form of CXCR4. The first mechanism comprises a shift in coreceptor usage from CXCR4 to CCR5, which is induced by selective pressure from CXCR4 antagonists. However, this is unlikely to occur frequently because coreceptor switching from CCR5 to CXCR4, and *vice versa*, requires multiple mutations throughout gp160 via transitional intermediates with poor replication fitness [77].

There is an evolutionary gap in viral fitness between viruses using CXCR4 and those using CCR5. However, an R5X4 dual-tropic virus can shift from X4-dominated tropism to R5-dominated tropism [83]. The R5X4 dual-tropic 89.6 mainly uses CXCR4 as a coreceptor, but after selection with the CXCR4 antagonist T140, coreceptor usage shifted from a phenotype that mainly used CXCR4 to one mainly using CCR5 due to a single amino acid substitution (R308S) in the V3 loop in vitro. These results indicated that the R5X4 virus could shift its main coreceptor usage due to a low genetic barrier to the development of resistance. In contrast, an outgrowth of the pre-existing minority of the R5 virus caused by CXCR4 antagonists, is expected to lead to virologic failure. AMD3100 is a small molecule compound called a bicyclam that has potent antiviral activity against a variety of X4-tropic strains [94-99]. However, it is not clinically available because of low oral bioavailability [100]. After treatment of clinical isolates *in vitro* with AM3100 for 28 days, the major population of viruses using CXCR4 was promptly replaced by the pre-existing minor population using CCR5 with multiple mutations in the V3 loop in vitro [101].

The third possible pathway results from accumulation of mutations in the viral envelope that allow interaction between gp120 and the coreceptor in the presence of the inhibitor. AMD3100-resistant viruses selected *in vitro* from NL4-3 strain still used CXCR4 as a coreceptor and contained several mutations in the V3 loop and showed poor fitness [102]. In contrast, other viruses resistant to POL3026, a specific β-hairpin mimetic CXCR4 antagonist, did not show any fitness cost

and contained four mutations (Q310H, I320T, N325D, and A329T) in the gp120 V3 loop [70]. These four mutations were shared by viral strains resistant to SDF-1α [103] and T134 [104], indicating that the V3 loop is a crucial region for the acquisition of CXCR4 antagonist resistance.

The fourth possible mechanism involves acquisition of the ability to utilize the inhibitor-bound form as well as the drug-free form of CXCR4 for viral entry. Several clinical isolates demonstrate infection through the AMD3100-bound form of CXCR4, indicating a noncompetitive mode of drug resistance [99]. The V1/V2 region of one of the isolates is responsible for this property, suggesting that baseline resistance to this kind of CXCR4 antagonist should be considered while developing CXCR4 antagonists. Recent advances have led to the development of orally-active CXCR4 antagonists, including AMD11070 [105], KRH-3955 [106], and GSK81297 [107]. Therefore, to prevent the possible emergence of pre-existing forms of the CCR5 virus, it is likely that CXCR4 antagonists will be effective only in combination with a CCR5 antagonist or other antiviral drugs.

## Fusion inhibitory peptides and their mechanisms of action

Fusion inhibitors: Enfuvirtide (T-20) was approved by the FDA in 2003 as the first fusion inhibitor that efficiently suppresses the replication of HIV-1 resistant to available classes of anti-HIV-1 drugs (Figure 1), such as reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs). Hence, it has been widely used for treatment of HIV-1 infected patients where treatment with other antiretroviral drugs has failed [108]. T-20 comprises a 36 amino acid peptide derived from the gp41 HIV-1 C-terminal heptad repeat (C-HR), as shown in Figure 7.

During HIV-1 entry, binding of gp120 to CD4 and either CCR5 or CXCR4 initiates penetration of the hydrophobic fusion peptide domain at the N-terminal heptad repeat (N-HR) of gp41 into the target
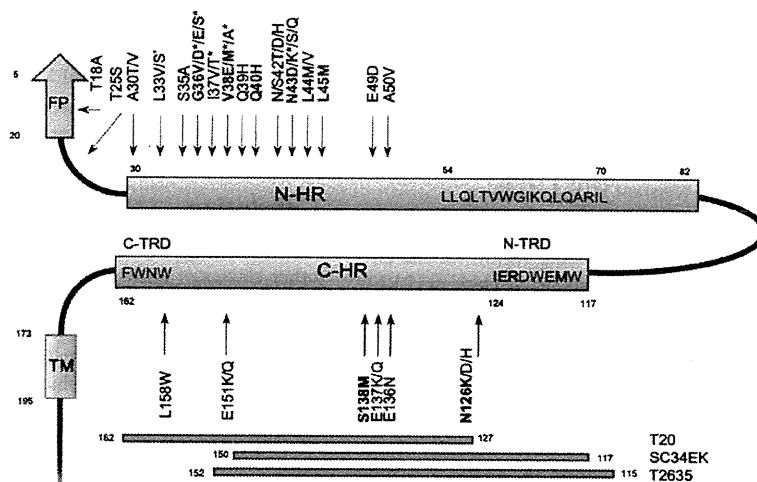


