyrosine residues

which are crucial in co-receptor function. These negative charged residues may involve in interaction with positive amino around the bridging sheet on gp120 [21].

3. GENETIC MANIPULATION OF CCR5 GENE

In many years of fight against HIV infection, Highly Active Anti-retroviral Treatment (HAART) has shown promises in many HIV positive individuals in reducing viral load and ameliorating the life-threatening condition. However, due to the increased drug resistance shown by the HIV-1 variants more effective therapeutic interventions are needed to combat the disease. Many novel host-based strategies that interfere with the entry pathway are being developed. With recent advances in genome editing technology successful CCR5 modifications in human cells using Transcription Activator-like Effector Nucleases (TALENs) [22-24], Zinc Finger Nucleases (ZFNs) [25], and CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated) system [26-30] have been reported. CRISPR/Cas9 system is more convenient, flexible, and readily produced than TALEN and ZFN. The possible reasons are: CRISPR/Cas9 utilizes a fixed nuclease and requires the design of only 20-nt sequence-matching gRNAs. Moreover, unlike TALENs and ZFNs, CRISPRs/Cas9 does not necessitate de novo engineering of proteins for each genomic target, thereby making it easier for multiplex genome engineering [31]. It has also been reported that the CRISPR/Cas9 system provides high specificity of genomic modification with low off-target effects [32-34].

Recently, CCR5 gene function was successfully disrupted in human pluripotent stem cells using zinc finger nucleases (ZFNs) [35]. In line with this study, Kang et al. [36] evaluated the efficacy of using CRISPR/Cas9 to edit the CCR5 gene in Induced pluripotent stem cells (iPSCs) and compared single with dual guide RNA (gRNA) strategies for CCR5 disruption to protect cells from HIV-1 using CCR5 for entry. They found that the dual gRNA approach significantly increased the frequency of biallelic CCR5 gene editing without compromising specificity. Furthermore, to ensure the homogeneity of gene modification within cells, they applied a single cell sorting approach for establishing clonal iPSC lines. These cell lines retained the typical characteristics of pluripotent stem cells and thus differentiated efficiently into hematopoietic cells [36]. These results are in agreement with those

obtained by several studies reporting that the use of dual gRNAs has higher editing efficiency in the mouse embryo [37], Human Embryonic Kidney 293 (HEK293) and human colon cancer (HCT116) cells [38], primary human CD4+ T cells, and CD34+ hematopoietic stem and progenitor cells [39]. Regarding the use of ZFNs, Tebas and Colleagues, (2014) carried out a site-specific modification of the CCR5 gene on the infusion of autologous CD4 T cells in which the CCR5 gene was rendered dysfunctional by a zinc-finger nuclease (ZFN) and reported the decrease in HIV DNA in most of the patients who received the treatment. However, they also reported a serious adverse event associated with infusion of the modified autologous CD4 T cells which was attributed to a transfusion reaction [25].

Alternatively, a number of si/shRNA constructs targeting CCR5 message are being assessed in small clinical trials. These constructs degrade transcribed mRNA while leaving the gene intact. Should these constructs be efficient, transcripts from both CCR5 alleles would be reduced, which may have an advantage over ZFNs. A recent study by Burke et al. [40] used dual-combination anti-HIV-1 lentiviral vector (LVsh5/C46) to downregulate CCR5 expression of transduced cells via RNAi and inhibits HIV-1 fusion through cell surface expression of antiviral peptide anchored by cell membrane. Currently, there exist new treatments, such as Hematopoietic Stem Cell Transplantation (HSCT) in Berlin patients, for $\Delta 32/\Delta 32$ mutation which has the potential to replace co-receptor antagonist and HAART drug problems such as toxicity, low safety, and side-effects. Based on this treatment, Esmailzadeh et al. [41] evaluated a new hypothetical mutation, CCR5 m303/m303 as autologous HSCT. They believed that their novel hypothesis could lead to the treatment for HIV/AIDS affected patients worldwide.

4. PHARMACOLOGICAL BLOCKADE OF CCR5

Since the identification of HIV co-receptors, CCR5 and CXCR4 and understanding their role in supporting HIV infection, drugs aimed at blocking envelope interactions with these co-receptors are being developed. They are termed chemokine inhibitors and are the first antiretroviral drugs that target host proteins. The apparent absence of a deficit in the immunologic functions among individuals with naturally occurring CCR5- $\Delta 32$ mutations provides some certainty that pharmacologic blockade of CCR5 may not have negative consequences [42]. It is presumed that redundancy in the chemokine network allows other chemokine receptors to perform the function of CCR5 [43]. By the same token, however, it is believed that pharmacologic blockade of a receptor in mature individuals may have different consequences than a congenital absence of the receptor. Therefore, the long-term safety of CCR5 blockade remains to be proven. The murine studies corroborated with studies in humans revealed that "CCR5 $\Delta 32$ heterozygotes have a six-fold increased risk for severe morbidity from West Nile virus infection and a five-fold increased risk of mortality" [44].

