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CCR5 Monoclonal Antibodies for HIV-1 Therapy

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Abstract

Purpose of review—This report summarizes emerging clinical and preclinical data pertaining to the use of CCR5 monoclonal antibodies (mAbs) as therapies for HIV-1 infection. The epitope specificity of CCR5 mAbs is discussed in relation to its critical impact on antiviral activity and CCR5 antagonism. We compare and contrast mAbs and small-molecule CCR5 antagonists in terms of their binding and antiviral properties. Two CCR5 mAbs have entered clinical testing and have successfully completed proof-of-concept studies in HIV-infected individuals, providing initial information on the potential therapeutic utility of these agents.

Recent findings—New studies support the view that the most potently antiviral CCR5 mAbs recognize the second extracellular loop of CCR5 either exclusively or in combination with the amino terminus. Studies have revealed fundamental differences in how mAbs and small molecules bind CCR5 and inhibit HIV-1. CCR5 mAbs and small-molecule CCR5 antagonists have demonstrated consistent antiviral synergy and limited or no viral cross-resistance in independent studies. Single intravenous infusions of CCR5 mAbs significantly reduced HIV-1 RNA levels in infected individuals for 2–3 weeks without appreciable toxicity.

Summary—CCR5 mAbs have demonstrated broad and potent antiviral activity *in vitro*. Clinical studies have established CCR5 mAbs as potent antiretroviral agents with prolonged activity following a single dose. CCR5 mAbs represent both a distinct class of CCR5 inhibitor and a novel approach to HIV-1 therapy.

Keywords

CCR5; monoclonal antibody; PRO 140; HGS004

Introduction

CCR5 monoclonal antibodies (mAbs) recently entered proof-of-concept trials in HIV-infected individuals with only CCR5-tropic (R5) virus detectable, and results from the first two studies are available. Single, well-tolerated infusions of CCR5 mAbs resulted in potent, rapid and prolonged reductions in viral load, and the single-dose antiviral effects compared favorably with those obtained after 10 to 14 days of treatment with small-molecule CCR5 antagonists. The emerging clinical and laboratory data support the view that CCR5 mAbs offer several potential advantages over existing antiretroviral therapies in terms of potency, tolerability, dosing frequency and other factors.

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CCR5 Structure and Roles in Immune Function and HIV-1 Infection

CCR5 biology was reviewed recently [1]. The following paragraphs summarize critical aspects and recent developments. HIV-1 entry into host cells proceeds through a coordinated series of events mediated by the viral envelope glycoproteins gp120/gp41 and host cell receptors. HIV-1 gp120 first binds its primary receptor, CD4 [2], and then undergoes a conformational change that exposes a binding site for a chemokine receptor [3;4], principally CCR5 [5–7] or CXCR4 [8], that acts as a fusion co-receptor. These events trigger gp41-mediated fusion of the viral and cellular membranes. CCR5 is the predominant co-receptor used by HIV-1 for transmission and during the early stages of infection. In contrast, CXCR4-using variants are rarely transmitted but can become more apparent later in disease [9–14].

CCR5 belongs to a family of G-protein-coupled receptors (GPCRs) that respond to stimuli ranging from photons to proteins. CCR5 is expressed on a number of cell types, including activated/memory $CD4^+$ and $CD8^+$ T cells, macrophages, NK cells, NKT cells, microglia and astrocytes. On $CD4^+$ T cells, CCR5 is a marker for a T helper 1 (Th1) phenotype. CCR5 regulates cell migration, activation and polarization through multiple kinase pathways. Its natural agonists are the chemokines CCL3 (MIP-1α), CCL3L1 (MIP-1αP), CCL4 (MIP-1β) and CCL5 (RANTES), which are soluble ~8 kDa cytokines. HIV-1 infection does not require CCR5 signaling.

CCR5 spans the plasma membrane seven times in a serpentine manner (Figure 1) [15]. The extracellular portions represent potential targets for HIV-inhibitory mAbs and comprise an amino-terminal domain (Nt) and three extracellular loops (ECL1, ECL2 and ECL3). Sulfation of Nt tyrosines promotes gp120 binding and facilitates HIV-1 entry [16;17].

