Novel Anti-Viral Therapy: Drugs that Block HIV Entry at Different Target Sites

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Abstract: Drugs that block the entry of human immunodeficiency virus type 1 (HIV-1) into host cells abrogate the establishment of a productive infection and should ideally diminish the chances of HIV-1 developing resistance. This review will give an overview of the mechanism by which the envelope glycoprotein mediates HIV-1 entry and will summarize current drug developments.

Keywords: HIV, entry, inhibitor.

HIV ENTRY PROCESS

The acquired immunodeficiency syndrome (AIDS) was first described about 20 years ago. Since then almost 20 million people have died from human immunodeficiency virus (HIV) infection and an enormous number of people are infected. Improved drugs are urgently needed and a new class of drugs, the entry inhibitors, are currently in clinical development and some have already entered the market. These drugs block the entry of HIV type 1 (HIV-1) into host cells and have clear advantages over the currently used drugs that act at later stages of the viral life cycle. Entry inhibitors should abrogate the establishment of a productive infection and therefore have the potential to diminish the chances of HIV-1 developing resistance. We will summarize the current developments by starting with a short introduction into the HIV entry process.

HIV entry into cells is a complex process accomplished by envelope glycoproteins (Env) on the surface of the virions (Fig. **1**). Envs attach virions to target cells and promote the thermodynamically unfavorable fusion of the viral and the target cell membrane. The HIV Env is synthesized as a homotrimeric gp160 precursor protein and is cleaved in the Golgi apparatus, during transport to the cell surface, into two subunits, the surface glycoprotein gp120 (SU) and the transmembrane domain gp41 (TM). The gp120 subunit remains non-covalently associated with gp41 after cleavage. Virus entry is accompanied by a series of conformational changes in these proteins which begin with the binding of the primary viral receptor CD4. CD4 binding stabilizes a conformation of gp120 in which a previously hidden site that can associate with the co-receptor is exposed. Co-receptor binding induces additional changes that lead to dissociation of gp120 from gp41, major refolding of gp41 and finally membrane fusion. A detailed understanding of these conformational rearrangements has been obtained from X-ray crystallographic studies performed with either CD4-bound HIV gp120 in complex with a monoclonal antibody fragment that recognizes the co-receptor binding site [1] or the unliganded simian immunodeficiency virus (SIV) gp120 resembling the prefusion structure [2].

As a result of the reverse transcription step during viral replication, HIV is highly variable and many different HIV strains exist. Based on genetic similarities, the numerous virus strains have been classified into types and subtypes. There are two types of HIV: HIV-1 and HIV-2, and several subtypes or clades.

HIV tropism is controlled at the level of co-receptor usage. M-tropic (R5) viruses infect monocytes and macrophages, are usually found early in infection (primary strains) and use the chemokine receptor CCR5. T-tropic (X4) viruses infect T cell lines, emerge late in disease and use the chemokine receptor CXCR4. Chemokine receptors belong to the family of seven-transmembrane-spanning receptors and are coupled to G-protein signaling pathways.

The different Env conformations that occur during the viral entry process present target sites for intervention and Fig. **1** summarizes the therapeutic strategies. HIV entry inhibitors are roughly divided into three classes: (1) CD4 attachment inhibitors, (2) co-receptor inhibitors and (3) fusion inhibitors which target gp41.

1. CD4 Attachment Inhibitors

The primary HIV receptor CD4 is a membrane-spanning surface molecule expressed by T-helper cells. Drugs that prevent gp120 binding to CD4 should only block the CD4 binding site in gp120 and should not induce the CD4-bound stable conformation of gp120 which can bind the co-receptor and initiate fusion. Initially, recombinant, soluble versions of CD4 (sCD4) were thought to have therapeutic potential by binding HIV as a competitor for membrane-bound CD4. Recombinant sCD4 has been shown to be efficient against laboratory strains of HIV *in vitro*, but primary M-tropic strains are relatively resistant. PRO 542 is the latest molecule in the line of soluble CD4 receptors. It consists of the two N-terminal extracellular immunoglobulin (Ig)-like domains of CD4, which contain the gp120 binding site, fused to human