In 2007, the FDA approved the first CCR5 inhibitor, maraviroc, for treatment of patients infected with an R5-using virus. Maraviroc is a CCR5 antagonist which shows potent *in vitro* and *in vivo* anti-HIV-1 activity. Maraviroc is an entry inhibitor (Fig. 3), specifically, a negative allosteric modulator of the CCR5 receptor. The drug binds to CCR5 and blocks the HIV protein gp120 from associating with the receptor. Thus, the entry of HIV into human macrophages and T-cells is prevented [45]. Because HIV can also use other co-receptors, such as CXCR4, an HIV tropism test such as a profile assay must be performed to determine if the drug will be effective [46].

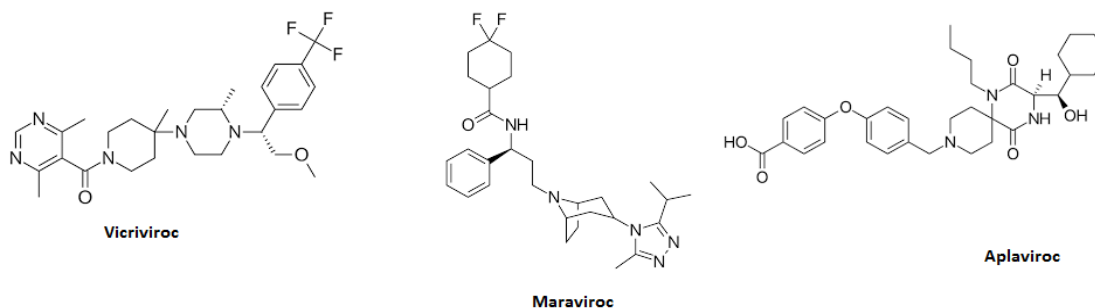


Fig. 2. Structures of CCR5 antagonists: Vicriviroc, maraviroc and aplaviroc

Vicriviroc is another drug 2- to 40-fold more potent *in vitro* than the first-generation compound, Schering C. Similar to maraviroc, this compound blocks signaling by the C-C chemokines at nanomolar concentrations. The drug has not shown any central nervous system (CNS) adverse effects upon testing in healthy volunteers or HIV-1-infected subjects, to date. A GlaxoSmithKline compound, Aplaviroc, also demonstrated antiviral activity with minimum side effects during the period short-term monotherapy studies [47].

Previously, some scientists thought the major advantage of CCR5 receptor antagonist is the elimination of resistance observed with HAART. However, resistant-mutants emerge due to the high error rate of HIV-1 reverse transcriptase and rapid turnover of the viral population [47].

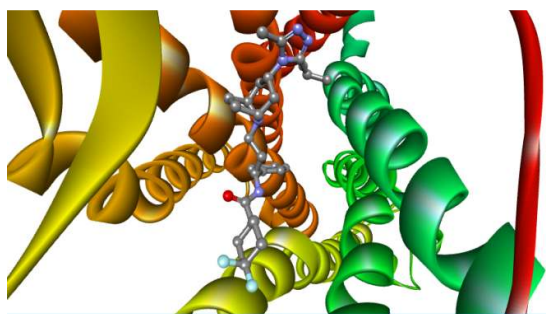


Fig. 3. Crystal structure of CCR5 in complex with maraviroc. The structure was retrieved from Protein Data Bank (PDB ID, 4 mbs) [48], water was removed and chain A extracted with maraviroc in the receptor's binding pocket and viewed in Biovia Discovery Studio molecular modelling program

5. CCR5 GENETIC MANIPULATION VERSUS PHARMACOLOGICAL BLOCKADE

Both the two approaches are proven to be effective, with few limitations, as described above. Therefore, understanding how advantageous one approach can be over the other would be based on promises and limitations. One of the greatest concerns in the implementation of both approaches is the possibility of selection of minority variants of CXCR4 or dual/mixed tropic virus in the presence of viral load. Even though CCR5 genetic manipulation can provide a lifelong HIV-1 control and be more efficacious than CCR5 pharmacological blockage, its greatest limitation

is the possibility of off-target effects due to DNA flexibility. The efficiency of CCR5 gene modification is dictated by the particular platform, the targeted sequence, the chromatin structure at the target site, and the vector used to express the genome-editing nuclease, meganucleases, ZFNs and TALENs. Mark and Bruce, [48] pointed to the fact that genome-editing technology is based on the efficiency of gene disruption and the propensity for off-target effects. Disruption of CCR5 gene requires cutting double-stranded DNA in a targeted fashion. Initially, this approach has employed zinc finger nucleases. Although the zinc fingers bind specific sequences within the double-stranded DNA, the double-strand break is created by the dimerized restriction endonuclease. As a mechanism to repair this damage, the DNA undergoes either homologous end joining, to maintain the original sequence (but remains a persisting target), or undergoes non-homologous recombination that results in the insertion or deletion of base pairs, leading to a frame-shift mutation, disallowing gene expression [49,50].

