Human immunodeficiency virus (HIV)-1 infection is initiated by the binding of gp120 envelope glycoprotein to its cell receptor (CD4) and a coreceptor (CXCR4 or CCR5), followed by a series of conformational changes in the gp41 transmembrane subunit. These changes include insertion of fusion peptide into the target cell membrane and association of C-heptad repeat (CHR) peptide with the N-heptad repeat (NHR) trimer, a pre-hairpin fusion intermediate. A stable six-helix bundle core is then formed, bringing the viral envelope and target cell membrane into close proximity for fusion. Peptides derived from the CHR region, such as T20 and C34, inhibit HIV-1 fusion by interacting with the gp41 fusion intermediate. A number of anti-HIV-1 peptides and small molecule compounds targeting the gp41 NHR-trimer have been identified. By combining HIV fusion/entry inhibitors targeting different sites in the gp41 fusion intermediate, a potent synergistic effect takes place, resulting in a potential new therapeutic strategy for the HIV infection/AIDS. Here, we present an overview of the current development of anti-HIV drugs, particularly those targeting the gp41 fusion intermediate. [J Formos Med Assoc 2010;109(2):94–105]

Key Words: fusion inhibitor, gp41, HIV-1, peptide, therapeutics

Since acquired immunodeficiency syndrome (AIDS) was first reported in 1981, almost 60 million people worldwide have been infected by human immunodeficiency virus (HIV), the causative agent for AIDS; and cumulatively 25 million patients have died from AIDS.1–3 By now, 28 anti-HIV drugs, including 15 reverse transcriptase inhibitors (RTIs) and 10 protease inhibitors (PIs), have been licensed by the US Food and Drug Administration (FDA) for treatments of HIV infection/AIDS.4 Clinical application of these antiretroviral drugs in combinations (known as highly active antiretroviral therapy) have been shown to reduce the morbidity and mortality of HIV infection/AIDS significantly.5 However, because of the emergence of multidrug resistance to RTIs and PIs, many patients failed to respond to the current antiretroviral therapeutics.6–8 Therefore, it is essential to develop new anti-HIV drugs, particularly targeting the fusion and entry steps of HIV infection.

HIV gp41 Fusion Intermediate

The HIV envelope (Env) glycoprotein surface subunit gp120 is responsible for virus binding to
its cell receptors, CD4; while its transmembrane subunit gp41 mediates virus fusion with and entry into the host cell. The gp41 consists of an extracellular domain, a transmembrane domain, and a cytoplasm domain. The extracellular domain contains a fusion peptide, a N-terminal heptad repeat (NHR), a loop region, a C-terminal heptad repeat (CHR), and a membrane-proximal external region (MPER) (Figure 1A). The NHR contains a pocket-forming domain (PFD), a heptad repeat (HR) sequence, and a glycine-isoleucine-valine (GIV) motif. CHR contains a pocket-binding domain (PBD) and an HR sequence. MPER contains a tryptophan-rich domain (TRD) or lipid-binding domain (LBD).9–13 Both NHR and CHR regions contain hydrophobic 4-3 HR sequences that can form coiled-coil structure (Figure 1B).14

HIV infection is initiated by the binding of gp120 to its primary receptor, CD4, and a chemokine coreceptor (CXCR4 or CCR5) on the host cell, triggering a series of conformational changes in gp41 and allowing the fusion peptide of gp41 inserts into the cell membrane, and the pre-hairpin fusion intermediate NHR-trimer forms. In each groove of the NHR-trimer, there is a highly conserved hydrophobic deep pocket formed by the PFD, which plays a critical role in maintaining the stability of the six-helix bundle (6-HB) and viral fusion.9,15,16 Subsequently, three molecules of CHR pack obliquely in an anti-parallel manner into the highly conserved hydrophobic grooves on the surface of the NHR-trimer to form a stable gp41 6-HB core structure,8 which brings the viral and host cell membranes into proximity for virus-cell fusion.19 Therefore, any molecules that block the 6-HB formation may inhibit the virus-cell fusion.10,20,21

Anti-HIV Peptides Targeting the gp41 Fusion Intermediate

In the early 1990s, our group discovered the first highly potent anti-HIV peptide, SJ-2176, which was derived from the HIV-1 gp41 CHR region (a.a. 630–659, the residue numbers correspond to their positions in gp160 of HIV-1HXB2).22,23 Later, Wild et al reported another CHR peptide, T20 (a.a. 638–673), with potent inhibitory activity against HIV fusion.24 Using the protein dissection strategy, Lu et al identified a series of NHR peptides, including N51 (a.a. 540–590), N36 (a.a. 546–581), and N34 (a.a. 546–579), as well as CHR peptides, such as C43 (a.a. 624–666), C34 (a.a. 628–661), and C28 (a.a. 628–655) (Figure 1A).14,25,26 C34 exhibits higher anti-HIV potency than SJ-2176 and T20.5

