Galaxy Publication

A Predictive Approach for Determining the Optimal Anti-CCR5 Monoclonal Antibody

Tatiana Hillman[1]*

[1]Department of Health Sciences, University of Walden, Minneapolis, Minnesota, United States.

*E-mail ✉ thillman@thel4binc.org

ABSTRACT

Inhibition of CCR5 (R5) has gained significant attention as a potential strategy for preventing, treating, and even curing diseases. R5, a transmembrane protein, plays a crucial role in HIV infection by interacting with the CD4 receptor and CXCR4 (X4) on T cells, facilitating HIV binding to lymphocytes. Given the success of drugs such as maraviroc, which target R5, researchers have hypothesized that monoclonal antibodies (mAbs) targeting R5 could provide an effective treatment for HIV. However, there is a knowledge gap regarding the structural interactions between monoclonal antibodies and R5. To address this, our study used a predictive model involving a sample of n = 160, using non-linear regression analysis to examine the impact of different inhibitor types (control, competitive inhibitor (CI), non-competitive inhibitor (NI), and uncompetitive inhibitor (UI)) on the R5-gp120 interaction, based on Michaelis-Menten enzyme kinetics. Our results, with a significant P-value < 0.05, indicate that non-competitive anti-R5 mAbs are the most effective inhibitors, as the NI variant reduced the R5E Vmax to 20 μM/min while reducing the gp120S Km to 5 nM. This suggests that non-competitive anti-R5 mAbs could more efficiently block CCR5 activity, offering insights into the design of more potent allosteric inhibitors for CCR5.

Keywords: Glycoproteins, Co-receptors, CCR5, Monoclonal antibodies, HIV

Introduction

When a mutation known as a 32-base pair deletion (Δ32) occurs in the CCR5 (R5) gene, resistance to HIV-1 infection may develop, as the CCR5 protein is absent from the surface of the cells [1-3]. Individuals who are heterozygous for this mutation experience a slower progression of HIV disease, with less rapid CD4 cell count decline and lower viral loads [1-3]. As a result, CCR5 has become a promising target for therapeutic interventions against HIV-1 [1]. Initial research centered on the CD4 receptor, as it was discovered that HIV entered cells by binding to it [1-3]. However, it was soon recognized that the CD4 receptor alone was insufficient for HIV to enter the host cell. A coreceptor, CCR5, was also essential for the virus to gain access. This discovery, along with the identification of another coreceptor, CXCR4, came shortly after the CD4 receptor was elucidated [1-3]. CCR5 is a G protein-coupled receptor with seven transmembrane segments. The HIV gp120 envelope protein binds to the N-terminus and extracellular loops of CCR5, facilitating the virus's entry into T cells **(Figure 1a)**.

CCR5's natural ligands, such as Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES), macrophage inflammatory protein-1 alpha (MIP-1α), and macrophage inflammatory protein-1 beta (MIP-1β), were found to effectively inhibit HIV entry into host cells [2, 3]. This led to the development of synthetic CCR5 inhibitors designed to block HIV entry [1]. CCR5 is present on various cell types, including T lymphocytes, dendritic cells, and macrophages [4], and CCR5-tropic HIV-1 strains utilize this receptor for viral transmission [5]. Several CCR5-targeted drugs have been developed, including maraviroc, aplaviroc, vicriviroc, INCB009471, and TBR 652. These initial anti-HIV agents focus on disrupting host cell pathways rather than viral enzymes [1]. Additional CCR5 inhibitors currently in development include monoclonal antibodies such as PRO 140 and

HGS004. PRO 140, a humanized monoclonal antibody, blocks CCR5 and prevents CCR5-tropic HIV-1 from entering cells [6]. Similarly, HGS004, an IgG4 monoclonal antibody, also functions as a CCR5 antagonist [1]. Monoclonal antibodies (mAbs) targeting CCR5 as antagonists have garnered increased attention in recent studies [7], offering potential solutions for therapeutic interventions [3, 8-20]. However, the structural interaction between these mAbs and CCR5 remains underexplored [21]. Consequently, the understanding of CCR5 blockade by mAbs is still insufficient. Furthermore, there is a need for improvements in the molecular design of maraviroc [22]. A noteworthy dichotomy exists between small chemical antagonists and protein-based CCR5 inhibitors, where interactions between these two approaches may enhance the development of more effective drug designs for both classes of CCR5 antagonists [23]. Various anti-CCR5 mAb isotypes can target distinct CCR5 regions, including the amino-terminal domain, the second extracellular loop (ECL2), and other extracellular domains, such as the first extracellular loop (ECL1) **(Figures 1b and 1d)** [24]. mAbs binding to the N-terminus and extracellular loops of CCR5 may block HIV binding as competitive inhibitors **(Figures 1b and 1d)**. Although less understood, mAbs that bind to CCR5's transmembrane regions are believed to function as non-competitive allosteric inhibitors **(Figure 1c)**. These non-competitive mAbs do not interact with the binding sites for HIV-1 envelope proteins, which are essential for the virus's entry into host cells **(Figure 1c)**. Another possible mode of CCR5 inhibition involves uncompetitive inhibition, where an anti-CCR5 mAb binds to both an extracellular loop and the gp120 envelope protein, mimicking the CD4 receptor **(Figure 1d)**.

In light of these considerations, the current study aims to determine the most effective structural and molecular isotype of an anti-CCR5 mAb in preventing HIV envelope protein binding to lymphocytes. To achieve this, a predictive model was developed using Michaelis-Menten enzyme kinetics to evaluate the efficacy of different anti-CCR5 mAb inhibition types—competitive, non-competitive, or uncompetitive. The study used non-linear regression analysis of enzyme kinetics data, incorporating known concentrations of CCR5 (R5E) and HIV-1 gp120 envelope proteins (gp120S), along with anti-CCR5 mAb concentrations and established reaction rate constants for R5-to-gp120 binding interactions.
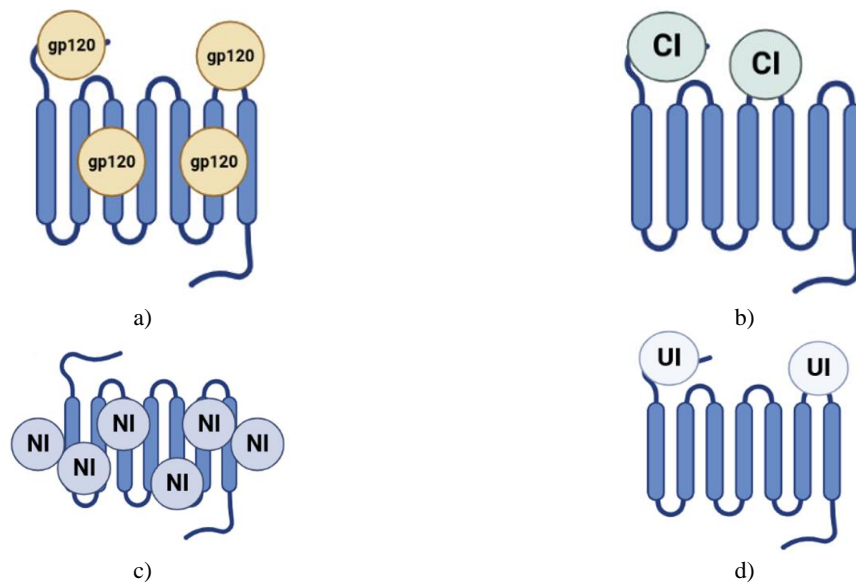