Similarly, receptor antagonist drugs are seen as problematic in view of drug toxicity; a possibility of drug binding to off-target chemokine receptors and the emergence of escape mutants. Although CCR5 antagonists target a host cell receptor, resistance to CCR5 antagonists can still occur through two different mechanisms. The first is through the selection of minority variants of CXCR4 or mixed tropic virus [51–53]. It has been proposed that switching to CXCR4 may not occur due to a reduced fitness of transitional variants and/or sensitivity to CCR5 antagonists [54-56]. The second mechanism is the development of mutations in the V3 loop, gp120, and gp41 that may lead to resistance to CCR5 antagonists. It is thought that these mutations may allow the resistant virus to bind to the cell's CCR5 receptor that is already bound to maraviroc [57]. However, others have questioned the role of these mutations in the V3 loop in the development of resistance to maraviroc [58]. Similarly, an evaluation of the viruses from 323 CCR5 antagonist-naïve patients showed that about 7.3% had mutation combinations previously described with maraviroc resistance [59,60].

Despite these limitations suffered by the use of CCR5 antagonists, it can still be a better approach, in terms of safety and feasibility, than CCR5 gene editing for two concrete reasons.

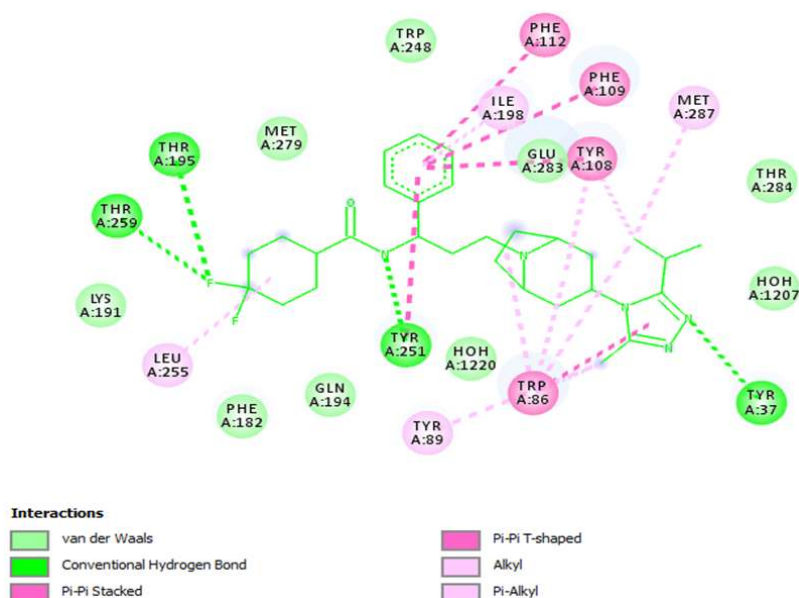


Fig. 4. 2D interaction diagram between maraviroc and the amino acids in the CCR5 binding pocket. The surface complementarity between the co-receptor and the drug allows the various interactions to be formed with the amino acids in the binding pocket of the protein

First, the side effects of the co-receptor antagonists due to off-target effects may be passive and, therefore, less severe than the lifelong effects of genetic modification of CCR5 associated with off-target effects. Second, most genome editing procedures require an individualized approach; where patient own tissue or cells are taken out, modified and returned back to the patient. Therefore, the cost of perfecting this procedure—ranging from pre-clinical, clinical trial to approval by regulatory agency could be quite high, which in turn increases the cost of treatment. This potentially makes CCR5 gene editing much less accessible than co-receptor antagonists-- which can be produced on a large scale. Hence, the affordability of the treatment options can be the main consideration towards ensuring the success of CCR5-based HIV-1 control strategies. Furthermore, the implementation of both the two strategies requires more refinements towards ensuring that the HIV-1 develops no viable mechanism in response to the scarcity or absence of CCR5.

6. CONCLUSIONS

Both the Genetic manipulation and pharmacological blockade of CCR5 have proven to be better in HIV-1 prevention, Control and Treatment than the use of HAART. However, these host-based strategies, with CCR5 as the

target, suffer technical challenges due to off targets effects. Another concern is the potential selection of CXCR4-tropic virus in response to CCR5 blockade. There is therefore need to take into consideration various viral potentials as well as the host's own off-targets while perfecting these strategies. Moreover, the use of CCR5 antagonist may have some advantages over genetic modification in terms of affordability and reduced severity associated with off-targets.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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