Crystallographic studies and biochemical analyses revealed that both SJ-2176 and C34 contain the PBD and HR sequence, and inhibit HIV-1 Env-mediated membrane fusion by interacting with the pocket region and HR sequence in the viral gp41 NHR and blocking gp41 6-HB core formation, a critical step in virus-cell fusion.17–19,21,27 T20, which contains the TRD/LBD but lacks the PBD, inhibits HIV fusion by interacting with the viral gp41 NHR domain through its N-terminal HR sequence and binding to the lipid membrane of the target cell via its C-terminal LBD (Figure 1).11,12,28,29 T20 (also called enfuvirtide or Fuzeon®) was developed by Trimeris, Inc. (Durham, NC, USA) and Hoffmann-La Roche, Inc. (Nutley, NJ, USA) as the first HIV fusion/entry inhibitors. T20 have been licensed by the US FDA since 2003 for the treatment of HIV patients who failed to respond to the current antiretroviral therapeutics.30 T20 is effective against a broad spectrum of HIV-1 strains, including those resistant to RTIs and PIs. However, T20 could induce drug-resistant mutations in the viral gp41 NHR region, resulting in an increased number of patients failing to respond to T20.31–34 In addition, T20 has several other pitfalls, including low potency and short half-life in vivo.35 To address these problem, T20 analogous peptides or recombinant proteins containing CHR sequence with improved production efficiency, half-life, and antiviral potency against a broad spectrum of HIV-1 strains, including the T20-resistant variants, have been developed.
Figure 1. Schematic representation of the HIV-1 gp41 fusion intermediate and its target sites for anti-HIV therapeutics. (A) Schematic view of the HIV-1HXB2 gp41 molecule and sequences of the NHR- and CHR-peptides. PFD in the NHR, the QIWNMT-motif and PBD in CHR, as well as LBD in the MPER are highlighted in green, pink, blue, and red, respectively. (B) Interaction between the NHR- and CHR-peptides. The dashed lines between the NHR and CHR domains indicate the interaction between the residues located at the e, g and a, d positions in the helical wheels of the NHR and CHR domains, respectively. The association between NHR and CHR results in formation of the 6-HB core, while the interaction between PFD in the NHR and PBD in the CHR is critical for stabilization of 6-HB. (C) The target sites in the gp41 fusion intermediate for HIV fusion/entry inhibitors. FP = fusion peptide; NHR = N-terminal heptad repeat; CHR = C-terminal heptad repeat; MPER = membrane-proximal external region; PFD = pocket-forming domain; PBD = pocket-binding domain; LBD = lipid-binding domain; HB = helix bundle; TM = transmembrane domain; CP = cytoplasmic domain.
T1249, designed by Trimeris Inc. as the second-generation HIV fusion inhibitor, is a 39-mer peptide consisting of a PBD, a CHR-binding domain, and a TRD (Figure 1). Pre-clinical and clinical studies have demonstrated that T1249 has a longer half-life in primates and greater anti-HIV-1 potency than T20. T1249 is active against most T20-resistant HIV-1 variants. However, the clinical development of T1249 was discontinued due to formulation difficulties.

T1144 was also designed by Trimeris Inc. as the third-generation HIV fusion inhibitor by modifying the amino acid sequence of C38 (a.a. 626–673) to increase the helicity and 6-HB stability. Like C34, it contains a PBD and a HR sequence (Figure 1). T1144 and its analogous peptides have shown more potent anti-HIV-1 activity than T20 and have exhibited improved pharmacokinetic properties. They are effective against T20-resistant virus strains with lower chances to induce drug-resistant mutations, suggesting that T1144 is an attractive candidate for further development.

Sifuvirtide is a new generation of HIV fusion inhibitor designed by FusoGen Pharmaceuticals Inc. (Tianjin, China) through modification of the CHR-peptide C36 (a.a. 627–672), based on the 3D structural information of HIV-1 gp41 and computer modeling analysis. Like C34 and T1144, Sifuvirtide contains the PBD and the HR sequence (Figure 1). We have demonstrated that Sifuvirtide is more potent than T20 against both primary- and laboratory-adapted HIV-1 strains and is effective against T20-resistant virus variants. It has a longer half-life than T20. In the Phase Ia clinical trial, Sifuvirtide showed good safety, tolerability, and pharmacokinetic profiles in healthy individuals. It is currently under Phase II clinical development.