Figure 1. Potential binding sites of anti-R5 mAbs; this figure illustrates the various epitopes where anti-R5 monoclonal antibodies may attach; a) the interaction between HIV envelope gp120 proteins and CCR5, b) the anti-R5 mAb binds as a competitive inhibitor, c) the non-competitive mAb binding to the transmembrane regions of CCR5, and d) the binding of uncompetitive inhibitors

Materials and Methods

Study design

In this study, 160 data points (n = 160) were used and split into two distinct datasets for processing. Four experimental conditions were tested: a control group with no R5 inhibitors, and three conditions involving R5 inhibitors—competitive, non-competitive, and uncompetitive. One dataset included the molar concentrations of the gp120 protein (gp120S), while the second dataset measured the enzymatic activity, expressed as velocities

(μM/min), of the R5 protein (R5E) that facilitated the reaction with gp120S. Michaelis-Menten enzyme kinetics were applied to these datasets, and the parameters involved in each condition were analyzed, including the molar concentrations of R5E, gp120S, as well as the constants for reaction rates, Km, Ki, and Vmax.

Data calculation and approximations
To determine the molar concentrations of R5 and gp120, the molecular weights of R5 (40.6 kDa) and gp120 (120 kDa) were taken into account. The R5 protein acted as the enzyme, with gp120 serving as the substrate in the kinetic analysis. The estimated molar concentrations for R5E and gp120S were based on the fact that 10,000 molecules of R5 are needed for one HIV infection in a T cell. The concentration of R5E and gp120S was assumed to fall within a range of 1 billion to 1 trillion molecules per T cell, simulating a high viral load condition. The study tested four different conditions: the control group (no inhibitors), competitive inhibitors (CI), non-competitive inhibitors (NI), and uncompetitive inhibitors (UI). The initial concentrations for R5E and gp120S were 30 nM and 20 nM, respectively.

Michaelis-Menten kinetics analysis
The molar concentrations of R5E, gp120S, and the inhibitors (CI, NI, UI) were utilized to calculate both Km and Ki values for the reactions. The catalytic rate constant (kcat) for the enzymatic reactions was set at 60 minutes, and this was combined with the molar concentrations to compute Vmax for each condition. The Michaelis-Menten equations (1-4) were applied to compute the reaction velocities (μM/s) under the four conditions: no inhibitor, competitive inhibitor, non-competitive inhibitor, and uncompetitive inhibitor. Graphs were constructed using Excel for each condition. The concentrations of the inhibitors used in the calculations were 200, 500, 2000, and 10,000 μM. These concentrations were plotted against the molar concentrations of gp120S (nM), allowing the reaction velocities (μM/min) to be predicted for each interaction of R5E, gp120S, and the inhibitors (CI, NI, UI).

Statistical Analysis
To analyze the data from the Michaelis-Menten enzyme kinetics, descriptive statistics and unpaired t-tests were conducted using GraphPad Prism. Statistical significance was determined with a P-value of < 0.05. Additionally, a non-linear regression model, developed in Excel, was employed to evaluate the enzyme kinetic data and forecast which anti-R5 mAb inhibitor (CI, NI, or UI) would most effectively impede the R5-gp120 interaction.

$$V = Vmax[S]/Km+[S] \tag{1}$$

$$V = Vmax[S]/(Km(1+[I]/Ki) + [S]) \tag{2}$$

$$V = Vmax/(1+[I]/Ki) [S]/Km+[S] \tag{3}$$

$$V = (Vmax/(1+[I]/Ki))[S]/Km(1+[I]/Ki)+[S] \tag{4}$$
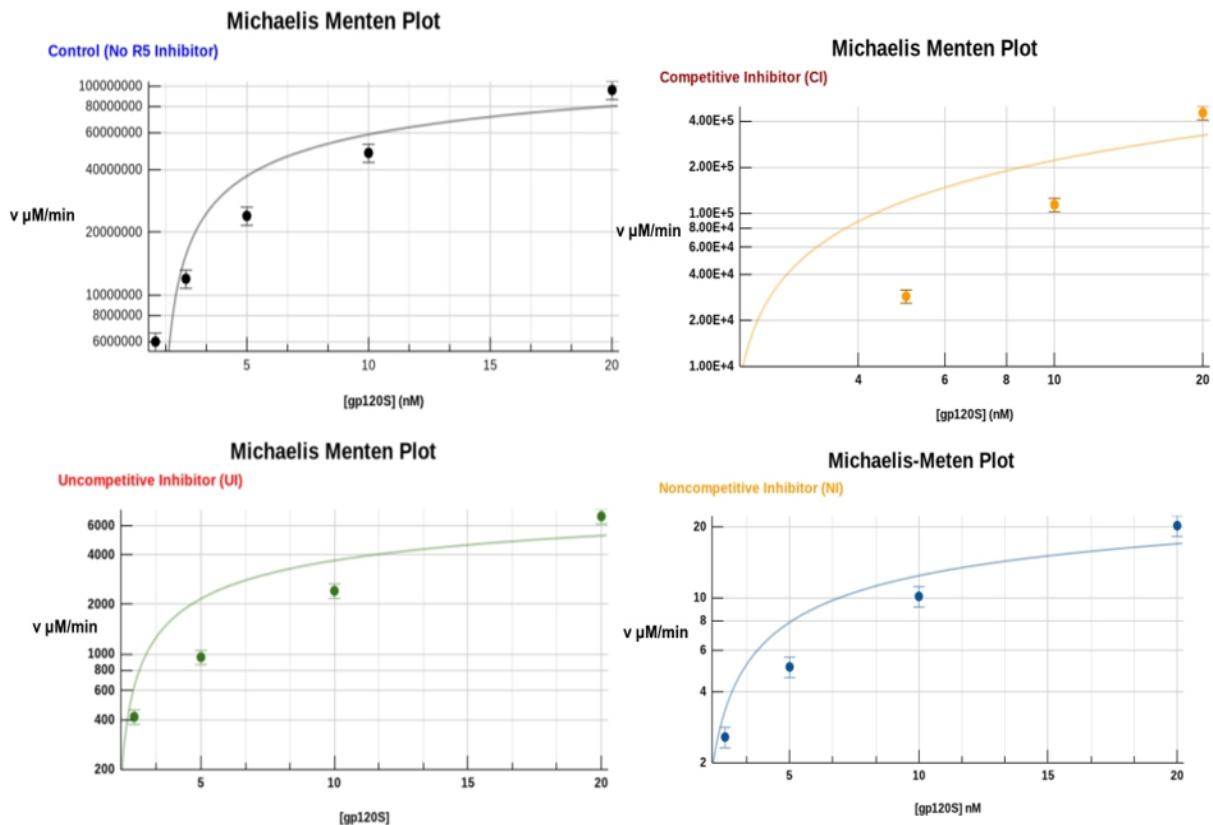
Results and Discussion