Numerous coding region, regulatory region and copy-number polymorphisms exist in the genes for CCR5 and its ligands. The geographic distributions of these polymorphisms suggest that these genes have evolved in response to differing environmental pathogens. Polymorphisms in CCR5 and its ligands critically affect HIV-1 transmission and disease progression [9–14; 18–20]. A number of polymorphisms have a greater disease-modifying effect than heterozygosity for the well-studied 32 basepair deletion in the CCR5 coding region (Δ32). Δ32 is an inactivating mutation that affords nearly complete protection from HIV-1 infection when present in two copies. Δ32 homozygotes lack a functional CCR5 receptor. This genetic absence of CCR5 has no obvious effect on human health, but it has been associated with increased risk for symptomatic disease from flavivirus infections [21;22]. The findings support development of agents that block HIV-1 without abrogating CCR5's role in normal immune function.

Generation of CCR5 mAbs

Numerous CCR5 mAbs have been described [23–33]. Most mAbs were generated by immunizing wild-type mice with rodent cell lines engineered to express human CCR5. Typically, hybridomas were generated and then screened for production of mAbs that bind CCR5. In other cases, hybridomas were initially screened for inhibition of HIV-1 envelope-mediated cell fusion. This latter approached yielded mAbs with potent antiviral activity [25; 32].

Alternatively, CCR5 mAbs were generated in mice transgenic for the heavy and light chain genes for human antibodies [31] or were isolated from a phage antibody library constructed from non-immunized human donors [28]. Another mAb was isolated from a phage antibody library derived from an immunized rabbit [30]. Additional mAbs were raised against an Nt peptide [29].

As a class, GPCRs are not highly immunogenic in their native states embedded within the cell membrane. The extracellular portion of CCR5 comprises just 90 amino acids distributed over four domains. The largest of these domains are the Nt and ECL2 at approximately 30 amino acids each. It perhaps is not surprising that significant efforts were expended to generate CCR5 mAbs: Wu *et al.* identified CCR5 mAbs in one of eight hybridoma fusions [23], while other groups reported screening between 10,000 and 25,000 hybridoma supernatants to identify six to seven novel CCR5 mAbs [25;32].

Epitope specificity

The most potent HIV-inhibitory mAbs described to date recognize conformational epitopes. For such mAbs, specificity has been mapped using CCR5 point mutants [25;27;33;34], CCR5 deletants, and/or CCR5 chimeras that contain extracellular regions from homologous chemokine receptors [26;27;33;34]. These approaches have yielded results that are broadly consistent, with CCR5 point mutants providing the greatest precision. For example, independent groups have mapped the epitope for mAb 2D7 to ECL2 using CCR5/CCR2b chimeras [24;27;33;34]. The 2D7 epitope was mapped to ECL2 residues K171 and E172 using CCR5 alanine point mutants [25;27;33;34].

Table 1 lists the epitopes recognized by mAbs that have been mapped using CCR5 point mutants, and the amino acids involved in mAb binding are illustrated in Figure 1. For these mAbs, the dominant epitopes lie within the Nt and ECL2, which are the largest extracellular regions and show significant divergence from mouse CCR5. As illustrated in Figure 1, ECL2 can be divided into amino-terminal and carboxy-terminal regions based on patterns of mAb reactivity [27;34].

Most potently antiviral mAbs bind residues in ECL2 alone or in combination with Nt residues [25;27;34]. Compared to ECL2 mAbs, mAbs that bind exclusively to the Nt typically have less potent antiviral activity [24;25;27]. In contrast, Nt mAbs are more potent than ECL2 mAbs in blocking binding of soluble gp120/CD4 complexes to CCR5 [25;27;34]. This finding presumably reflects the multiple roles of CCR5 in binding gp120 and triggering membrane fusion.