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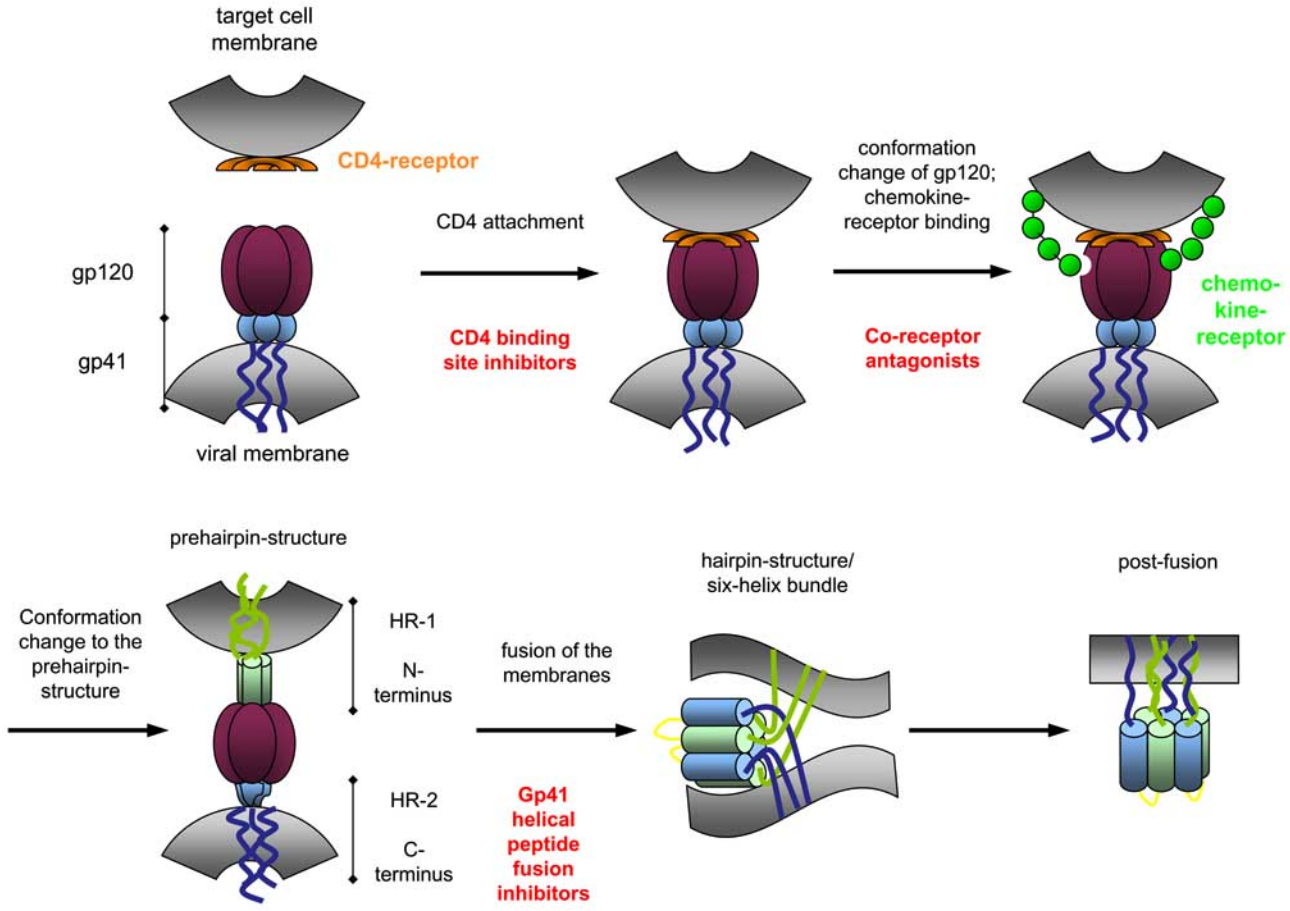
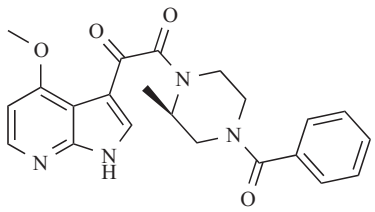


Fig. (1). Schematic presentation of the HIV entry process and possible target sites of interference.

IgG heavy and light chain, and is a tetravalent, high affinity gp120 binder [3]. Clinical trials in children demonstrated *in vivo* efficiency [4].