The molecular weights and concentrations of CCR5 and gp120 envelope protein were used to extract data, with reaction rate constants calculated from the known binding rates of gp120 to CCR5. Michaelis-Menten enzyme kinetics were applied using the molarities and distributions of R5 and gp120 on T cells to determine both initial and subsequent velocities. In this study, CCR5 acted as the enzyme (R5E), while gp120 was the substrate (gp120S). Anti-CCR5 monoclonal antibodies (mAbs) were classified as competitive (CI), non-competitive (NI), or uncompetitive inhibitors (UI). The kinetic data were analyzed using nonlinear regression to create a predictive model. For the control group, the Vmax was over 1 billion μMmin-1, while the competitive inhibitor reduced it to 2E + 7 μMmin-1, and the uncompetitive inhibitor brought it down to 2 million μMmin-1. The most effective inhibitor was the non-competitive mAb, which reduced the Vmax to 20 μMmin-1 **(Figures 2a-5a)**. The Km values for the control, CI, NI, and UI were recorded as 9.5 nM, 5 nM, 7 nM, and 8 nM, respectively **(Figures 2a-5a)**.
The P-values for enzyme kinetic data at 200 μM of inhibitors were 0.047 for CI, 0.046972 for NI, and 0.046978 for UI, determined by a one-tailed T-test **(Figure 2b)**. At 500 μM, the P-values were 0.049 for CI, 0.04697 for NI, and 0.04698 for UI **(Figure 3b)**. At 2000 μM, the P-values were 0.051 for CI, 0.046970 for NI, and 0.047 for UI **(Figure 4b)**. At 10,000 μM, the P-values were 0.0786 for CI, 0.04697 for NI, and 0.0471 for UI **(Figure 5b)**. Based on these results, the non-competitive anti-CCR5 mAb inhibitor was identified as the most effective at
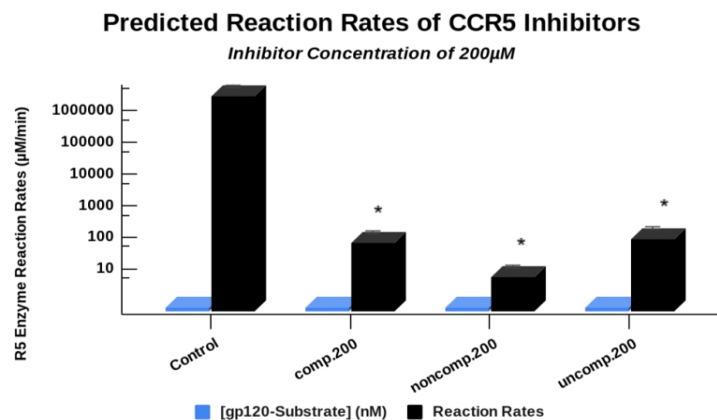
reducing the R5 reaction rate, with a P-value < 0.05, and requiring a lower dose compared to competitive and uncompetitive inhibitors **(Table 1)**.

Table 1. Displayed the Vmax and Km for each inhibitor at 200, 500, 2000, and 10,000 µM; the P-values are significant at $P < 0.05$

Inhibitor (µM)	Vmax/Km	P-values
200		
Control	1E + 8/10	
CI	3E + %/6	$P < 0.05$
NI	18/5.7	
UI	5E + 3/6	
500		
Control	1E + 8/10	
CI	1E + 6/7	$P < 0.05$
NI	20/5	
UI	1E + 5/10	
2000		
Control	1E + 8/10	
CI	4E + 6/7	$P < 0.05$
NI	19/5	
UI	4E + 4/6	
10000		
Control	1E + 8/10	
CI	2E + 7/8	$P < 0.05$
NI	19/5	
UI	2E + 5/9	

a)

Predicted Reaction Rates of CCR5 Inhibitors
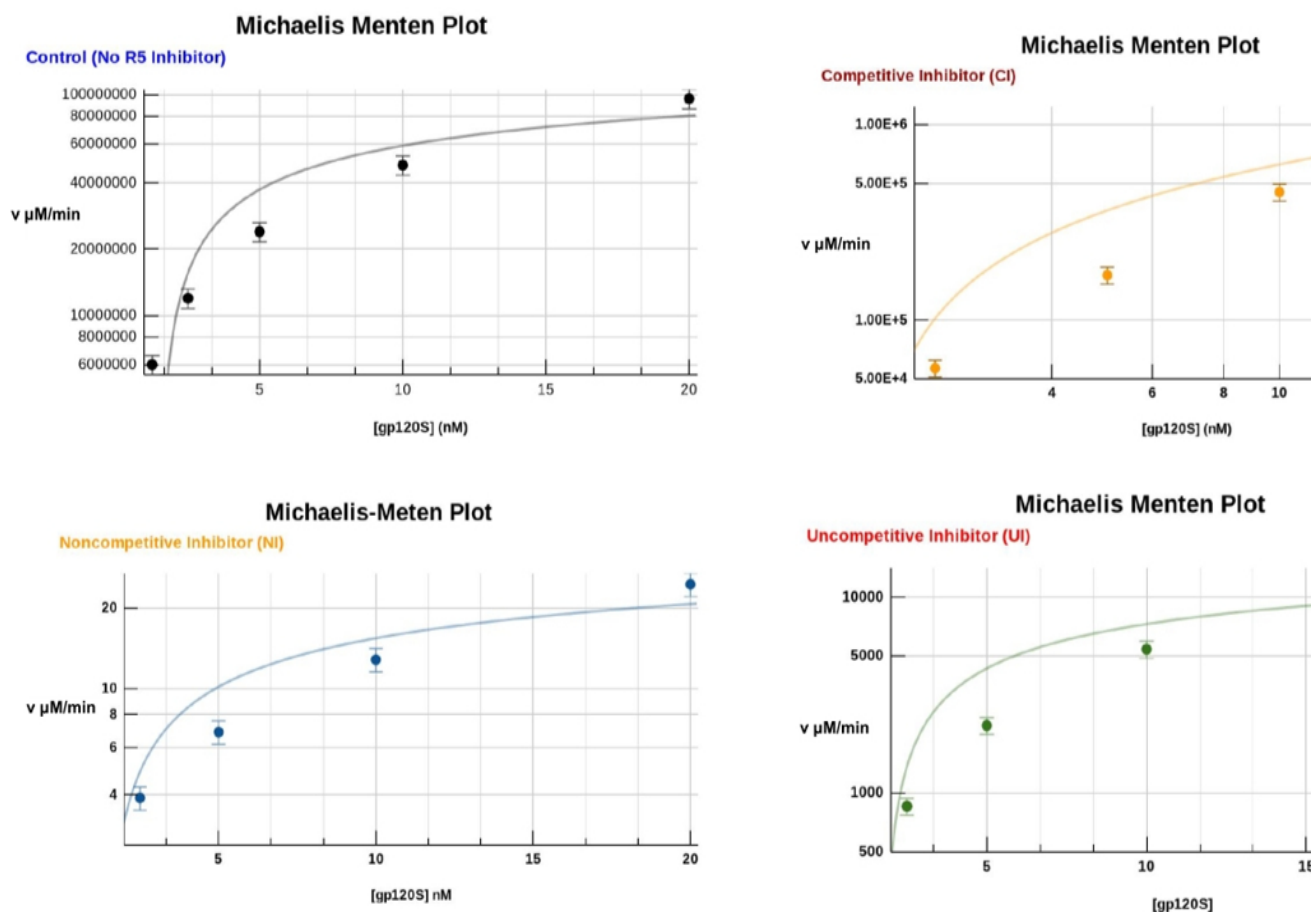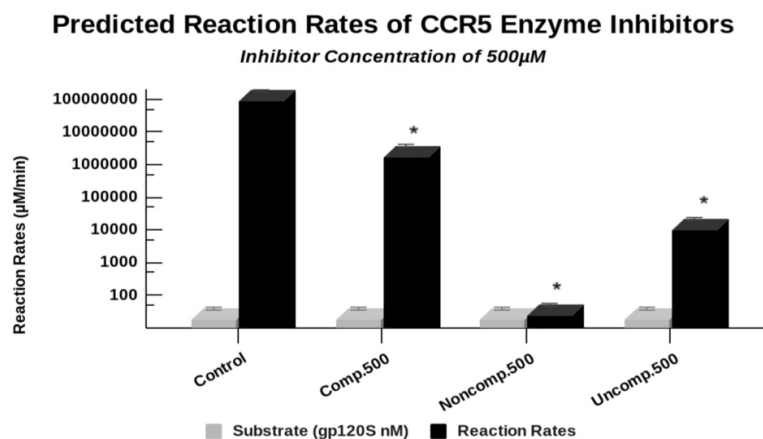### Inhibitor Concentration of 200µM