CP32 is a 32-mer peptide derived from a CHR sequence (a.a. 621–652) which contains the HR sequence, PBD, and a QIWNMT motif (Figure 1). This motif, which is located at the upstream region of the CHR and immediately adjacent to the PBD, is critical for the gp41 6-HB core stabilization. This peptide can interact with the NHR-peptide T21 (a.a. 553–590) to form 6-HB with high thermostability ($T_m=81^\circ C$) and significantly inhibit the gp41 fusogenic core formation between the viral NHR and CHR, thereby blocking HIV-1 Env-mediated cell-cell fusion and HIV-1 replication. Notably, CP32 exhibited potent antiviral activity against HIV-1 strains which is resistant to T20 and C34, because it lacks the sequence that binds to GIV motif, the determinant of T20-resistance in NHR.

Interestingly, further modification of CP32 results in the generation of an analogous peptide, CP32M, which has improved thermostability ($T_m=94^\circ C$) and enhanced antiviral potency against HIV-1 variants resistant to T20, C34, and T1249.

SC34EK(Nle) is a C34 analogous peptide with improved solubility and anti-HIV-1 efficacy. The non-conserved residues located at the solvent-accessible sites in the 4-3 HR sequence, including those at the "b", "c", "f", and "g" positions in the $\alpha$-helical wheel, are restituted with the positively and negatively charged residues (Lys and Glu, respectively) at the $i$ and $i+4$ positions to introduce intrahelical salt bridges, which are expected to enhance the solubility and $\alpha$-helicity of the CHR-peptides. Indeed, SC34EK(Nle) exhibits higher HIV fusion inhibitory activity and solubility in aqueous solutions than T20 and C34.

C14 linkmid was designed based on a 14-mer CHR-peptide, C14 (a.a. 626–639) by substituting the residues at the "b" position (a.a. 629 and 636) in the helical wheel with Glu and crosslinking these residues via a ($\alpha$, $\omega$)-diaminoalkane group, which stabilizes the helix of C14. This short CHR-peptide can bind to the hydrophobic pocket on the NHR-trimer and inhibit the HIV-1 Env-induced cell-cell fusion with IC$_{50}$ of 35 $\mu$M, while the unmodified C14 peptide has no anti-HIV-1 activity.

D10-p1-2K, D10-p5-2K, and PIE7 are short cyclic peptides consisting of all D-amino acid residues which are resistant to proteolytic enzymes. As determined by X-ray crystallography and nuclear magnetic resonance, these peptides specifically bind to the hydrophobic pocket of the NHR-peptide T21 (a.a. 553–590) to form 6-HB with high thermostability ($T_m=81^\circ C$) and significantly inhibit the gp41 fusogenic core formation between the viral NHR and CHR, thereby blocking HIV-1 Env-mediated cell-cell fusion and HIV-1 replication. Notably, CP32 exhibited potent antiviral activity against HIV-1 strains which is resistant to T20 and C34, because it lacks the sequence that binds to GIV motif, the determinant of T20-resistance in NHR.

Interestingly, further modification of CP32 results in the generation of an analogous peptide, CP32M, which has improved thermostability ($T_m=94^\circ C$) and enhanced antiviral potency against HIV-1 variants resistant to T20, C34, and T1249.
presented on NHR-trimers, such as IQN17. D10-p5-2K and PIE7 can inhibit HIV-1 infection with IC$_{50}$, ranging from low μM to nM levels. These short D-peptides are expected to be resistant to proteases, which may significantly increase their serum half-lives and may be absorbed systemically when taken orally, suggesting an anti-HIV therapeutic potential.

C52L is a recombinant protein consisting of the PBD, the HR sequence and the TRD, overlapping the gp41 CHR sequence (a.a. 624–675). It binds to the gp41 NHR region and potently inhibits in vitro infection by diverse primary HIV-1 isolates with nanomolar (nM) IC$_{50}$ values. C52L is also very effective against T20-resistant variants. Since it is expressed in Escherichia coli, C52L is expected to be more economical to manufacture on a large scale than T20-like peptides produced by chemical synthesis and have a potential to be developed as an effective and inexpensive microbicide for prevention of sexual transmission of HIV.