These findings are consistent with a two-site model for gp120-CCR5 interactions [35;36]. In the model for subtype B viruses, the bridging sheet and V3 stem on gp120 bind to tyrosine-sulfated forms of the CCR5 Nt, whereas the V3 crown interacts with ECL2 [16;37–40]. The model suggests that optimal inhibition of HIV-1 may be obtained with a mAb that occludes HIV-1's access to both ECL2 and Nt, either by directly binding a multidomain epitope or by steric hindrance.

The binding sites for CCR5 mAbs are distinct from those for small-molecule CCR5 antagonists. These differences in CCR5 binding translate into important differences in antiviral properties, as described below. The available small-molecule CCR5 inhibitors bind the hydrophobic cavity formed by the transmembrane helices. Notably, E283 in the seventh transmembrane region is a principal site of interaction for small molecules. A recent study mapped the binding sites for maraviroc and vicriviroc to an identical set of amino acids [41], as indicated in Figure 1.

Antiviral activity in vitro

Although numerous CCR5 mAbs have been described, few broadly and potently inhibit HIV-1. As discussed above, epitope specificity critically influences antiviral activity. Antiviral activity did not correlate with CCR5 binding affinity for mAbs to unrelated epitopes [31]; however, antiviral activity tracked CCR5 binding affinity for mAbs to similar epitopes [34;42].

Given the multitude of CCR5 mAbs developed by independent groups, no systematic comparison of antiviral activities has been performed. However, a number of mAbs were tested using PhenoSense™ HIV-1 Entry (Monogram Biosciences, South San Francisco, CA), a single-cycle assay that utilizes HIV-1 envelope-complemented reporter viruses [43–46]. The validated, reproducible nature of this assay enables some limited cross-study generalizations. Based on the published information, the most potent CCR5 mAbs demonstrated 50% inhibitory concentrations (IC50s) in the range of 0.1–1.0 μg/mL (0.67–6.7 nM), with an approximately 1 $\log_{10}$ variation across diverse viral isolates. The mAbs afford essentially complete inhibition at higher concentrations [43–46].

CCR5 mAbs have demonstrated similar potencies for viruses derived from different genetic subtypes [32;47;48], stages of disease [49], and adult and pediatric infections [50]. CCR5 expression levels show considerable person-to-person variation [23;51;52] and have been reported to affect both HIV-1 infectivity [23] and the potency of CCR5 inhibitors in vitro [52]. CCR5 mAbs efficiently inhibited CCR5-mediated entry of dual/mixed (R5X4) viruses in cell lines that express CCR5 but not CXCR4 [25;32;50]; however, limited inhibition of R5X4 viruses was observed in cultures of peripheral blood mononuclear cells [47–49].

CCR5 antagonism

In contrast to the two-site model for gp120, chemokines principally bind CCR5 via ECL2 [24;53;54]. The binding sites for gp120 and chemokines on CCR5 therefore are overlapping but distinct, and the antiviral and antagonist activities of CCR5 mAbs are dissociable. ECL2 mAbs often inhibit HIV-1 and chemokine signaling with similar efficiencies [24;25;32]; Nt mAbs typically display minimal CCR5 antagonism but less potent antiviral activity [25;32;33;55]. Rarely, CCR5 mAbs have been reported to possess agonist or partial agonist activity [33]; most mAbs do not activate CCR5 at any concentration.

Amongst the mAbs described in the published literature, the mAb PA14 binds a unique epitope spanning ECL2 and Nt [25]. PA14 preferentially inhibited HIV-1 at concentrations that did not block the natural activity of CCR5 *in vitro*, although CCR5 antagonism was observed at higher concentrations [25]. When compared with the ECL2 mAb 2D7 in parallel testing, PA14 was a more potent HIV-1 inhibitor and a less potent CCR5 antagonist. The findings indicate that PA14 can distinguish fine differences in the binding sites for HIV-1 and chemokines on CCR5.