b)

Figure 2. Michaelis-Menten enzyme kinetics with non-linear regression for 200 µM inhibitors; a) this figure illustrates the Michaelis-Menten curve plots for each inhibitor (control, CI, UI, and NI), showcasing the Vmax and Km values for each condition, and b) statistical analysis of the enzyme kinetic data revealed P-values of 0.047 for CI, 0.046972 for NI, and 0.046978 for UI, calculated using a one-tailed T-test, with a significance threshold of $P < 0.05$
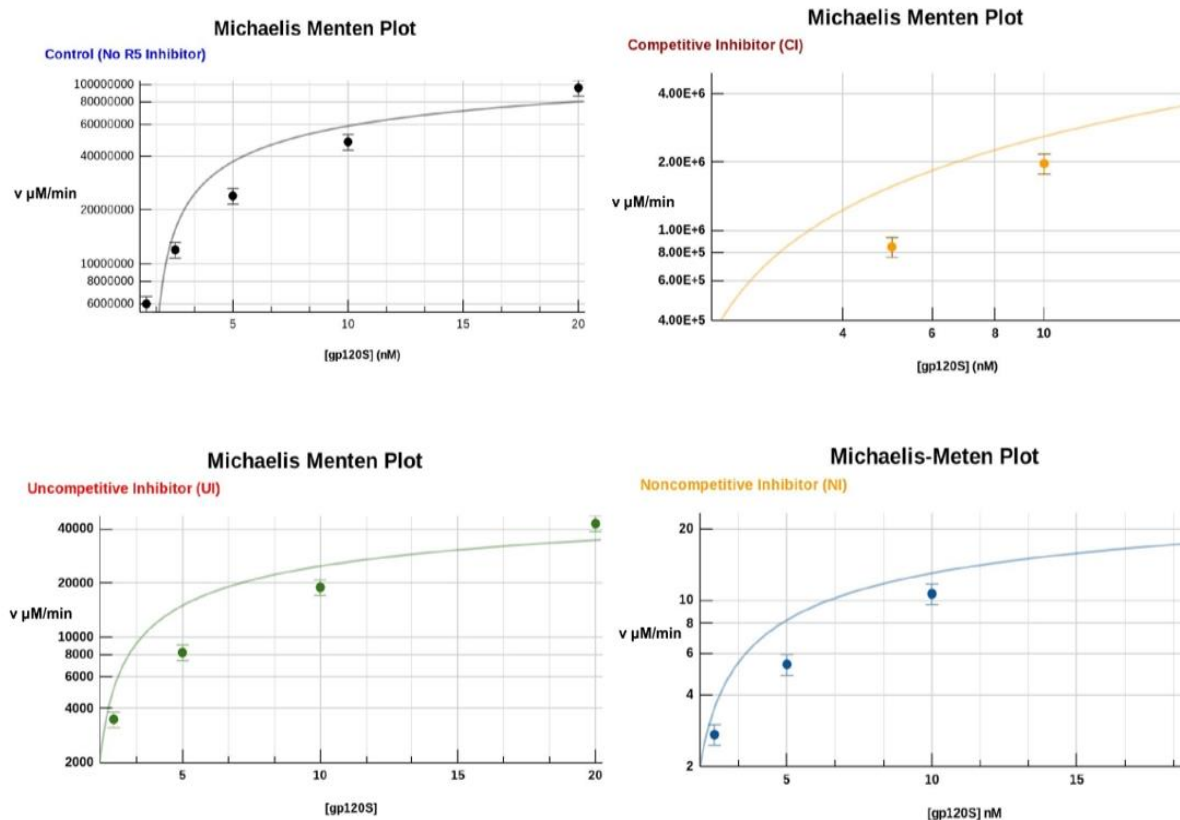

a)

b)

Figure 3. Michaelis-Menten enzyme kinetics with non-linear regression for 500 µM inhibitors; a) the figure displays the Michaelis-Menten curves for each inhibitor (control, CI, UI, and NI), indicating the Vmax and Km for each condition, b) statistical analysis of the enzyme kinetics revealed p-values of 0.049 for CI, 0.04697 for NI, and 0.04698 for UI, obtained using a one-tailed T-test, with statistical significance defined at $P < 0.05$.
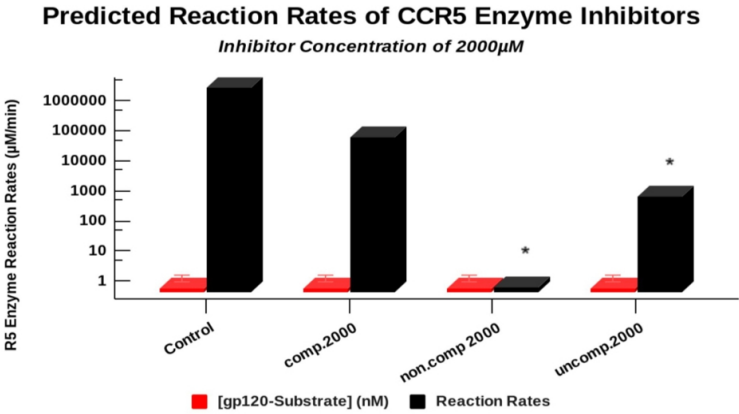

a)

b)