HR212, another recombinant protein expressed in E. coli, consists of two molecules of HR2-peptide (C34: a.a. 628–661) and one molecule of HR1-peptide (N34: a.a. 546–579) linked in the order of HR2-HR1-HR2. In aqueous solution, HR212 can form a stable 6-HB with three HR2-peptides exposed, which may bind to the NHR region in the viral gp41, resulting in inhibition of gp41-mediated membrane fusion. HR212 exhibits potent inhibitory activity on infection by primary and laboratory-adapted HIV-1 strains with IC$_{50}$ values in the nM range. These data suggested that HR212 can be potentially developed as a therapeutic agent in a manner analogous to synthetic CHR-peptides, but may be much less expensive than the synthetic peptides since HR212 can be easily expressed and purified from the bacterial culture.

In the pre-hairpin fusion intermediate, the hydrophobic face of CHR-helix is also accessible to the peptidic HIV fusion inhibitors, such as T21, N51, and N36. HR121 is a recombinant protein containing two molecules of HR1-peptide N34 and one molecule of HR2-peptide C34 connected by linkers in the order of HR1-HR2-HR1. In aqueous solution, HR121 forms a stable 6-HB with the HR1-trimer exposed, which can bind to the CHR region in the viral gp41 and block the viral gp41 fusion core formation. It is also effective in inhibiting HIV-1 infection, although its anti-HIV-1 potency is lower than that of HR212.

NCCG-gp41 was designed by linking an NHR-peptide N35 (a.a. 546–580) to the N-terminus of N34(L6)C28 polypeptide and substituting Leu576, Gln577, and Ala578 of N35 with Cys, Cys, and Gly, respectively. In NCCG-gp41, the three N34(L6)C28 polypeptides form 6-HB whereas the three N35 peptides form an NHR-trimer stabilized by three intermolecular disulfide bonds. Like HR121, NCCG-gp41 can inhibit HIV-1 Env-mediated membrane fusion by targeting the gp41 CHR-helices in the fusion intermediate state.

5-Helix contains three NHR-peptides N40 (a.a. 543–582) and two CHR-peptides C38 (a.a. 625–662) connected by linkers in the order of N40-C38-N40-C38-N40. In aqueous solution, it forms a stable and soluble 5-HB with one exposed hydrophobic groove, which can attract one of the viral gp41 CHR-helices to form 6-HB core. Therefore, 5-helix exhibits highly potent anti-HIV-1 activity with IC$_{50}$ at low nM level. 5-Helix conjugated with Pseudomonas, an exotoxin protein, can serve as a “biological missile” by specifically binding to the CHR region of the viral gp41 on the HIV-1-infected cells, thereby killing these infected cells with its toxin component or blocking fusion between HIV-1-infected and uninfected cells.

Virus-inhibitory peptide (VIRIP) is a 20-mer peptide corresponding to the C-proximal region of α1-antitrypsin, the most abundant circulating serine PIs in human blood. This peptide inhibits infection by a wide variety of primary HIV-1 isolates. Changes of a few amino acid residues in the peptide result in increase of its ant-HIV-1 potency by two orders of magnitude. Therefore, this natural HIV fusion/entry
inhibitor targeting the gp41 fusion peptide may be further developed as a new class of antiretroviral drugs.

Small Molecule Anti-HIV Compounds Targeting the gp41 Fusion Intermediate

Although T20 is effective against HIV-1 strains resistant to RTIs and PIs, it has a short half-life in vivo because the peptide can be easily degraded by proteolytic enzymes in the blood. To maintain constant high concentration of T20 in vivo, T20 must be administered by injection twice a day at 90 mg/dose, resulting in painful injection-site reactions in most patients and high cost to the patients (>US$20,000/year/patient). Because of these problems, it is essential to develop small molecule HIV fusion/entry inhibitors targeting the gp41 fusion intermediate with oral bioavailability and low production cost.

ADS-J1 is the first small molecule HIV fusion/entry inhibitor identified with a molecular modeling-based virtual screening in combination with a sandwich enzyme-linked immunosorbent assay using the NHR-peptide N36, the CHR-peptide C34, and a conformation-specific monoclonal antibody, NC-1. ADS-J1 inhibits HIV-1-mediated membrane fusion and HIV-1 replication with IC₅₀ in the low μM range. Computer-aided molecular docking analysis suggested that ADS-J1 is able to dock into the deep hydrophobic pocket on the gp41 N-helix trimer through the interaction between hydrophobic groups (phenyl and naphthalene) of ADS-J1 and the hydrophobic residues in the pocket. Furthermore, one of the sulfonic acid groups of ADS-J1 is in close proximity to a basic residue (K574) in the pocket region of the NHR domain. Binding of ADS-J1 to K574 may block the interaction between the K574 in NHR and the D632 in CHR in the viral gp41 to form a salt bridge, which is important for the stability of the fusion-active gp41 core. Although ADS-J1 possesses properties as an HIV fusion/entry inhibitor targeting gp41, it is not an ideal lead for drug development since it contains several sulfonic acid groups and an azo group that may potentially induce tumorigenesis.