**Figure 7:** Schematic view of HIV-1 gp41 functional domains and mutation map for T-20. Putative hydrophobic pocket region of the N-HR is shown (green) and may form a leucine-zipper-like domain. In the C-HR, two tryptophan-rich domains (TRD; pink) are located at the N- and C-terminal regions (N-TRD and C-TRD, respectively). The N-TRD binds to the hydrophobic pocket in the N-HR, whereas the C-TRD plays a key role in membrane association. FP; fusion peptide domain, which penetrates into the target cell membrane. TM; transmembrane region. The amino acid sequence of the HXB2 clone is shown as a representative HIV-1 sequence. Only mutations located in the extracellular domain of gp41 are shown. Mutations observed in in vitro and in vivo selections are indicated by an asterisk (*). I37T was only selected in vitro. Primary and secondary mutations were most frequently associated with T-20 resistance (red and blue, respectively). In addition, T25S/A, S35A/T, R46K, L55F, Q56R/K, V72L, A101I/T/V/G, L108Q, N109D, D113G/N, E119Q, L130V, I135L, N140I, and L158W were selected in patients under T-20 containing regimens, but observed in some drug-naïve HIV-1 strains (Los Alamos HIV Sequence Data Bank, http://www.hiv.lanl.gov/content/index (natural polymorphisms). Corresponding regions of T-20, SC34EK, and T2635 are shown. T-20 is comprised of the original sequence but others are extensively modified.

cell membrane [6]. In the gp41 extra-cellular domain, the α-helical region at the C-HR begins to fold and interact with a trimeric form of the N-HR in an anti-parallel manner. This intramolecular folding forms a stable six-helix bundle and facilitates the fusion of the virus envelope and cellular membranes. During the fusion step of HIV-1 replication, T-20 can interfere with the formation of the six-helix bundle consisting of a trimeric N-HR/C-HR complex.

In the C-HR, two tryptophan-rich domains (TRDs) are located in close proximity to the connection loop (N-TRD) and the membrane-spanning or transmembrane region (C-TRD). Both TRDs resemble a leucine zipper structure and are believed to be important for interactions of the N-HR and the C-HR. T-20 contains the amino acid sequence of the C-TRD, whereas C34-based peptides, such as SC34EK and T2635, contain the N-TRD. T-20 is believed to bind to the N-HR as a decoy and prevents the formation of the six-helix bundle [109], resulting in the inhibition of HIV-1 entry. This mode of action has been well documented with another fusion inhibitory peptide, C34, and remains controversial whether the mechanisms of action of T-20 and C34 are in fact the same.

**Primary and secondary mutations for fusion inhibitors:** Although some fusion peptides, such as N36 [110] and IQN17 [111], are designed using the N-HR sequence, most have been designed using the C-HR sequence. Primary mutations for a representative C-HR derived peptide, T-20, are generally introduced within the N-HR, a putative binding site of T-20 [112,113]. Mutations frequently reported *in vivo* are located at amino acid positions 36–45 of the gp41, including G36D/S/E/V, V38A/M/E, Q40H, N42T, and N43D/K (Figure 7) [114]. Using circular dichroism analysis, others and we clearly demonstrated that these primary mutations reduce the binding affinity of C-peptides with the N-HR [112,115]. This mutation also impairs physiological intra-molecular binding of the C-HR with the N-HR, providing a replication cost [116]. Therefore, HIV-1 develops secondary or compensatory mutations in the C-HR to restore the reduced stabilities of the six-helix bundle by the introduction of primary mutations. N126K, E137K/Q, and S138A [115,117] have been reported *in vivo*, usually in combination with N-HR mutations. Mutations in the C-HR restore the intra-molecular folding/interaction of the C-HR with the N-HR. The enhanced binding affinity by the secondary mutations can be applied to peptide design, such as C34 with N126K and T-20 with S138A, which maintain anti-HIV-1 activity, even to drug-resistant HIV-1 [115].

Secondary mutations of the N-HR are not only non-synonymous, but also synonymous. A part of the RNA coding region for the env gene, including gp41, also encodes the Rev-responsible element (RRE), which is an RNA secondary structure important for unspliced RNA export from the nucleus that is required for efficient viral protein synthesis and packaging of genomic RNA [118,119]. Primary mutations at positions 36 and 38 for stem II and at 43 for stem III affect the RRE structure. Synonymous and non-synonymous mutations introduced into the gp41 compensate for RRE structure stability, such as T18A for V38A [120] and A30V for G36D [116], and Q41 (CAG to CAA) and L44 (UUG to CUG) for N43D [121]. This association between the gp41 and RRE results in some genetic restrictions.

**Impact of mutations on clinical potency:** Only one or two amino acid substitutions in gp41 appear to be sufficient for clinical treatment failure, where after the emergence of mutations, viral load gradually increases [122]. For example, G36E, V38A, Q40H, and N43D were shown to confer 39.3-, 16-, 21-, and 18-fold reductions in susceptibility to T-20, respectively [123]. Double or triple substitutions have also been identified in clinical isolates from patients undergoing ther-

apy with T-20. Mutations such as N42T+N43S, V38A+N42D, and Q40H+L45M confer 61-, 140-, and 67-fold reductions in susceptibility to T-20, respectively [123]. Mutations at codons 36 (G36E/D/S) and 38 (V38A/G/M) seem to emerge relatively rapidly *in vivo*, whereas Q40H and N43D emerge more slowly [122]. After prolonged therapy, HIV-1 has been shown to develop secondary mutations and may confer more apparent resistance with improved replication kinetics. Therefore, combination regimens with other inhibitors, such as RTIs and PIs, are indispensable for sufficient positive viral responses.