A small compound drug that also fulfills the requirement is 1-(4-benzoyl-2-methyl-piperazin-1-yl)-2-(2-methoxy-5,7-diazabicyclo[4. 3. 0]nona-1,3,6,8-tetraen-9-yl)-ethane-1,2-dione (BMS-378806) (Fig. **2**), which is being developed by Bristol-Myers Squibb. It binds to gp120 and inhibits Env interaction with the CD4 receptor with a binding stoichiometry of 1:1 [5]. The drug-binding site in gp120 has been defined by resistance mutations and determination of the structure of the unliganded gp120. It lies in the deep, hydrophobic channel of the CD4 binding site and suggests that BMS-378806 inhibits entry by stabilizing the unliganded gp120 conformation [2]. BMS-378806 is active against most HIV-1 strains with a median EC_{50} of 40 nM against subtype B isolates, however it is inactive against HIV-2 and SIV [6]. It exhibits no significant cytotoxicity and has highly favourable pharmacological properties, such as low protein binding *in vitro*, minimal human serum effect on anti-HIV-1 potency, good oral bioavailability in animal species and a clean safety profile in initial animal toxicology studies. Although BMS-378806-resistant mutations also arise, this compound or derivatives thereof have prospects of becoming useful drugs.

CD4 attachment inhibitor



BMS-378806

Fig. (2). Structure of the CD4 attachment inhibitor BMS-378806.

2. Co-Receptor Inhibitors

CCR5 Antagonists

The value of co-receptors as drug targets has been made clear by the appearance of resistance to HIV-1 infection in individuals homozygous for an inactivating 32 base pair deletion in CCR5. This demonstrated that the lack of CCR5 causes resistance to HIV infection but no significant side effects [7]. The HIV co-receptors belong to the family of seven-transmembrane-spanning chemokine receptors and their ligands, the chemokines are inhibitors of the gp120-co-receptor interaction. The physiological ligands of CCR5 are the chemokines Macrophage Inflammatory Protein-1α,

(MIP-1α), MIP-1ß and Regulated upon Activation, Normal T Expressed, and Secreted (RANTES). Takeda scientists used the CCR5-RANTES interaction for high-throughput screening of inhibitors, which resulted in the identification of lead compounds with a quaternary ammonium or phosphonium moiety. A series of anilide derivatives with a quaternary ammonium moiety were designed and synthesized, and Dimethyl-[[4-[[10-(4-methylphenyl)-3-bicyclo[5. 4. 0]undeca-2,8,10,12-tetraenyl]carbonylamino] phenyl]methyl]-tetrahydropyran-4-yl-ammonium chloride (TAK-779) (Fig. **3**) was identified as a highly potent and selective nonpeptide CCR5 antagonist with a IC_{50} value of 1. 4 nM. TAK-779 inhibits the replication of M-tropic HIV-1 with EC_{50} values of 1. 2 to 3. 7 nM [8]. It binds in a cavity formed among several of the transmembrane helices of CCR5 but does not result in CCR5 signaling or internalization [9]. However, due to its charged nature, TAK-779 is pharmacological unfavourable. Cationic molecules are known to be membrane impermeable and, in addition, TAK-779 caused significant irritation at the injection site during clinical testing. This prevented this compound from advancing into further studies. Further modifications, eliminating the positively charged moieties, led to a new candidate for clinical studies, TAK-220 (the structure of which has not been disclosed). TAK-220 is orally available and inhibits clinical HIV isolates with an EC_{50} of 1. 1 nM [10].

Schering has developed a large series of piperidine and piperazine derivatives with very tight CCR5 binding and potent CCR5 antagonistic activity. One of the N-oxide derivatives thereof is SCH-C (Fig. **3**), which inhibits HIV infection mediated by CCR5 with a mean IC_{50} between 0. 4 and 9 nM, and has been shown to inhibit HIV replication in a SCID-hu Thy/Liv mouse model [11]. Further adjustment of the substitution pattern produced SCH-D (Fig. **3**), which is more potent than SCH-C (EC_{50} in the range of 0. 1-3 nM) and is currently undergoing phase I clinical trials [10].

E913 (Fig. **3**) is a spirodiketopiperazine derivative and is another specific CCR5 inhibitor (IC_{50} of 2 nM) which appears to bind to the C-terminal half of the second extracellular loop of CCR5 [12]. GlaxoSmithKline currently have this compound in clinical development.