Figure 4. Michaelis-Menten enzyme kinetics with non-linear regression for 2000 µM inhibitors; a) the figure illustrates the Michaelis-Menten plots for each inhibitor (control, CI, UI, and NI), showing the Vmax and Km for each condition, and b) the enzyme kinetic analysis revealed P-values of 0.051 for CI, 0.046970 for NI, and 0.047 for UI, determined through a one-tailed T-test, with statistical significance considered at $P < 0.05$

a)

Predicted Reaction Rates of CCR5 Enzyme Inhibitors
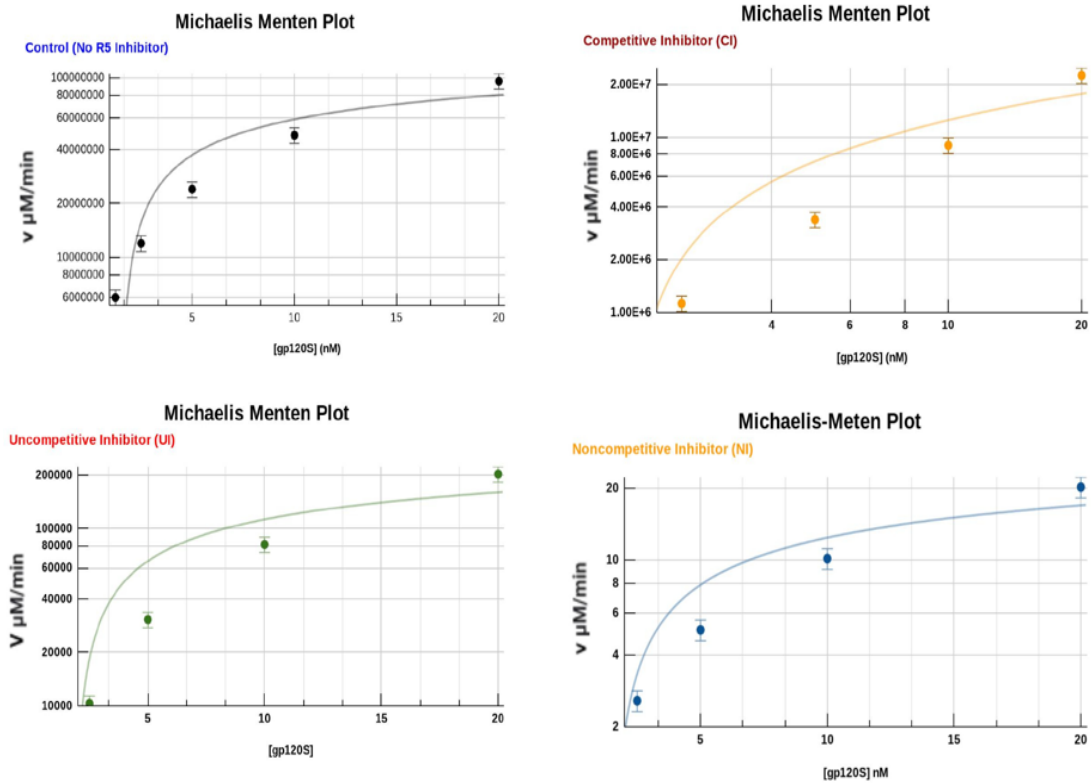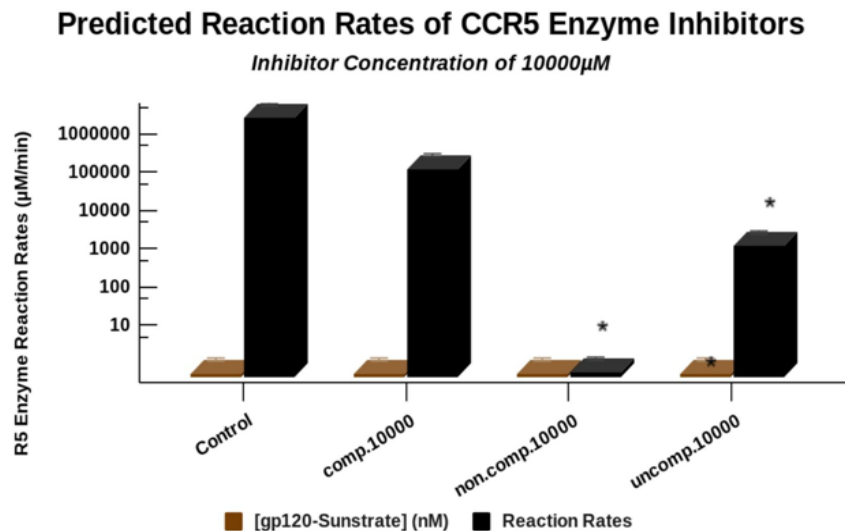### *Inhibitor Concentration of 10000μM*

b)

Figure 5. Michaelis-Menten enzyme kinetics with non-linear regression for 10,000 μM inhibitors; a) the figure presents the Michaelis-Menten plots for each inhibitor (control, CI, UI, and NI), showing the Vmax and Km for each condition; b) the enzyme kinetic analysis produced P-values of 0.0786 for CI, 0.04697 for NI, and 0.0471 for UI, calculated using a one-tailed T-test, with statistical significance set at $P < 0.05$

Monoclonal antibody (mAb) therapy targeting CCR5 has shown potential for improving treatment outcomes in HIV patients. This study utilized Michaelis-Menten enzyme kinetic data to assess the interaction between the HIV envelope protein gp120 and CCR5, predicting the effectiveness of competitive, non-competitive, and uncompetitive mAb inhibitors against CCR5. With a statistically significant result ($P < 0.05$), the findings suggest that non-competitive mAbs (NI) are the most potent, reducing the R5E Vmax to 20 μM/min and maintaining a gp120S Km of 5 nM. Additionally, antibodies targeting the first extracellular loop (ECL1) of CCR5 have not shown adverse effects on immune function, as evidenced by healthy individuals producing natural anti-CCR5 antibodies [22], as well as animal models like mice and macaques, where CCR5 immunization triggered anti-CCR5 antibody production [23-33]. On the other hand, mAbs directed at the ECL2 region of CCR5 can block chemokine binding and signaling [34]. Unlike these, mAbs that bind the N-terminus, where HIV-1 enters the cell, can prevent HIV-1 entry into CD4+ T cells, such as the 2D7 mAb targeting ECL2. However, 2D7 does not hinder transcytosis by epithelial cells [35-37]. Antibody-based treatments, in contrast to chemokines or standard antiretroviral drugs, may help reduce complications from drug resistance and unwanted interactions with other CCR receptors [38].