We then screened a chemical library consisting of 33,040 “drug-like” compounds using a fluorescent-linked immunosorbent assay-based high-throughput screening assay and identified two N-substituted pyroles, NB-2 and NB-64, which have potent anti-HIV-1 activities. These compounds inhibited infection by both laboratory-adapted and primary HIV-1 strains with distinct genotypes (clades A to G and group O) and phenotypes (R5, X4, and R5X4) at low μM levels. Furthermore, both NB-2 and NB-64 effectively inhibited HIV-1 Env-mediated cell-cell fusion. They interfere with the gp41 conformational changes by blocking the formation of the fusion-active gp41 6-HB. Computer-aided molecular docking analysis has shown that both compounds fit inside the hydrophobic pocket, and their -COOH group interacts with a positively charged residue (K574) around the pocket to form a salt bridge, thereby blocking the formation of the 6-HB and ultimately inhibiting HIV-1-mediated membrane fusion. The -COOH group in NB-2 and NB-64 is critical to their inhibitory activities since NB-177 and NB-178 which have the same structure as NB-2 and NB-64, respectively, but lack the -COOH group have no anti-HIV-1 activities.

Based on the structures of NB-64, Xie and colleagues recently designed and synthesized 42 N-carboxyphenylpyrrole derivatives in two categories (A and B series). We tested these compounds and found that 11 of them exhibited promising anti-HIV-1 activity, which is correlated with their inhibitory activities on gp41 6-HB formation, suggesting that these compounds block HIV fusion and entry by disrupting gp41 core formation. The most active compound, N-(3-carboxy-4-hydroxy)phenyl-2,5-dimethylpyrrole (A₁₂) (Figure 2), could inhibit HIV-1 IIIB infection with IC₅₀ of 0.69 μM. The molecular docking analysis has revealed that the carboxyl group of A₁₂ could interact with either K574 or R579 in the gp41 NHR to form salt bridges and that two methyl groups on the pyrrole ring are...
favorable for interaction with the hydrophobic residues in the gp41 pocket. Like NB-2 (molecular weight = 231) and NB-64 (molecular weight = 222), A12 is also a low molecular size compound (molecular weight = 231). Therefore, it only partially occupies the deep hydrophobic pocket, which can hold a molecule of >600 dalton. We therefore proposed that enlarging the molecular size of A12 may improve its binding affinity and anti-HIV-1 activity.

Accordingly, we designed a series of 2-aryl-5-(4-oxo-3-phenethyl-2-thioxothiazolidinylidenemethyl) furans with larger molecular size (437–515). Computer-based molecular docking

Figure 2. Chemical structures of the small molecule anti-HIV compounds targeting the gp41 fusion intermediate.
analysis indicates that these compounds could occupy most of the space in the deep hydrophobic pocket on the gp41 NHR-trimer. Katritzky and colleagues synthesized 15 compounds (11a-o) of this series by Suzuki-Miyaura cross coupling and Knoevenagel condensation. We tested these compounds for their anti-HIV-1 activity and found that all 15 compounds had improved anti-HIV-1 activity. One of these compounds, NB-293, inhibited infection by the laboratory HIV-1 strain IIIB and a primary HIV-1 isolate 94UG103 with IC_{50} of 44 nM and 74 nM, respectively, >20-fold more potent than NB-2, NB-64, and A_{12}, suggesting that this compound can serve as a lead for development of novel small molecule HIV fusion inhibitors targeting the gp41 fusion intermediate.