The Merck group has discovered a large number of chemokine antagonists with a 1, 3, 5-trisubstituted five-membered ring as the main structural element (usually of the pyrrolidine or cyclopentane type). These compounds bind with affinities of <1 μM to CCR5 [13, 14].

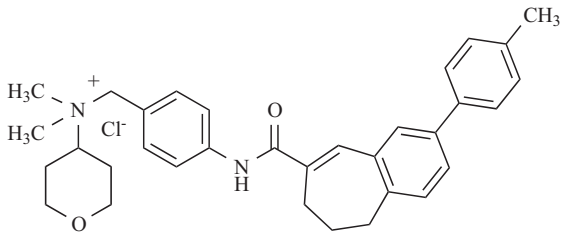
A general concern in blocking CCR5 is the appearance of HIV variants that utilize CXCR4. Since these variants have been associated with progression to AIDS, CCR5 antagonists could accelerate disease progression. However, the adaptation of R5 HIV strains in the presence of CCR5 antagonists did not lead to a switch to CXCR4 but rather changed the mode of interaction of Env with CCR5 [15], demonstrating the usefulness of blocking CCR5 for therapy.

CXCR4 Antagonists

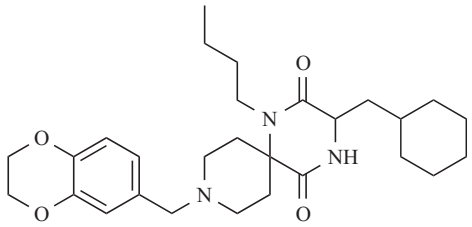
In contrast to CCR5, long term blocking of CXCR4 may have negative side effects, because mice that lack CXCR4 or its ligand, SDF-1 (stromal cell-derived factor 1α), have clear developmental defects [16, 17].

The bicyclam AMD3100 (Fig. **4**) was the first small molecular compound found that inhibited the gp120-CXCR4 interaction and SDF-1 signal transduction [18]. AMD3100 inhibits X4 HIV strains with an IC_{50} of 2-20 nM and it is inactive against R5 strains. Effectiveness has been shown in murine models, but unfortunately, human clinical trials were halted due to cardio-toxicity in some patients and failure to meet the efficiency goals. AMD3100 is therefore not being

CCR5 antagonists



TAK-779



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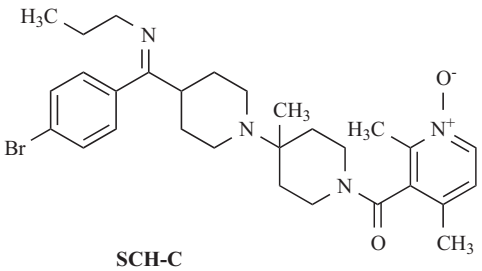
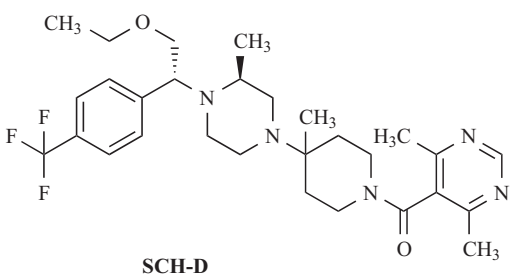
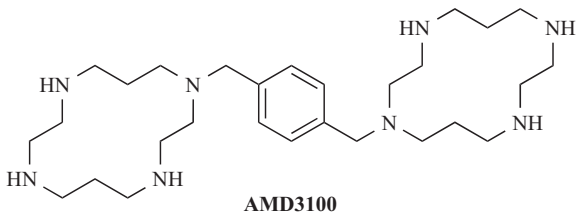


SCH-C

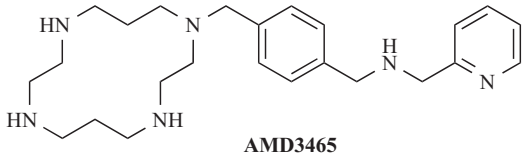


SCH-D

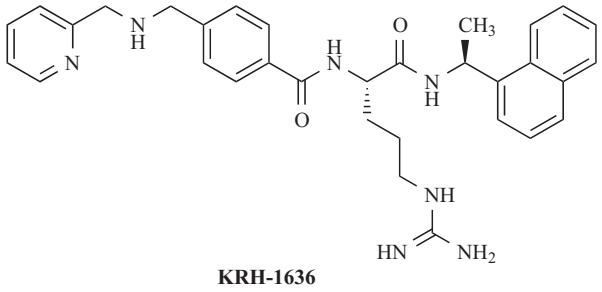
Fig. (3). Structure of CCR5 antagonists.