Competitive inhibition
A monoclonal antibody (mAb) targeting CCR5 as a competitive inhibitor could bind to the same site as the CCR5 antagonist, 5P12 CCL5, a key CCL5 5M derivative that interacts with the N-terminus of CCR5, converting CCL5 5M into an antagonist [39]. When the structure of CCL5 5P12 5M is examined, it is evident that its N-terminus differs from that of 5P7 by an amino acid exchange—leucine at position 7 in 5P12 versus threonine in 5P7. Due to the hydrophobic environment of CCR5, the leucine residue in 5P12 better fits this location compared to threonine in 5P7 [23]. Additionally, a mutation within the CCL5-CCR5 complex has been identified, where the interaction between the tyrosine residue of CCR5 S272 and a water molecule leads to enhanced anti-HIV-1 activity [39]. However, utilizing CCL5 derivatives for developing competitive anti-R5 mAb inhibitors poses challenges due to mutations in wild-type chemokines that can reduce receptor affinity and immune tolerance, diminishing their therapeutic effectiveness in clinical settings [23]. This study's modeling of enzyme kinetics for competitive inhibitors predicted a decrease in efficacy, as evidenced by the CI inhibitors' Vmax exceeding 1E + 7 μM/min, a Km of 7 nM, and diminishing statistical significance as the inhibitor dose increased. PRO140 is an example of a competitive anti-R5 mAb that prevents HIV from entering host cells, inhibiting viral replication while leaving CCR5's function intact in vitro [40-44]. PRO140 is considered a competitive inhibitor because it targets extracellular sites rather than transmembrane regions of CCR5 [45].

Non-competitive inhibition

An anti-R5 mAb functioning as a non-competitive allosteric antagonist binds to the intracellular and transmembrane regions of CCR5 [23]. By binding to intracellular regions of CCR5, these mAbs block G protein coupling, while binding to the transmembrane domains prevents conformational shifts necessary for G protein activation, including those for CCR5 [46-62]. GPCRs, such as CCR5, are susceptible to allosteric modulation, where molecules binding outside the orthosteric site regulate receptor activity. These modulators are classified as negative allosteric modulators (NAM) or positive allosteric modulators (PAM) [46]. Although fewer allosteric antibody modulators have been developed, they present promising therapeutic potential [46].

Anti-R5 mAbs acting as NAMs for therapeutic purposes offer substantial benefits, as these modulators tend to be more selective and can influence the activity of endogenous ligands, providing additional therapeutic advantages [46]. This study's predictive model demonstrates that non-competitive inhibition by anti-R5 mAbs is likely to be highly effective, as the Vmax of R5E was reduced to 20 µM/min and the Km of gp120S to 5 nM. Endogenous molecules like lipids and ions regulate GPCR activity; however, they lack the required properties for designing synthetic allosteric modulators [46]. As endogenous molecules, anti-R5 mAbs may outperform synthetic allosteric modulators in effectively inhibiting CCR5. The development of allosteric modulators can be accelerated by integrating computational methods with available crystal structures to identify and refine potential allosteric sites [2, 63, 64].

Uncompetitive inhibition

Monoclonal antibodies (mAbs) have been proposed as potential anti-CCR5 inhibitors, acting similarly to receptor-to-co-receptor-mimetic peptides. Given that HIV entry requires the interaction between CCR5 and CD4 on the host cell surface, the use of such mimetic peptides could offer a novel means of preventing viral entry. However, their use in clinical trials has been limited [41]. In the current study, uncompetitive inhibitors showed some efficacy, with the R5E Vmax predicted to be 200,000 µM/min and a gp120S Km of 8 nM. That said, the use of antibodies in this capacity does come with challenges. mAb production remains a complicated process involving cell lines from bacteria, yeast, or mammals, using hybridoma technology [7]. This method often results in low yields, extensive purification difficulties, and contamination risks. Alternative production systems, such as plant-based systems, have been considered [7]. Additionally, the study's predictions may have been constrained by not fully accounting for variations in the structures, affinities, and sizes of anti-R5 monoclonal antibodies, all of which influence the pharmacokinetics of these antibodies. On the plus side, mAbs tend to be more tolerable in humans compared to smaller synthetic molecules, with fewer associated risks [7].

Conclusion

While antiretroviral (ARV) medications effectively treat and prevent HIV, they are associated with various side effects, particularly at higher doses [7]. CCR5-targeting antibodies offer a promising alternative for HIV treatment. By binding to the CCR5 receptor, mAbs can block HIV entry without fostering the resistance typically seen with small molecule drugs [7]. However, the development of resistance to mAbs remains a topic that warrants further study. Additionally, due to their long elimination half-lives, mAbs would require infrequent dosing, which could be beneficial for patients who must undergo long-term therapy. Future research will be crucial for patients who exhibit resistance to conventional ARVs [7]. The key finding of this study is the identification of non-competitive mAbs as the most effective inhibitors of CCR5, making them more promising than competitive and uncompetitive alternatives. These findings could significantly contribute to the design of more efficient monoclonal antibodies for CCR5 inhibition.

References

1. Gilliam BL, Riedel DJ, Redfield RR. Clinical use of CCR5 inhibitors in HIV and beyond. J Transl Med. 2011;9 Suppl 1(Suppl 1):S9. doi:10.1186/1479-5876-9-S1-S9

2. Qi B, Fang Q, Liu S, Hou W, Li J, Huang Y, et al. Advances of CCR5 antagonists: from small molecules to macromolecules. Eur J Med Chem. 2020;208:112819.

3. Brelot A, Chakrabarti LA. CCR5 Revisited: How Mechanisms of HIV Entry Govern AIDS Pathogenesis. J Mol Biol. 2018;430(17):2557-89.

4. Lee B, Sharron M, Montaner LJ, Weissman D, Doms RW. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc Natl Acad Sci U S A. 1999;96(9):5215-20. doi:10.1073/pnas.96.9.5215

5. Michael NL, Chang G, Louie LG, Mascola JR, Dondero D, Birx DL, et al. The role of viral phenotype and CCR-5 gene defects in HIV-1 transmission and disease progression. Nat Med. 1997;3(3):338-40. doi:10.1038/nm0397-338

6. Jacobson JM, Saag MS, Thompson MA, Fischl MA, Liporace R, Reichman RC, et al. Antiviral activity of single-dose PRO 140, a CCR5 monoclonal antibody, in HIV-infected adults. J Infect Dis. 2008;198(9):1345-52. doi:10.1086/592169

7. Prathipati PK, Mandal S, Destache CJ. A review of CCR5 antibodies against HIV: Current and future aspects. Ther Deliv. 2019;10(2):107-12. doi:10.4155/tde-2018-0072

8. Chang XL, Webb GM, Wu HL, Greene JM, Abdulhaqq S, Bateman KB, et al. Antibody-based CCR5 blockade protects Macaques from mucosal SHIV transmission. Nat Commun. 2021;12(1):3343. doi:10.1038/s41467-021-23697-6

9. Hartley O, Martins E, Scurci I. Preventing HIV transmission through blockade of CCR5: rationale, progress and perspectives. Swiss Med Wkly. 2018;148:w14580.