T-20 appears to inhibit replication of HIV-1 subtype independently [124-126], since T-20 has mainly been used for subtype B HIV-1 infected patients. Based on the mechanism of action of T-20, interference of N-and C-HR interactions may be expected, where amino acid sequences are highly conserved across all subtypes. However, in non-B subtype HIV-1, N42S predominantly emerged as a resistance-related mutation [124,125].

**Resistance to the next generation inhibitors:** Next generation inhibitors have been designed using several strategies, such as the introduction of specific amino acid motifs and secondary mutations into the sequence of the original peptide inhibitors [115] to enhance the stability of the α-helical structure between inhibitors and fusion domain at the N-HR. In contrast to T-20, primary mutations to third generation inhibitors were not selected *in vitro* [127,128]; therefore, the accumulation of multiple mutations is likely necessary for the development of resistance. In the case of SC34EK, 13 amino acid substitutions (D36G, Q41R, N43K, A96D, N126K, E151K, H132Y, V182I, P203S, L204I, S241F, H258Q, and A312T) were introduced and single amino acid substitutions only conferred weak resistance (<6-fold) [127]. For another peptide, T-2635, 12 amino acids in 10 positions (A6V, L33S, Q66R/L, K77E/N, T94N, N100D, N126K, H132Q, E136G, and E151G) were selected, and single mutations did not confer resistance to T-2635 [128]. Interestingly, some of these mutations were located outside the N-HR and C-HR. Cross-resistance between SC34EK and T-2635 was only examined for the SC34EK-resistant virus and revealed little cross-resistance [127]. Further studies of resistance profiles might be helpful in defining new strategies for the design of fusion inhibitors that can suppress the replication of resistant variants of HIV-1.

## Conclusion

The emergence of viruses resistant to entry inhibitors, as well as other classes of antiviral agents (reverse transcriptase or protease inhibitors), has been reported *in vitro* and *in vivo*. Resistance to entry inhibitors, including attachment inhibitors and coreceptor antagonists, is mainly conferred as a result of missense mutations within the gp120 subunit of the *env* gene, which differ from one inhibitor to another. Alternatively, treatment failure can occur through the expansion of pre-existing CXCR4-using virus for CCR5 antagonists, and vice versa. Agents that target gp41-dependent fusion select for HIV-1 variants with mutationswithin the gp41 envelope gene. These results indicate the incredible flexibility of the HIV-1 genome to escape from a variety of entry inhibitors. Therefore, the development of novel entry inhibitors for clinical use is needed to limit escape mutants by effective combination therapy.

## References

1. Potter SJ, Chew CB, Steain M, Dwyer DE, Saksena NK (2004) Obstacles to successful antiretroviral treatment of HIV-1 infection: problems &amp; perspectives. Indian J Med Res 119: 217-237.

2. Shafer RW, Schapiro JM (2008) HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev 10: 67-84.

3. Gupta RK, Gibb DM, Pillay D (2009) Management of paediatric HIV-1 resistance. Curr Opin Infect Dis 22: 256-263.

4. Wild C, Greenwell T, Matthews T (1993) A synthetic peptide from HIV-1 gp41 is a potent inhibitor of virus-mediated cell-cell fusion. AIDS Res Hum Retroviruses 9: 1051-1053.

5. Chan DC, Fass D, Berger JM, Kim PS (1997) Core structure of gp41 from the HIV envelope glycoprotein. Cell 89: 263-273.

6. Chan DC, Kim PS (1998) HIV entry and its inhibition. Cell 93: 681-684.

7. Westby M, van der Ryst E (2010) CCR5 antagonists: host-targeted antiviral agents for the treatment of HIV infection, 4 years on. Antivir Chem Chemother 20: 179-192.

8. Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, et al. (2005) Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob Agents Chemother 49: 4721-4732.

9. Fatkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, et al. (2005) Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. Nat Med 11: 1170-1172.

10. Moore JP, Kuritzkes DR (2009) A piece de resistance: how HIV-1 escapes small molecule CCR5 inhibitors. Curr Opin HIV AIDS 4: 118-124.

11. Wyatt R, Sodroski J (1998) The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science 280: 1884-1888.

12. Sattentau QJ, Moore JP (1993) The role of CD4 in HIV binding and entry. Philos Trans R Soc Lond B Biol Sci 342: 59-66.

13. Ugolini S, Mondor I, Sattentau QJ (1999) HIV-1 attachment: another look. Trends Microbiol 7: 144-149.

14. ES, Li XL, Moudgil T, Ho DD (1990) High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc Natl Acad Sci U S A 87: 6574-6578.

15. Orloff SL, Kennedy MS, Belperron AA, Maddon PJ, McDougal JS (1993) Two mechanisms of soluble CD4 (sCD4)-mediated inhibition of human immunodeficiency virus type 1 (HIV-1) infectivity and their relation to primary HIV-1 isolates with reduced sensitivity to sCD4. J Virol 67: 1461-1471.

16. McKeating J, Balfe P, Clapham P, Weiss RA (1991) Recombinant CD4-selected human immunodeficiency virus type 1 variants with reduced gp120 affinity for CD4 and increased cell fusion capacity. J Virol 65: 4777-4785.

17. Yoshimura K, Harada S, Shibata J, Hatada M, Yamada Y, et al. (2010) Enhanced exposure of human immunodeficiency virus type 1 primary isolate neutralization epitopes through binding of CD4 mimetic compounds. J Virol 84: 7558-7568.