AMD3100



AMD3465



KRH-1636

H_2N-Arg-Arg-Trp-Cys-Tyr-Arg-Lys-Cys-Tyr-Lys-Gly-Tyr-Cys-Tyr-Arg-Lys-Cys-Arg-$CONH_2$ **T22**

H_2N-Arg-Arg-Trp-Cys-Tyr-Arg-Lys-D-Lys-Pro-Tyr-Arg-Cit-Cys-Arg-COOH **T134**

H_2N-Arg-Arg-Nal-Cys-Tyr-Arg-Lys-D-Lys-Pro-Tyr-Arg-Cit-Cys-Arg-COOH **T140**

AcNH-D-Arg-(D-Arg)8-COOH **ALX40-4C**

Fig. (4). Structure of CXCR4 antagonists.

further developed as an anti-HIV drug: however, it is going to be used for stem cell mobilization, which is another mode of action of this compound [19].

AMD3100 was used as a lead structure for new derivatives with only one cyclam moiety, which should have better pharmacological profiles when given orally. One derivative (AMD3465) (Fig. **4**) was further modified to bis-amines and retains only the central 1,4-bisphenylene moiety, whereas different heterocyclic groups were attached to the two terminal positions. As a consequence of their less polar character, these compounds should possess better bioavailability [20]. However, they have been withheld from clinical development as anti-HIV drugs due to the cardio-toxicity observed with AMD3100.

Several other CXCR4 antagonists are in development, including oligopeptides, such as T22 [21] and its derivatives T134 [22] and T140 [23] (derived from defensin peptides in the blood of horseshoe crabs), as well as ALX40-4C [24] (Fig. **4**). They all inhibit X4-HIV infection by binding to CXCR4. T22 is an 18-residue peptide amide, possessing an antiparallel ß-sheet structure maintained by two disulfide bridges between the four cysteine residues. This structure could be downsized to the more potent compounds T134 (des-[Cys(8, 13), Tyr(9,12)]-[D-Lys10, Pro11, L-citrul-line16]-T22 without C-terminal amide) and T140 [[L-3-(2-naphthyl)alanine3]-T134]. ALX40-4C (N-α-acetyl-nona-D-arginine) was evaluated before co-receptors were known (Fig. **4**). It acts on the second extracellular loop of CXCR4 and was well tolerated in a Phase I clinical trial [25].

A structural intermediate between small compound and small peptide is KRH-1636, which also efficiently blocks X4 HIV [26].

3. Viral Fusion Inhibitors

The transmembrane domain of the HIV Env, gp41 has three domains: an extracellular (ectodomain), a transmembrane and an intercellular. Gp41 anchors the Env complex within the viral membrane and mediates membrane fusion. The ectodomain itself can be divided into three functional domains: a fusion peptide (FP) at the N-terminus and two heptad repeat regions (HR-1 and HR-2) (Fig. **5**). The fusion progress is initiated by the interaction of gp120 with CD4 and the co-receptor. The resulting conformational changes in gp120 and gp41 lead to the exposure of the FP and its insertion into the target cell membrane. This process is mediated by the formation of a triple-stranded coiled-coil structure of the N-terminal HR-1 region. Subsequently, gp41 folds back on itself and the C-terminal HR-2 region is packed into grooves on the outside of the triple-stranded HR-1 coiled-coil, and a six-helix bundle is formed. The two membranes are thereby brought into close proximity and the change in free energy associated with the structural changes is predicted to be sufficient to cause lipid mixing and membrane fusion [27].

The exposed groove between the helices that is formed during the fusion process has been identified as a potential target structure [28, 29]. Drug design started with the use of