10. Chang XL, Reed JS, Webb GM, Wu HL, Le J, Bateman KB, et al. Suppression of human and simian immunodeficiency virus replication with the CCR5-specific antibody Leronlimab in two species. PLoS Pathogens. 2022;18(3):e1010396.

11. Weichseldorfer M, Tagaya Y, Reitz M, DeVico AL, Latinovic OS. Identifying CCR5 coreceptor populations permissive for HIV-1 entry and productive infection: implications for in vivo studies. J Transl Med. 2022;20(1):1-2.

12. Claireaux M, Robinot R, Kervevan J, Patgaonkar M, Staropoli I, Brelot A, et al. Low CCR5 expression protects HIV-specific CD4+ T cells of elite controllers from viral entry. Nat Commun. 2022;13(1):1-9.

13. Gonzalo-Gil E, Rapuano PB, Ikediobi U, Leibowitz R, Mehta S, Coskun AK, et al. Transcriptional down-regulation of ccr5 in a subset of HIV+ controllers and their family members. Elife. 2019;8:e44360.

14. Mehlotra RK. New Knowledge About CCR5, HIV infection, and disease progression: Is "Old" Still Valuable? AIDS Res Hum Retroviruses. 2020;36(10):795-9.

15. Wiredja DD, Tabler CO, Schlatzer DM, Li M, Chance MR, Tilton JC. Global phosphoproteomics of CCR5-tropic HIV-1 signaling reveals reprogramming of cellular protein production pathways and identifies p70-S6K1 and MK2 as HIV-responsive kinases required for optimal infection of CD4+ T cells. Retrovirology. 2018;15(1):1-3.

16. Kulkarni S, Lied A, Kulkarni V, Rucevic M, Martin MP, Walker-Sperling V, et al. CCR5AS lncRNA variation differentially regulates CCR5, influencing HIV disease outcome. Nat Immunol. 2019;20(7):824-34.

17. Chang XL, Wu HL, Webb GM, Tiwary M, Hughes C, Reed JS, et al. CCR5 receptor occupancy analysis reveals increased peripheral blood CCR5+CD4+ T cells following treatment with the Anti-CCR5 Antibody Leronlimab. Front Immunol. 2021;12:794638. doi:10.3389/fimmu.2021.794638

18. Mohamed H, Gurrola T, Berman R, Collins M, Sariyer IK, Nonnemacher MR, et al. Targeting CCR5 as a component of an HIV-1 therapeutic strategy. Front Immunol. 2022;12:816515.

19. Vangelista L, Vento S. The expanding therapeutic perspective of CCR5 blockade. Front Immunol. 2018;8:1981.

20. Ellwanger JH, Kaminski VL, Rodrigues AG, Kulmann-Leal B, Chies JAB. CCR5 and CCR5Δ32 in bacterial and parasitic infections: thinking chemokine receptors outside the HIV box. Int J Immunogenet. 2020;47(3):261-85.

21. Scurci I, Martins E, Hartley O. CCR5: established paradigms and new frontiers for a 'celebrity' chemokine receptor. Cytokine. 2018;109:81-93.

22. Boncompain G, Herit F, Tessier S, Lescure A, Del Nery E, Gestraud P, et al. Targeting CCR5 trafficking to inhibit HIV-1 infection. Sci Adv. 2019;5(10):eaax0821.

23. Amerzhanova Y, Vangelista L. Filling the gaps in antagonist CCR5 binding, a retrospective and perspective analysis. Front Immunol. 2022;13:826418. doi:10.3389/fimmu.2022.826418

24. Peng P, Chen H, Zhu Y, Wang Z, Li J, Luo RH, et al. Structure-based design of 1-Heteroaryl-1,3-propanediamine derivatives as a novel series of CC-Chemokine receptor 5 antagonists. J Med Chem 2018;61(21):9621-36. doi:10.1021/acs.jmedchem.8b01077

25. Ellwanger JH, Kulmann-Leal B, Kaminski VL, Rodrigues AG, Bragatte MAS, Chies JAB. Beyond HIV infection: neglected and varied impacts of CCR5 and CCR5Δ32 on viral diseases. Virus Res. 2020;286:198040.

26. Zhang H, Chen K, Tan Q, Shao Q, Han S, Zhang C, et al. Structural basis for chemokine recognition and receptor activation of chemokine receptor CCR5. Nat Commun. 2021;12(1):4151.

27. Wasilko DJ, Johnson ZL, Ammirati M, Che Y, Griffor MC, Han S, et al. Structural basis for chemokine receptor CCR6 activation by the endogenous protein ligand CCL20. Nat Commun. 2020;11(1):1-9.

28. Scurci I, Akondi KB, Pinheiro I, Paolini-Bertrand M, Borgeat A, Cerini F, et al. CCR5 tyrosine sulfation heterogeneity generates cell surface receptor subpopulations with different ligand binding properties. Biochim Biophys Acta Gen Subj. 2021;1865(1):129753.

29. Saha S, Shukla AK. The inside story: crystal structure of the chemokine receptor CCR7 with an intracellular allosteric antagonist. Biochemistry. 2019;59(1):12-4.

30. Larsen O, Lückmann M, van der Velden WJC, Oliva-Santiago M, Brvar M, Ulven T, et al. Selective allosteric modulation of N-Terminally cleaved, but not full length CCL3 in CCR1. ACS Pharmacol Transl Sci. 2019;2(6):429-41.

31. Heredia JD, Park J, Brubaker RJ, Szymanski SK, Gill KS, Procko E. Mapping interaction sites on human chemokine receptors by deep mutational scanning. J Immunol. 2018;200(11):3825-39.

32. Zhu Y, Zhao YL, Li J, Liu H, Zhao Q, Wu BL, et al. Molecular binding mode of PF-232798, a clinical anti-HIV candidate, at chemokine receptor CCR5. Acta Pharmacologica Sinica. 2019;40(4):563-8.