At the same time, several other groups have also put extensive effort into the search for lead compounds that target the gp41 NHR-trimer, especially the pocket region. Harrison’s group at Harvard University used a structure-based combinatorial approach to screen a biased combinatorial library consisting of 61,275 compounds, each of which was linked to the N-terminal residue of a CHR-peptide P30 (a.a. 636–665) that lacks the PBD. They found one hybrid molecule, C7-Mn34-Mn42-P30, with HIV-1 fusion inhibitory activity about 20-fold over the P30 peptide alone. However, the small non-peptide moiety itself (C7-Mn34-Mn42) has no detectable anti-HIV-1 activity, although it could bind to the hydrophobic pocket on the gp41 NHR-trimer as shown by X-ray crystallography (Figure 2). Its low binding affinity to the gp41 hydrophobic pocket may be attributed to its inability to inhibit HIV-1 infection. Later, the same group screened several libraries consisting of 34,800 small molecules (<500) with a high-throughput screening assay using a metal-ligated dye-conjugated NHR-peptide and a fluorophore-labeled CHR-peptide, which can form 6-HB in liquid. Cai et al identified three new low-molecular-weight compounds that could inhibit the gp41 6-HB formation. Among these, the best compound is 11 (Figure 2) which inhibits HIV-1 Env-mediated syncytium-formation in the low μM range (IC_{50} ~ 8 μM). Although these compounds lack potent anti-HIV-1 activity, they provide new molecular scaffolds for the development of HIV fusion/entry inhibitors targeting the gp41 fusion-intermediate.
HIV Fusion/Entry Inhibitor-based Combination Therapy

Using antiretroviral drugs with different targets in combination is able to increase the antiviral potency because of the synergistic effect resulting from the combination, reduce adverse effects and delay the emergence of drug resistance. It is well known that combining HIV-1 entry inhibitors targeting gp120 (e.g. PRO 542), gp41 (e.g. T20), and the coreceptor, CXCR4 (e.g. AMD3100) or CCR5 (e.g. SCH-C) exhibits strong synergistic anti-HIV-1 activity. We therefore proposed that combining HIV fusion/entry inhibitors targeting different sites in the gp41 fusion intermediate could also result in a similar phenomenon. Consequently, we first tested the combination of T20, which contains an HR-binding sequence and LBD, with Sifuvirtide that consists of the PBD and an HR-binding sequence (Figure 1). We found that this combination indeed exhibited strong synergic antiviral activity against both laboratory-adapted and primary HIV-1 strains.

Then, we tested the combinations of T20, the first generation HIV fusion inhibitor, with T1249 and T1144, the second and third generation HIV fusion inhibitors, respectively, against HIV-1-induced cell-cell fusion. Very surprisingly, double combination of T20 with T1249 or T1144 leads to highly potent synergism with two orders of dose reduction, while triple combination (T20+T1249+T1144) exhibits exceptional synergistic effect with three orders of dose reduction. Strong synergism was also observed in the combinations of T20 with T1249 and/or T1144 against infection by both laboratory-adapted strains and primary HIV-1 isolates. Strikingly, combining T20 with T1249 and/or T1144 leads to strong synergism against infection by T20- and T1249-resistant variants.

Previous in vitro and in vivo studies have demonstrated that the GIV motif in the gp41 NHR region is the primary determinant of HIV-1 resistance to T20. Unlike T20, both T1249 and T1144 have a primary binding site in the hydrophobic pocket on the gp41 NHR-trimer. Therefore, the mutations in the GIV motif in the viral gp41 NHR region may have much less effect on the binding of T1249 and T1144 to the viral gp41 NHR than that of T20. Therefore, the combination of T1249 or T1144 with T20 may overcome the T20-resistance of the mutant viruses. Because T20, T1249 and T1144 contain different functional domains and have distinct primary binding sites in the gp41 fusion intermediate, binding of one CHR-peptide to the viral gp41 NHR domain may extend the temporal window period of the fusion intermediate, which would then become more accessible to other CHR-peptides, resulting in synergistic effect against HIV-1 infection. These findings suggest a new therapeutic strategy for treatment of HIV/AIDS patients who have failed to respond to the first generation HIV fusion inhibitor or other anti-HIV drugs targeting different steps of HIV replication cycle.

Concluding Remarks

Since the discovery of the peptidic HIV fusion inhibitors in the early of 1990s, a series of anti-HIV peptides and small molecule compounds targeting the gp41 fusion intermediate have been studied. One of them, T20 has been licensed by the US FDA for use in clinics to treat HIV-infected patients who fail to respond to current antiretroviral therapeutics, while others are in pre-clinical or clinical development. These studies have provided knowledge, techniques, and experience for development of viral fusion/entry inhibitors against HIV and other viruses with class I membrane fusion proteins. We are the first to show that combining different generations of HIV fusion/entry inhibitors targeting the distinct target sites in the gp41 fusion intermediate results in highly potent synergy, thus providing a new therapeutic strategy for treatment of HIV infection and other diseases.
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