18. Jacobson JM, Israel RJ, Lowy I, Ostrow NA, Vassilatos LS, et al. (2004) Treatment of advanced human immunodeficiency virus type 1 disease with the viral entry inhibitor PRO 542. Antimicrob Agents Chemother 48: 423-429.

19. Bodart V, Anastassov V, Darkes MC, Idzan SR, Labrecque J, et al. (2009) Pharmacology of AMD3465: a small molecule antagonist of the chemokine receptor CXCR4. Biochem Pharmacol 78: 993-1000.

20. Kuritzkes DR, Jacobson J, Powderly WG, Godofsky E, DeJesus E, et al. (2004) Antiretroviral activity of the anti-CD4 monoclonal antibody TNX-355 in patients infected with HIV type 1. J Infect Dis 189: 286-291.

21. Toma J, Weinheimer SP, Stawiski E, Whitcomb JM, Lewis ST, et al. (2011) Loss of asparagine-linked glycosylation sites in variable region 5 of human immunodeficiency virus type 1 envelope is associated with resistance to CD4 antibody ibalizumab. J Virol 85: 3872-3880.

22. Zwick MB, Jensen R, Church S, Wang M, Stiegler G, et al. (2005) Anti-human immunodeficiency virus type 1 (HIV-1) antibodies 2F5 and 4E10 require surprisingly few crucial residues in the membrane-proximal external region of glycoprotein gp41 to neutralize HIV-1. J Virol 79: 1252-1261.

23. Calarese DA, Lee HK, Huang CY, Best MD, Astronomo RD, et al. (2005) Dissection of the carbohydrate specificity of the broadly neutralizing anti-HIV-1 antibody 2G12. Proc Natl Acad Sci U S A 102: 13372-13377.

24. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, et al. (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326: 285-289.

25. DR, Pyati J, Koduri R, Sharp SJ, Thornton GB, et al. (1994) Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 266: 1024-1027.

26. T, Xu L, Dey B, Hessell AJ, Van Ryk D, et al. (2007) Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature 445: 732-737.

27. H, Stamatatos L, Ip JE, Barbas CF, Parren PW, et al. (1997) Human immunodeficiency virus type 1 mutants that escape neutralization by human monoclonal antibody IgG1b12. off. J Virol 71: 6869-6874.

28. Wu X, Zhou T, O'Dell S, Wyatt RT, Kwong PD, et al. (2009) Mechanism of human immunodeficiency virus type 1 resistance to monoclonal antibody B12 that effectively targets the site of CD4 attachment. J Virol 83: 10892-10907.

29. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, et al. (2010) Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 329: 856-861.

30. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, et al. (2010) Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 329: 811-817.

31. Li Y, O'Dell S, Walker LM, Wu X, Guenaga J, et al. (2011) Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. J Virol 85: 8954-8967.

32. Guo Q, Ho HT, Dicker I, Fan L, Zhou N, et al. (2003) Biochemical and genetic characterizations of a novel human immunodeficiency virus type 1 inhibitor that blocks gp120-CD4 interactions. J Virol 77: 10528-10536.

33. Lin PF, Blair W, Wang T, Spicer T, Guo Q, et al. (2003) A small molecule HIV-1 inhibitor that targets the HIV-1 envelope and inhibits CD4 receptor binding. Proc Natl Acad Sci U S A 100: 11013-11018.

34. Fransen S, Bridger G, Whitcomb JM, Toma J, Stawiski E, et al. (2008) Suppression of dualtropic human immunodeficiency virus type 1 by the CXCR4 antagonist AMD3100 is associated with efficiency of CXCR4 use and baseline virus composition. Antimicrob Agents Chemother 52: 2608-2615.

35. Zhou N, Nowicka-Sans B, Zhang S, Fan L, Fang J, et al. (2011) *In vivo* patterns of resistance to the HIV attachment inhibitor BMS-488043. Antimicrob Agents Chemother 55: 729-737.

36. A, Madani N, Klein JC, Hubicki A, Ng D, et al. (2006) Thermodynamics of binding of a low-molecular-weight CD4 mimetic to HIV-1 gp120. Biochemistry 45: 10973-10980.

37. Q, Ma L, Jiang S, Lu H, Liu S, et al. (2005) Identification of N-phenyl-N'-(2,2,6,6-tetramethyl-piperidin-4-yl)-oxalamides as a new class of HIV-1 entry inhibitors that prevent gp120 binding to CD4. Virology 339: 213-225.

38. Lalonde JM, Elban MA, Courter JR, Sugawara A, Soeta T, et al. (2011) Design, synthesis and biological evaluation of small molecule inhibitors of CD4-gp120 binding based on virtual screening. Bioorg Med Chem 19: 91-101.

39. Madani N, Schon A, Princiotto AM, Lalonde JM, Courter JR, et al. (2008) Small-molecule CD4 mimics interact with a highly conserved pocket on HIV-1 gp120. Structure 16: 1689-1701.

40. Narumi T, Ochiai C, Yoshimura K, Harada S, Tanaka T, et al. (2010) CD4 mimics targeting the HIV entry mechanism and their hybrid molecules with a CXCR4 antagonist. Bioorg Med Chem Lett 20: 5853-5858.

41. Yamada Y, Ochiai C, Yoshimura K, Tanaka T, Ohashi N, et al. (2010) CD4 mimics targeting the mechanism of HIV entry. Bioorg Med Chem Lett 20: 354-358.