Synergy with other antiretroviral agents

Three *in vitro* studies examined the antiviral activity of CCR5 mAbs in combination with small-molecule CCR5 antagonists [56–58]. The antibodies examined were PA14, PRO 140 (humanized PA14), 2D7, RoAb13, RoAb14, 2D7 and 45523. The small-molecule CCR5 antagonists included maraviroc, vicriviroc, aplaviroc, SCH-C and TAK-779. Antiviral synergy was reported by each group for most studied combinations of CCR5 mAbs and small-molecule antagonists, and the synergy was attributed to co-binding of CCR5 [56;57]. One notable exception was mAb 45523 used in combination with either maraviroc or aplaviroc, where synergy was not observed due to competition for CCR5 binding [57]. In parallel studies, additive rather than synergistic effects were observed for combinations of small-molecule CCR5 inhibitors [56;57]. The findings provide a rationale to combine CCR5 mAbs and small-molecule antagonists in the clinic and further underscore the mechanistic differences between these classes of CCR5 inhibitors.

Synergy also was reported for combinations of CCR5 mAbs that bind distinct epitopes, with the highest synergy observed between Nt and ECL2 mAbs [25;57]. Additive to synergistic effects were reported between CCR5 mAbs and enfuvirtide, a peptide inhibitor of gp41

membrane fusion [56;57]. Additivity was observed between CCR5 mAbs that bind similar or overlapping epitopes.

Cross-resistance between CCR5 mAbs and small-molecule CCR5 antagonists

Viruses resistant to small-molecule CCR5 antagonists were generated by serial passage of virus in the presence of increasing concentrations of inhibitor in vitro. These viruses typically retained an R5 phenotype and acquired the ability to utilize inhibitor-bound receptor [59–64]. *In vivo* resistance has reflected the emergence of resistant R5 viruses as well as the outgrowth of pre-existing R5X4 viruses [65;66]. In single-cycle antiviral assays, viral resistance to small-molecule antagonists was manifest as a reduction in the maximum percent inhibition at high inhibitor concentrations rather than a change in IC50 [59–63;66], consistent with the view that small-molecule CCR5 antagonists act as allosteric inhibitors [67–69].

Several small-molecule resistant viruses were tested for susceptibility to CCR5 mAbs. Despite demonstrating high-level resistance to the small-molecule CCR5 antagonists, the viruses remained susceptible or even hyper-susceptible to inhibition by CCR5 mAbs [59–61;66;70]. The lack of cross-resistance between mAbs and small-molecule CCR5 antagonists likely reflects differences in their modes of CCR5 binding (Figure 1) and mechanisms of HIV-1 inhibition (competitive v. allosteric).

There is limited information at present regarding forced viral resistance to CCR5 mAbs. Additional studies are needed to determine whether such viruses retain an R5 phenotype and are susceptible to inhibition by small-molecule CCR5 antagonists.

Human clinical studies

Two CCR5 mAbs have been tested in HIV-infected individuals [42;51]. Both mAbs are of the human IgG4 isotype, and the studies shared several design similarities. In each case, the mAbs were studied as single, escalating intravenous infusions to HIV-infected subjects with HIV-1 RNA >5,000 copies/mL, CD4 > 250 cells/μL, only CCR5-tropic (R5) virus detectable, and no concurrent antiretroviral therapy. Co-receptor tropism was assessed using the first-generation Trofile™ assay (Monogram Biosciences) [43]. In both studies, subjects were followed for 56–58 days post-treatment to assess tolerability, pharmacokinetics (PK) and antiviral effects.

Unlike the development programs for small-molecule CCR5 antagonists [71–73], the phase 1 programs for the CCR5 mAbs did not examine drug-drug or food interactions. Such studies were not necessary given that mAbs are injected and are catabolized by proteolysis within cells of the reticuloendothelial system. This process is distinct from and does not interfere with the typical metabolic pathways for small-molecule drugs.

HGS004 (CCR5mAb004, Human Genome Sciences, Rockville, MD) [42]—HGS004 is a human mAb that binds ECL2 and inhibits R5 HIV-1 entry and chemokine signaling with similar efficiencies [31]. In a phase 1 clinical trial, 63 subjects were randomized to receive placebo or HGS004 at doses of 0.4, 2, 8, 20 and 40 mg/kg. Subjects were mostly male (86%) with a mean age of 41 years and mean HIV-1 RNA levels of 25,100 copies/mL. All subjects completed the study. Two 2mg/kg subjects experienced infusion-related uticarial rash that responded to diphenhydramine, and all subsequent subjects were pre-treated with diphenhydramine prior to infusion.