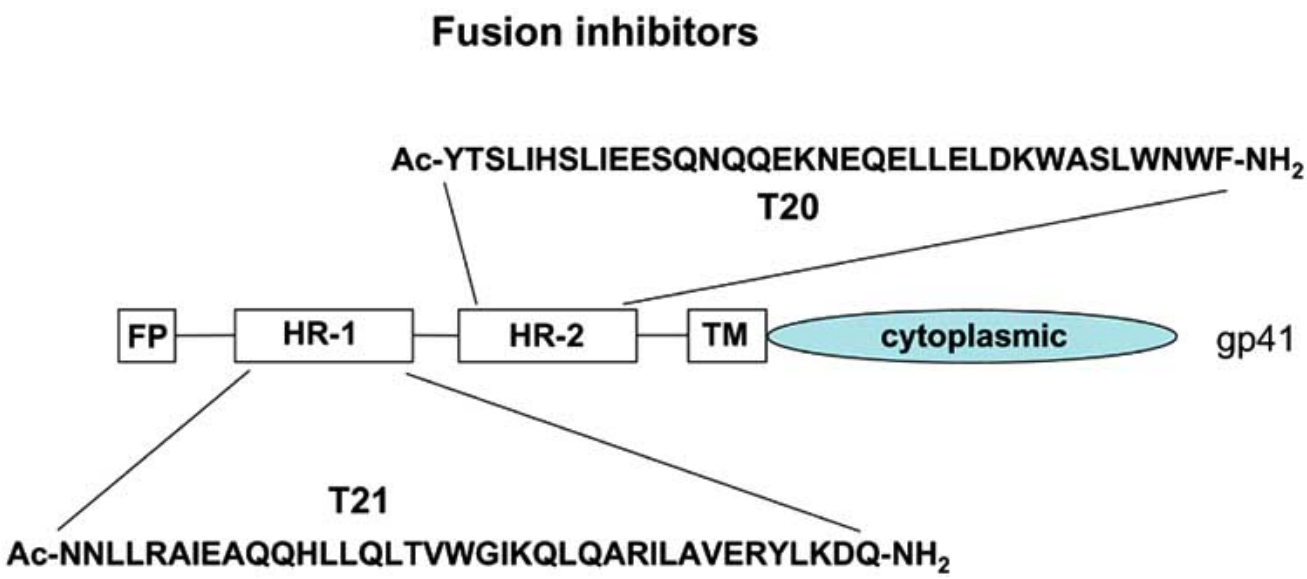


Fig. (5). Schematic presentation of the HIV Env gp41 domain.
FP = fusion peptide; HR-1 = N-terminal heptad repeat region; HR-2 = C-terminal heptad repeat region; TM = transmembrane region. The sequences and locations within gp41 of the fusion inhibitors T20 and T21 are given.

a synthetic N-peptide, DP107 (or T21), which had anti-viral activity. A C-terminal peptide DP178, later called T20, was however 1000-fold more potent. This is now known as enfuvirtide and is in clinical use in the USA. Enfuvirtide – also called Ro 29-9800 by the clinical developers Trimeris and Roche (trade name: FUZEON^{TM}) – is a linear, 36 amino acid synthetic peptide composed of naturally occurring L-amino acid residues. There is a carboxamide at the C-terminus and the N-terminus is acetylated. Its sequence corresponds exactly to that of the HR-2 domain in gp41. Enfuvirtide binds to the complementary region in HR-1 preventing the interaction with HR-2 and the formation of the six-helix bundle, resulting in an inhibition of fusion. Clinical trials have shown that enfuvirtide is active against laboratory and primary isolates of HIV-1, with an IC_{50} in the range of 4-280 nM. However, its use may be limited by complex manufacturing and a very high price [30, 31].

Shorter C-peptides have a low binding affinity for gp41 and poor inhibitory activity, which creates an obstacle for the development of small drugs. Extension of the enfuvirtide sequence to 39 amino acids derived from HR-2 (T1249) generated an oligopeptide that is 2-100 times more active [32].

Root *et al*. [33] reversed this inhibitory strategy with a small protein, 5-Helix, which lacks a third C-peptide and thereby created a high affinity binding site for the HR-2 region. This protein has potent inhibitory activity (nanomolar range) against diverse HIV variants.

CONCLUDING REMARKS

Retroviruses, particularly HIV, rapidly respond to a changed environment by the appearance of viral variants. Although entry inhibitors should ideally prevent infection and the appearance of resistant variants, viral escape from entry inhibitors was expected and has been observed. As was recognized with HAART (highly active anti-retroviral therapy), an effective antiviral therapy requires the combined application of drugs that target different sites of the viral life cycle. Entry inhibitors should therefore be given in combination with each other or with existing drugs to avoid resistance, and will become new tools to control HIV infections.

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Received: August 9, 2005 **Revised: October 4, 2005** **Accepted: October 4, 2005**