42. EE, Lin X, Li W, Cotter R, Klein MT, et al. (2006) Inhibition of highly productive HIV-1 infection in T cells, primary human macrophages, microglia, and astrocytes by Sargassum fusiforme. AIDS Res Ther 3: 15.

43. Lee DY, Lin X, Paskaleva EE, Liu Y, Puttamadappa SS, et al. (2009) Palmitic Acid Is a Novel CD4 Fusion Inhibitor That Blocks HIV Entry and Infection. AIDS Res Hum Retroviruses 25: 1231-1241.

44. Paskaleva EE, Xue J, Lee DY, Shekhtman A, Canki M (2010) Palmitic acid analogs exhibit nanomolar binding affinity for the HIV-1 CD4 receptor and nanomolar inhibition of gp120-to-CD4 fusion. PLoS One 5: e12168.

45. Lin X, Paskaleva EE, Chang W, Shekhtman A, Canki M (2011) Inhibition of HIV-

1 infection in ex vivo cervical tissue model of human vagina by palmitic acid; implications for a microbicide development. PLoS One 6: e24803.

46. Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR (1997) Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. J Exp Med 185: 621-628.

47. Liu R, Paxton Wa, Choe S, Ceradini D, Martin SR, et al. (1996) Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 86: 367-377.

48. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, et al. (1996) Resistance to HIV-1 infection in Caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382: 722-725.

49. Baba M, Nishimura O, Kanzaki N, Okamoto M, Sawada H, et al. (1999) A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci U S A 96: 5698-5703.

50. Imamura S, Ichikawa T, Nishikawa Y, Kanzaki N, Takashima K, et al. (2006) Discovery of a piperidine-4-carboxamide CCR5 antagonist (TAK-220) with highly potent Anti-HIV-1 activity. J Med Chem 49: 2784-2793.

51. Maeda K, Yoshimura K, Shibayama S, Habashita H, Tada H, et al. (2001) Novel low molecular weight spirodiketopiperazine derivatives potently inhibit R5 HIV-1 infection through their antagonistic effects on CCR5. J Biol Chem 276: 35194-35200.

52. Strizki JM, Xu S, Wagner NE, Wojcik L, Liu J, et al. (2001) SCH-C (SCH 351125), an orally bioavailable, smallmolecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 98: 1-6.

53. Tagat JR, McCombie SW, Nazareno D, Labroli MA, Xiao Y, et al. (2004) Piperazine-Based CCR5 Antagonists as HIV-1 Inhibitors. IV. Discovery of 1-[(4,6-Dimethyl-5-pyrimidinyl)carbonyl]- 4-[4-{2-methoxy-1(R)-4-(trifluoromethyl)phenyl}ethyl-3(S)-methyl-1-piperazinyl]- 4-methylpiperidine (Sch-417690/Sch-D), a Potent, Highly Sele. J Med Chem 47: 2405-2408.

54. Dragic T, Trkola a, Thompson Da, Cormier EG, Kajumo Fa, et al. (2000) A binding pocket for a small molecule inhibitor of HIV-1 entry within the transmembrane helices of CCR5. Proc Natl Acad Sci USA 97: 5639-5644.

55. Maeda K, Nakata H, Koh Y, Miyakawa T, Ogata H, et al. (2004) Spirodiketopiperazine-based CCR5 inhibitor which preserves CC-chemokine/CCR5 interactions and exerts potent antiviral activity against R5 human immunodeficiency virus type 1 in vitro. J Virol 78: 8654-8662.

56. Imamura S, Nishikawa Y, Ichikawa T, Hattori T, Matsushita Y, et al. (2005) CCR5 antagonists as anti-HIV-1 agents. Part 3: Synthesis and biological evaluation of piperidine-4-carboxamide derivatives. Bioorg Med Chem 13: 397-416.

57. Seibert C, Ying W, Gavrilov S, Tsamis F, Kuhmann SE, et al. (2006) Interaction of small molecule inhibitors of HIV-1 entry with CCR5. Virology 349: 41-54.

58. Tsamis F, Gavrilov S, Kajumo F, Seibert C, Kuhmann S, et al. (2003) Analysis of the mechanism by which the small-molecule CCR5 antagonists SCH-351125 and SCH-350581 inhibit human immunodeficiency virus type 1 entry. J Virol 77: 5201-5208.

59. Baba M, Miyake H, Wang X, Okamoto M, Takashima K (2007) Isolation and characterization of human immunodeficiency virus type 1 resistant to the small-molecule CCR5 antagonist TAK-652. Antimicrobial agents and chemotherapy 51: 707-715.

60. Trkola A, Kuhmann SE, Strizki JM, Maxwell E, Ketas T, et al. (2002) HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. Proc Natl Acad Sci U S A 99: 395-400.

61. Westby M, Smith-Burchnell C, Mori J, Lewis M, Mosley M, et al. (2007) Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. J Virol 81: 2359-2371.

62. Yuan Y, Maeda Y, Terasawa H, Monde K, Harada S, et al. (2011) A combination of polymorphic mutations in V3 loop of HIV-1 gp120 can confer noncompetitive resistance to maraviroc. Virology 413: 293-299.

63. Yusa K, Maeda Y, Fujioka A, Monde K, Harada S (2005) Isolation of TAK-779-resistant HIV-1 from an R5 HIV-1 GP120 V3 loop library. J Biol Chem 280: 30083-30090.