Significant reductions in HIV-1 RNA were observed at doses of 8 mg/kg and higher. Plasma HIV-1 RNA reductions of >1 $\log_{10}$ were observed in 14 of 26 subjects (54%) treated with 8, 20 or 40 mg/kg HGS004. Mean viral load reductions of 1 $\log_{10}$ were observed at day 14 for the 8 and 20 mg/kg groups. At 40 mg/kg, the mean viral load reduction was approximately 0.8

log_{10} at days 14 and 21. Three of ten 40 mg/kg subjects experienced a change in co-receptor tropism to dual/mixed virus on study. One of these individuals experienced a transient 1 log_{10} reduction in HIV-1 RNA; the others had no significant antiviral response. Co-receptor tropism changes also were observed in one subject each in the 0.4 and 20 mg/kg groups but not in other groups.

PK data were non-linear. Although the maximum serum concentrations were dose proportional, overall exposure (area under the concentration-time curve, AUC) increased more than proportionally with dose. The mean terminal serum half-life ranged from 4.7 to 7.9 days across the different dose levels. The mean CCR5 receptor occupancy was approximately 80% at day 28 for each of the three highest dose groups. Significant increases in CD4 and CD8 cell counts were observed in all HGS004 groups, and this finding was hypothesized to reflect redistribution of CCR5-expressing cells from peripheral tissues into the blood.

PRO 140 (Progenics Pharmaceuticals, Inc., Tarrytown, NY) [51]—PRO 140 is a humanized form of the mouse mAb PA14, which binds an epitope spanning ECL2 and Nt. PA14 and PRO 140 have been characterized for breadth and potency of antiviral activity in several preclinical studies [25;47;49;50;56;74].

The first HIV trial of PRO 140 was a randomized, double-blind, placebo-controlled study in 39 individuals with early-stage disease. Cohorts were randomized 3:10 to receive a single infusion of placebo or PRO 140 at doses of 0.5, 2 or 5 mg/kg. PRO 140 was generally well tolerated, and no dose-limiting toxicity or pattern of toxicity was observed. There was no requirement to pre-medicate with antihistamines. There were no remarkable laboratory or electrocardiogram findings.

Rapid, dose-dependent and highly significant reductions in HIV-1 RNA were observed (Figure 2). Mean maximum (nadir) viral load reductions of 0.39±0.20, 0.58±0.30, 1.20±0.63 ($p=0.0002$) and 1.83±0.41 log_{10} ($p<0.0001$) were observed for the placebo, 0.5 mg/kg, 2 mg/kg and 5 mg/kg groups, respectively. At day 10, the mean log_{10} declines in viral load were 0.13±0.24, 0.37±0.54, 1.04±0.45 ($p=0.0001$) and 1.70±0.49 ($p<0.0001$) for the placebo and ascending dose groups. Mean viral load reductions of >1 log_{10} persisted for 2–3 weeks post-treatment in the 5 mg/kg group. All 5 mg/kg subjects had an antiviral response of ≥1.0 log_{10} reduction in HIV-1 RNA (Figure 2). These single-dose antiviral effects are the largest reported for any HIV-1 drug and compare favorably with those observed following 10 to 14 days of treatment with small-molecule CCR5 antagonists [75–77].

The area under the PRO 140 concentration-time curve from time zero to infinity (AUC_∞) values increased more than proportionally with dose, averaging 11.1, 74.3 and 278 mg × day/L for the ascending dose groups. Mean terminal serum half-lives were 3.9 and 3.5 days for the 2 and 5 mg/kg dose groups, respectively. All tests for anti-PRO 140 antibodies were negative with the exception of a single low-titer result at Day 59 for a 5 mg/kg subject. The antibodies had no obvious effect on PK metrics or antiviral response. At 5 mg/kg PRO 140, there was a trend ($p=0.055$) towards increased $CD4^+$ cells over baseline. There was no depletion of $CCR5^+$ cells following treatment; however, significant receptor occupancy ($p<0.05$) was observed for 2–4 weeks in all PRO 140 groups. This study established PRO 140 as a potent antiretroviral agent with prolonged activity.