33. Blanpain C, Vanderwinden JM, Cihak J, Wittamer V, Le Poul E, Issafras H, et al. Multiple active states and oligomerization of CCR5 revealed by functional properties of monoclonal antibodies. Mol Biol Cell. 2002;13(2):723-37. doi:10.1091/mbc.01-03-0129

34. Panda S, Ding JL. Natural antibodies bridge innate and adaptive immunity. J Immunol. 2015;194(1):13-20. doi:10.4049/Immunol.1400844

35. Pastori C, Diomede L, Venuti A, Fisher G, Jarvik J, Bomsel M, et al. Induction of HIV-blocking anti-CCR5 IgA in Peyers's patches without histopathological alterations. J Virol. 2014;88(7):3623-35. doi:10.1128/JVI.03663-13

36. Van Rompay KK, Hunter Z, Jayashankar K, Peabody J, Montefiori D, LaBranche CC, et al. A vaccine against CCR5 protects a subset of macaques upon intravaginal challenge with simian immunodeficiency virus SIVmac251. J Virol. 2014;88(4):2011-24. doi:10.1128/JVI.02447-13

37. Chackerian B, Briglio L, Albert PS, Lowy DR, Schiller JT. Induction of autoantibodies to CCR5 in macaques and subsequent effects upon challenge with an R5-tropic simian/human immunodeficiency virus. J Virol. 2004;78(8):4037-47.

38. Lee B, Sharron M, Blanpain C, Doranz BJ, Vakili J, Setoh P, et al. Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. J Biol Chem. 1999;274(14):9617-26.

39. Bomsel M, Pastori C, Tudor D, Alberti C, Garcia S, Ferrari D, et al. Natural mucosal antibodies reactive with first extracellular loop of CCR5 inhibit HIV-1 transport across human epithelial cells. AIDS. 2007;21(1):13-22. doi:10.1097/QAD.0b013e328011049b

40. Wu L, LaRosa G, Kassam N, Gordon CJ, Heath H, Ruffing N, et al. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. J Exp Med. 1997;186(8):1373-81. doi:10.1084/jem.186.8.1373

41. Venuti A, Pastori C, Lopalco L. The role of natural antibodies to CC chemokine receptor 5 in HIV infection. Front Immunol. 2017;8:1358. doi:10.3389/fimmu.2017.01358

42. Secchi M, Grampa V, Vangelista L. Rational CCL5 mutagenesis integration in a lactobacilli platform generates extremely potent HIV-1 blockers. Sci Rep. 2018;8(1):1890.

43. Zheng Y, Han GW, Abagyan R, Wu B, Stevens RC, Cherezov V, et al. Structure of CC chemokine receptor 5 with a potent chemokine antagonist reveals mechanisms of chemokine recognition and molecular mimicry by HIV. Immunity. 2017;46(6):1005-17.

44. Trkola A, Ketas TJ, Nagashima KA, Zhao L, Cilliers T, Morris L, et al. Potent, broad-spectrum inhibition of human immunodeficiency virus type 1 by the CCR5 monoclonal antibody PRO 140. J Virol. 2001;75(2):579-88.

45. Li L, Tian JH, Yang K, Zhang P, Jia WQ. Humanized PA14 (a monoclonal CCR5 antibody) for treatment of people with HIV infection. Cochrane Database Syst Rev. 2014;2014(7):CD008439. doi:10.1002/14651858.CD008439.pub3

46. Wacker D, Stevens RC, Roth BL. How ligands illuminate GPCR molecular pharmacology. Cell. 2017;170(3):414-27. doi:10.1016/j.cell.2017.07.009

47. Vass M, Kooistra AJ, Yang D, Stevens RC, Wang MW, de Graaf C. Chemical diversity in the G protein-coupled receptor superfamily. Trends Pharmacol Sci. 2018;39(5):494-512.

48. Chan HCS, Li Y, Dahoun T, Vogel H, Yuan S. New binding sites, new opportunities for GPCR drug discovery. Trends Biochem Sci. 2019;44(4):312-30.

49. Yang D, Zhou Q, Labroska V, Qin S, Darbalaei S, Wu Y, et al. G protein-coupled receptors: structure- and function-based drug discovery. Signal Transduct Target Ther. 2021;6(1):7. doi:10.1038/s41392-020-00435-w

50. Wold EA, Chen J, Cunningham KA, Zhou J. Allosteric modulation of class a GPCRs: targets, agents, and emerging concepts. J Med Chem. 2019;62(1):88-127.

51. Tan L, Yan W, McCorvy JD, Cheng J. Biased ligands of G protein-coupled receptors (GPCRs): structure-functional selectivity relationships (SFSRs) and therapeutic potential. J Med Chem. 2018;61(22):9841-78.

52. Seyedabadi M, Ghahremani MH, Albert PR. Biased signaling of G protein coupled receptors (GPCRs): molecular determinants of GPCR/transducer selectivity and therapeutic potential. Pharmacol Ther. 2019;200:148-78.

53. Sriram K, Insel PA. G Protein-coupled receptors as targets for approved drugs: How many targets and how many drugs? Mol Pharmacol. 2018;93(4):251-8.

54. Wright SC, Bouvier M. Illuminating the complexity of GPCR pathway selectivity - advances in biosensor development. Curr Opin Struct Biol. 2021;69:142-9.

55. Wingler LM, Lefkowitz RJ. Conformational basis of G protein-coupled receptor signaling versatility. Trends Cell Biol. 2020;30(9):736-47.

56. Jones EM, Lubock NB, Venkatakrishnan AJ, Wang J, Tseng AM, Paggi JM, et al. Structural and functional characterization of G protein-coupled receptors with deep mutational scanning. Elife. 2020;9:e54895.

57. Thal DM, Glukhova A, Sexton PM, Christopoulos A. Structural insights into G-protein-coupled receptor allostery. Nature. 2018;559(7712):45-53. doi:10.1038/s41586-018-0259-z

58. Marti-Solano M, Crilly SE, Malinverni D, Munk C, Harris M, Pearce A, et al. Combinatorial expression of GPCR isoforms affects signalling and drug responses. Nature. 2020;587(7835):650-6.

59. Gusach A, Maslov I, Luginina A, Borshchevskiy V, Mishin A, Cherezov V. Beyond structure: emerging approaches to study GPCR dynamics. Curr Opin Struct Biol. 2020;63:18-25.

60. Yen HY, Hoi KK, Liko I, Hedger G, Horrell MR, Song W, et al. PtdIns(4,5)P2 stabilizes active states of GPCRs and enhances selectivity of G-protein coupling. Nature. 2018;559(7714):423-7.

61. Erlandson SC, McMahon C, Kruse AC. Structural basis for G protein-coupled receptor signaling. Annu Rev Biophys. 2018;47:1-18.

62. Patwardhan A, Cheng N, Trejo J. Post-translational modifications of G protein-coupled receptors control cellular signaling dynamics in space and time. Pharmacol Rev. 2021;73(1):120-51.

63. Wootten D, Christopoulos A, Marti-Solano M, Babu MM, Sexton PM. Mechanisms of signalling and biased agonism in G protein-coupled receptors. Nat Rev Mol Cell Biol. 2018;19(10):638-53.

64. Sutkeviciute I, Vilardaga JP. Structural insights into emergent signaling modes of G protein-coupled receptors. J Biol Chem. 2020;295(33):11626-42.