64. Ogert RA, Wojcik L, Buontempo C, Ba L, Buontempo P, et al. (2008) Mapping resistance to the CCR5 co-receptor antagonist vicriviroc using heterologous

chimeric HIV-1 envelope genes reveals key determinants in the C2-V5 domain of gp120. Virology 373: 387-399.

65. Ogert RA, Hou Y, Ba L, Wojcik L, Qiu P, et al. (2010) Clinical resistance to vicriviroc through adaptive V3 loop mutations in HIV-1 subtype D gp120 that alter interactions with the N-terminus and ECL2 of CCR5. Virology 400: 145-155.

66. Tilton JC, Wilen CB, Didigu Ca, Sinha R, Harrison JE, et al. (2010) A maraviroc-resistant HIV-1 with narrow cross-resistance to other CCR5 antagonists depends on both N-terminal and extracellular loop domains of drug-bound CCR5. J Virol 84: 10863-10876.

67. Tsibris AMN, Sagar M, Gulick RM, Su Z, Hughes M, et al. (2008) *In vivo* emergence of vicriviroc resistance in a human immunodeficiency virus type 1 subtype C-infected subject. J Virol 82: 8210-8214.

68. Gulick RM, Su Z, Flexner C, Hughes MD, Skolnik PR, et al. (2007) Phase 2 study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-Infected, treatment-experienced patients: AIDS clinical trials group 5211. J Infect Dis 196: 304-312.

69. Marozsan AJ, Kuhmann SE, Morgan T, Herrera C, Rivera-Troche E, et al. (2005) Generation and properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH-417690 (SCH-D). Virology 338: 182-199.

70. Moncunill G, Armand-Ugón M, Pauls E, Clotet B, Esté Ja (2008) HIV-1 escape to CCR5 coreceptor antagonism through selection of CXCR4-using variants in vitro. Aids 22: 23-31.

71. Briggs DR, Tuttle DL, Sleasman JW, Goodenow MM (2000) Envelope V3 amino acid sequence predicts HIV-1 phenotype (co-receptor usage and tropism for macrophages). Aids 14: 2937-2939.

72. Hu Q, Trent JO, Tomaras GD, Wang Z, Murray JL, et al. (2000) Identification of ENV determinants in V3 that influence the molecular anatomy of CCR5 utilization. J Mol Biol 302: 359-375.

73. N, Haraguchi Y, Takeuchi Y, Soda Y, Kanbe K, et al. (1999) Changes in and discrepancies between cell tropisms and coreceptor uses of human immunodeficiency virus type 1 induced by single point mutations at the V3 tip of the env protein. Virology 259: 324-333.

74. Verrier F, Borman AM, Brand D, Girard M (1999) Role of the HIV type 1 glycoprotein 120 V3 loop in determining coreceptor usage. AIDS Res Hum Retroviruses 15: 731-743.

75. Resch W, Hoffman N, Swanstrom R (2001) Improved success of phenotype prediction of the human immunodeficiency virus type 1 from envelope variable loop 3 sequence using neural networks. Virology 288: 51-62.

76. Maeda Y, Foda M, Matsushita S, Harada S (2000) Involvement of both the V2 and V3 regions of the CCR5-tropic human immunodeficiency virus type 1 envelope in reduced sensitivity to macrophage inflammatory protein 1alpha. J Virol 74: 1787-1793.

77. Pastore C, Ramos A, Mosier DE (2004) Intrinsic obstacles to human immunodeficiency virus type 1 coreceptor switching. J Virol 78: 7565-7574.

78. Kuhmann S, Pugach P, Kunstman K (2004) Genetic and phenotypic analyses of human immunodeficiency virus type 1 escape from a small-molecule CCR5 inhibitor. J Virol 78: 2790-2807.

79. Pugach P, Marozsan AJ, Ketas TJ, Landes EL, Moore JP, et al. (2007) HIV-1 clones resistant to a small molecule CCR5 inhibitor use the inhibitor-bound form of CCR5 for entry. Virology 361: 212-228.

80. Anastassopoulou CG, Marozsan AJ, Matet A, Snyder AD, Arts EJ, et al. (2007) Escape of HIV-1 from a small molecule CCR5 inhibitor is not associated with a fitness loss. PLoS pathogens 3: e79.

81. Ogert RA, Ba L, Hou Y, Buontempo C, Qiu P, et al. (2009) Structure-function analysis of human immunodeficiency virus type 1 gp120 amino acid mutations associated with resistance to the CCR5 coreceptor antagonist vicriviroc. J Virol 83: 12151-12163.

82. Huang C-c, Tang M, Zhang M-Y, Majeed S, Montabana E, et al. (2005) Structure of a V3-Containing HIV-1 gp120 Core. Science 310: 1025-1028.

83. Maeda Y, Yusa K, Harada S (2008) Altered sensitivity of an R5X4 HIV-1 strain 89.6 to coreceptor inhibitors by a single amino acid substitution in the V3 region of gp120. Antiviral Res 77: 128-135.

84. Berro R, Sanders RW, Lu M, Klasse PJ, Moore JP (2009) Two HIV-1 variants

resistant to small molecule CCR5 inhibitors differ in how they use CCR5 for entry. PLoS pathogens 5: e1000548.