Conclusions

CCR5 mAbs broadly and potently inhibit R5 HIV-1 in vitro, and potent antiviral activity has been demonstrated in HIV-infected individuals. CCR5 mAbs represent a novel approach to HIV-1 therapy and offer several potential advantages over existing therapies in terms of

infrequent (e.g., weekly to monthly) dosing, favorable tolerability, and limited drug-drug or food interactions. CCR5 mAbs are distinct from small-molecule CCR5 antagonists in terms of their binding sites on CCR5 and mechanisms of HIV-1 inhibition. CCR5 mAbs and small-molecule antagonists can be considered distinct classes of CCR5 inhibitors based on their potent antiviral synergy and lack of viral cross-resistance. Clinical proof of concept has been obtained using intravenously administered CCR5 mAbs. Additional studies of intravenously and subcutaneously administered CCR5 mAbs have been initiated.

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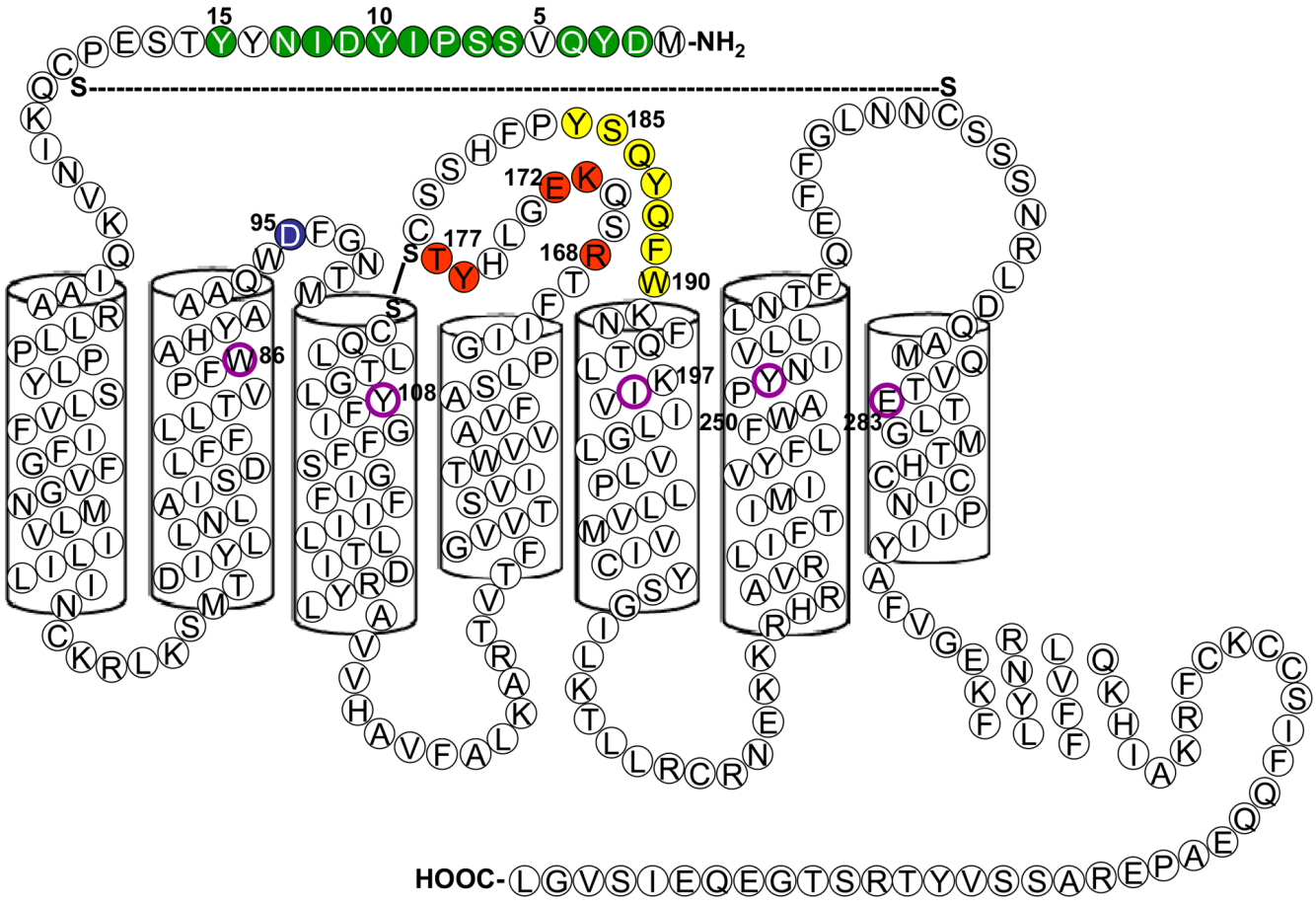

Figure 1. Schematic diagram of CCR5 illustrating the binding sites for mAbs and small molecules

Amino acids are indicated in the single-letter code. Residues implicated in mAb binding are color-coded by filled symbols in the extracellular domain or sub-domain. Green = Nt; blue = ECL1; red = amino-terminal portion of ECL2; yellow = carboxy-terminal portion of ECL2. The specificities of individual mAbs are listed in Table 1. A putative binding site for maraviroc and vicriviroc in the transmembrane helices (residues W86, Y108, I198, Y251 and E283) is illustrated with magenta circles. The assignment of the seven transmembrane helices [15] is indicated with cylinders.

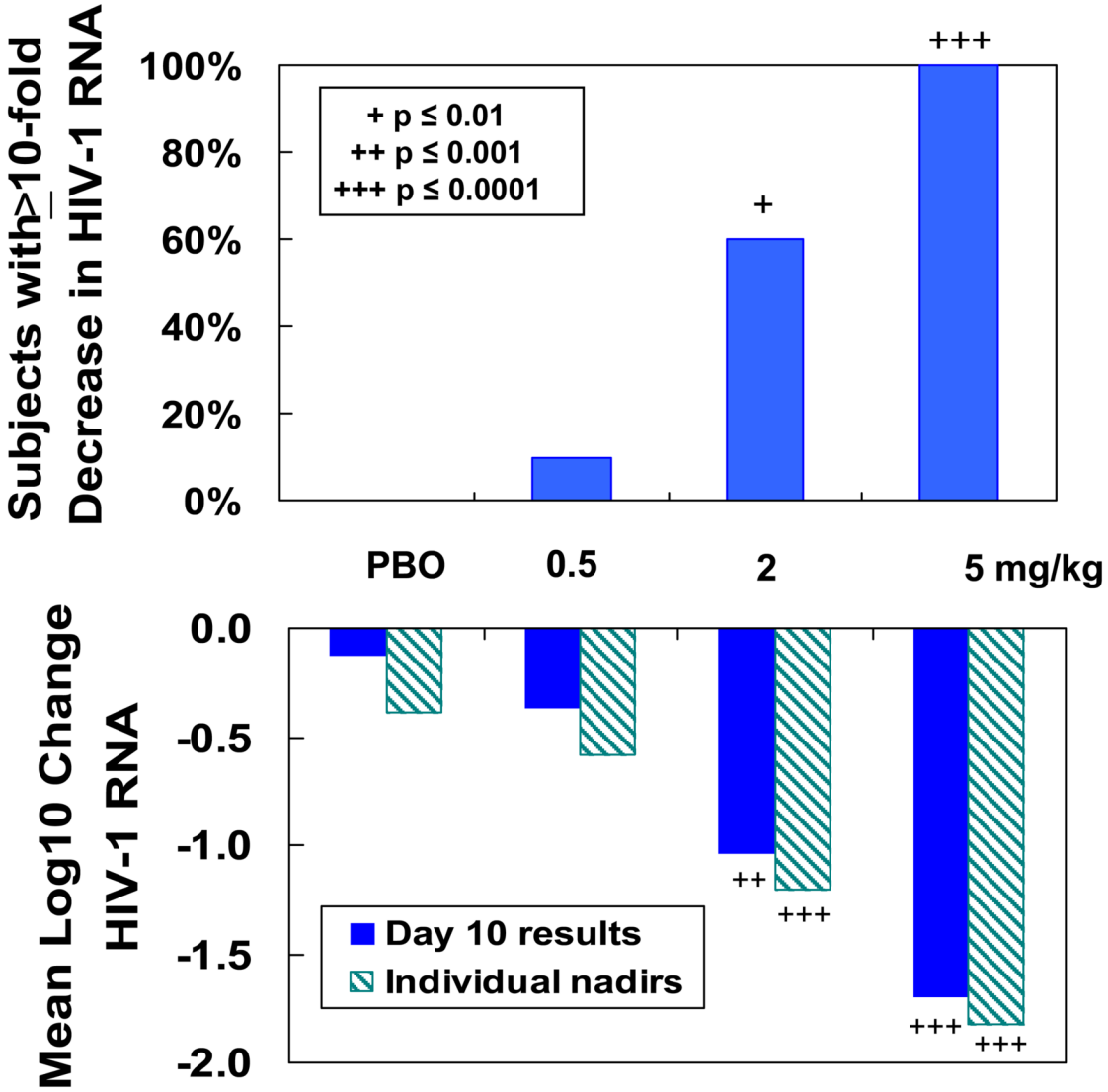

Figure 2. Antiviral activity of single-dose PRO 140 in HIV-infected adults

Subjects received single intravenous infusions of placebo (PBO) or PRO 140 at doses of 0.5 mg/kg, 2 mg/kg or 5 mg/kg. Plasma HIV-1 RNA levels were monitored for 58 days. ***Top panel:*** Percentage of subjects in each treatment group who experienced a ≥ 10-fold reduction in HIV-1 RNA at any timepoint post-treatment. ***Bottom panel:*** Mean $\log_{10}$ changes in HIV-1 RNA for each treatment group. Study Day 10 represents nine days post-treatment. An individual nadir represents the maximum reduction experienced by a subject at any time-point post-treatment.