85. Anastassopoulou CG, Ketas TJ, Klasse PJ, Moore JP (2009) Resistance to CCR5 inhibitors caused by sequence changes in the fusion peptide of HIV-1 gp41. Proc Natl Acad Sci USA 106: 5318-5323.

86. Lee B, Sharron M, Blanpain C, Doranz BJ, Vakili J, et al. (1999) Epitope mapping of CCR5 reveals multiple conformational states and distinct but overlapping structures involved in chemokine and coreceptor function. J Biol Chem 274: 9617-9626.

87. Berro R, Klasse PJ, Lascano D, Flegler A, Nagashima KA, et al. (2011) Multiple CCR5 conformations on the cell surface are used differentially by human immunodeficiency viruses resistant or sensitive to CCR5 inhibitors. J Virol 85: 8227-8240.

88. Feng Y, Broder CC, Kennedy PE, Berger EA (1996) HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 272: 872-877.

89. Salazar-Gonzalez JF, Salazar MG, Keele BF, Learn GH, Giorgi EE, et al. (2009) Genetic identity, biological phenotype, and evolutionary pathways of transmitted/founder viruses in acute and early HIV-1 infection. J Exp Med 206: 1273-1289.

90. Moore JP, Kitchen SG, Pugach P, Zack JA (2004) The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses 20: 111-126.

91. Schuitemaker H, Koot M, Kootstra NA, Dercksen MW, de Goede RE, et al. (1992) Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. J Virol 66: 1354-1360.

92. Gorry PR, Sterjovski J, Churchill M, Witlox K, Gray L, et al. (2004) The role of viral coreceptors and enhanced macrophage tropism in human immunodeficiency virus type 1 disease progression. Sex Health 1: 23-34.

93. Lapidot T (2001) Mechanism of human stem cell migration and repopulation of NOD/SCID and B2mnull NOD/SCID mice. The role of SDF-1/CXCR4 interactions. Ann N Y Acad Sci 938: 83-95.

94. De Clercq E, Yamamoto N, Pauwels R, Balzarini J, Witvrouw M, et al. (1994) Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. Antimicrob Agents Chemother 38: 668-674.

95. Donzella GA, Schols D, Lin SW, Este JA, Nagashima KA, et al. (1998) AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. Nat Med 4: 72-77.

96. Labrosse B, Labernardiere JL, Dam E, Trouplin V, Skrabal K, et al. (2003) Baseline susceptibility of primary human immunodeficiency virus type 1 to entry inhibitors. J Virol 77: 1610-1613.

97. Schols D, Este JA, Henson G, De Clercq E (1997) Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4. Antiviral Res 35: 147-156.

98. Schols D, Struyf S, Van Damme J, Este JA, Henson G, et al. (1997) Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. J Exp Med 186: 1383-1388.

99. Harrison JE, Lynch JB, Sierra LJ, Blackburn LA, Ray N, et al. (2008) Baseline resistance of primary human immunodeficiency virus type 1 strains to the CXCR4 inhibitor AMD3100. J Virol 82: 11695-11704.

100. Hendrix CW, Collier AC, Lederman MM, Schols D, Pollard RB, et al. (2004) Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. J Acquir Immune Defic Syndr 37: 1253-1262.

101. Este JA, Cabrera C, Blanco J, Gutierrez A, Bridger G, et al. (1999) Shift of clinical human immunodeficiency virus type 1 isolates from X4 to R5 and prevention of emergence of the syncytium-inducing phenotype by blockade of CXCR4. J Virol 73: 5577-5585.

102. Armand-Ugon M, Quinones-Mateu ME, Gutierez A, Barretina J, Blanco J, et al. (2003) Reduced fitness of HIV-1 resistant to CXCR4 antagonists. Antivir Ther 8: 1-8.

103. Schols D, Este JA, Cabrera C, De Clercq E (1998) T-cell-line-tropic human immunodeficiency virus type 1 that is made resistant to stromal cell-derived factor 1alpha contains mutations in the envelope gp120 but does not show a switch in coreceptor use. J Virol 72: 4032-4037.

104. Kanbara K, Sato S, Tanuma J, Tamamura H, Gotoh K, et al. (2001) Biological and genetic characterization of a human immunodeficiency virus strain resistant to CXCR4 antagonist T134. AIDS Res Hum Retroviruses 17: 615-622.

105. Moyle G, DeJesus E, Boffito M, Wong RS, Gibney C, et al. (2009) Proof of activity with AMD11070, an orally bioavailable inhibitor of CXCR4-tropic HIV type 1. Clin Infect Dis 48: 798-805.

106. Murakami T, Kumakura S, Yamazaki T, Tanaka R, Hamatake M, et al. (2009) The novel CXCR4 antagonist KRH-3955 is an orally bioavailable and extremely potent inhibitor of human immunodeficiency virus type 1 infection: comparative studies with AMD3100. Antimicrob Agents Chemother 53: 2940-2948.

107. Jenkinson S, Thomson M, McCoy D, Edelstein M, Danehower S, et al. (2010) Blockade of X4-tropic HIV-1 cellular entry by GSK812397, a potent noncompetitive CXCR4 receptor antagonist. Antimicrob Agents Chemother 54: 817-824.

108. Lazzarin A, Clotet B, Cooper D, Reynes J, Arasteh K, et al. (2003) Efficacy of enfuvirtide in patients infected with drug-resistant HIV-1 in Europe and Australia. N Engl J Med 348: 2186-2195.