Table 1

Amino acids implicated in mAb binding to CCR5 as determined using CCR5 point mutants

Mutation of the indicated amino acids was reported to reduce mAb binding to CCR5 as determined by flow cytometry.

Antibody	Epitope	Reference(s)
2D7	Q170, K171, E172, W190	[25;27;33;34]
502	D2, Y3	[27]
519	D2, Y3, Q4	[27]
45501	K171, E172	[27]
45517	K171, E172	[27]
45523	K171, E172, D95	[27]
45529	Y184, S185, Q186, Y187, Q188, F189	[27]
45531	Y184, S185, Q186, Y187, Q188, F189	[27]
45533	K171, E172	[27]
45549	K171, E172	[27]
CTC2	S6, S7, Y10, D11	[27]
CTC5	D2	[27]
CTC8	Y10, D11, I12, N13	[27]
CTC9	D2, Y3	[27]
CTC12	S7, I9, Y10, D11	[27]
MC-6	K171, E172	[33]
MC-7	S7, P8, Y10, D11	[33]
MCR35.4	Y184, S185, Q186, Y187, Q188, F189	[27]
MCR40.3	Y184, S185, Q186, Y187, Q188, F189	[27]
PA8	N13, Y15	[25]
PA9	D2, Y3, Q4, S7, P8, N13, Y176, T177	[25]
PA10	D2, Y3, Q4, P8, N13, Y176, T177	[25]
PA11	Q4	[25]
PA12	Q4	[25]
PA14	D2, R168, Y176	[25]
RoAb12	K171, E172, W190	[34]
RoAb14	K171, E172, W190	[34]
RoAb18	K171, E172, W190	[34]