109. Wild C, Oas T, McDanal C, Bolognesi D, Matthews T (1992) A synthetic peptide inhibitor of human immunodeficiency virus replication: correlation between solution structure and viral inhibition. Proc Natl Acad Sci U S A 89: 10537-10541.

110. Chen CH, Matthews TJ, McDanal CB, Bolognesi DP, Greenberg ML (1995) A molecular clasp in the human immunodeficiency virus (HIV) type 1 TM protein determines the anti-HIV activity of gp41 derivatives: implication for viral fusion. J Virol 69: 3771-3777.

111. Eckert DM, Malashkevich VN, Hong LH, Carr PA, Kim PS (1999) Inhibiting HIV-1 entry: discovery of D-peptide inhibitors that target the gp41 coiled-coil pocket. Cell 99: 103-115.

112. Rimsky LT, Shugars DC, Matthews TJ (1998) Determinants of human immunodeficiency virus type 1 resistance to gp41-derived inhibitory peptides. J Virol 72: 986-993.

113. Sista PR, Melby T, Davison D, Jin L, Mosier S, et al. (2004) Characterization of determinants of genotypic and phenotypic resistance to enfuvirtide in baseline and on-treatment HIV-1 isolates. AIDS 18: 1787-1794.

114. Marcial M, Lu J, Deeks SG, Ziermann R, Kuritzkes DR (2006) Performance of human immunodeficiency virus type 1 gp41 assays for detecting enfuvirtide (T-20) resistance mutations. J Clin Microbiol 44: 3384-3387.

115. Izumi K, Kodama E, Shimura K, Sakagami Y, Watanabe K, et al. (2009) Design of peptide-based inhibitors for human immunodeficiency virus type 1 strains resistant to T-20. J Biol Chem 284: 4914-4920.

116. Nameki D, Kodama E, Ikeuchi M, Mabuchi N, Otaka A, et al. (2005) Mutations conferring resistance to human immunodeficiency virus type 1 fusion inhibitors are restricted by gp41 and Rev-responsive element functions. J Virol 79: 764-770.

117. Xu L, Pozniak A, Wildfire A, Stanfield-Oakley SA, Mosier SM, et al. (2005) Emergence and evolution of enfuvirtide resistance following long-term therapy involves heptad repeat 2 mutations within gp41. Antimicrob Agents Chemother 49: 1113-1119.

118. Fischer U, Meyer S, Teufel M, Heckel C, Luhrmann R, et al. (1994) Evidence that HIV-1 Rev directly promotes the nuclear export of unspliced RNA. EMBO J 13: 4105-4112.

119. Daugherty MD, Liu B, Frankel AD (2010) Structural basis for cooperative RNA binding and export complex assembly by HIV Rev. Nat Struct Mol Biol 17: 1337-1342.

120. Svicher V, Aquaro S, D'Arrigo R, Artese A, Dimonte S, et al. (2008) Specific enfuvirtide-associated mutational pathways in HIV-1 Gp41 are significantly correlated with an increase in CD4(+) cell count, despite virological failure. J Infect Dis 197: 1408-1418.

121. Ueno M, Kodama EN, Shimura K, Sakurai Y, Kajiwara K, et al. (2009) Synonymous mutations in stem-loop III of Rev responsive elements enhance HIV-1 replication impaired by primary mutations for resistance to enfuvirtide. Antiviral Res 82: 67-72.

122. Lu J, Deeks SG, Hoh R, Beatty G, Kuritzkes BA, et al. (2006) Rapid emergence of enfuvirtide resistance in HIV-1-infected patients: results of a clonal analysis. J Acquir Immune Defic Syndr 43: 60-64.

123. Mink M, Mosier SM, Janumpalli S, Davison D, Jin L, et al. (2005) Impact of human immunodeficiency virus type 1 gp41 amino acid substitutions selected during enfuvirtide treatment on gp41 binding and antiviral potency of enfuvirtide in vitro. J Virol 79: 12447-12454.

124. Hanna SL, Yang C, Owen SM, Lal RB (2002) Variability of critical epitopes within HIV-1 heptad repeat domains for selected entry inhibitors in HIV-infected populations worldwide [corrected]. AIDS 16: 1603-1608.

125. Pessoa LS, Valadao AL, Abreu CM, Calazans AR, Martins AN, et al. (2011) Genotypic analysis of the gp41 HR1 region from HIV-1 isolates from enfuvirtide-treated and untreated patients. J Acquir Immune Defic Syndr 57 Suppl 3: S197-201.

126. Roman F, Gonzalez D, Lambert C, Deroo S, Fischer A, et al. (2003) Uncommon mutations at residue positions critical for enfuvirtide (T-20) resistance in enfuvirtide-naive patients infected with subtype B and non-B HIV-1 strains. J Acquir Immune Defic Syndr 33: 134-139.

127. Shimura K, Nameki D, Kajiwara K, Watanabe K, Sakagami Y, et al. (2010) Resistance profiles of novel electrostatically constrained HIV-1 fusion inhibitors. J Biol Chem 285: 39471-39480.

128. Eggink D, Bontjer I, Langedijk JP, Berkhout B, Sanders RW (2011) Resistance of Human Immunodeficiency Virus Type 1 to a Third-Generation Fusion Inhibitor Requires Multiple Mutations in gp41 and Is Accompanied by a Dramatic Loss of gp41 Function. J Virol 85: 10785-19797.