NEW TARGETS AND NOVEL ANTIRETROVIRALS

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Highly active antiretroviral therapy (HAART) has to date been based on use of a triple combination of drugs chosen from three classes of antiretrovirals (ARVs), nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). These ARV classes target two of the three virally encoded enzymes necessary for viral replication after entry of HIV into the host cell. A greater understanding of the viral-host interactions necessary for new infection of CD4 cells has led to the development of compounds able to inhibit viral entry. The novel fusion inhibitor T-20 has entered clinical use and has a particular role in salvage therapy of patients with HIV strains resistant to present classes of ARVs. Co-receptor inhibitors have shown *in vivo* antiviral activity and are now entering phase III development. Integrase (IN), the third viral enzyme encoded by the *pol* gene, has remained an elusive target. Compounds with low toxicity and able to inhibit (IN) are now entering phase II clinical development. These new HIV treatment modalities represent a significant advance in and addition to our anti-HIV armamentarium. This review will outline the mechanisms of action of entry inhibitors and IN inhibitors and discuss the lead compounds within each class.

ENTRY INHIBITORS

HIV infection of new human target cells is a process involving complex interactions between both viral and human receptors, resulting in fusion of viral and cellular membranes. There are at least three components of the initial HIV entry process, shown in Fig. IA: viral-cell attachment, mediated by interaction between viral gp120 and host cell CD4; attachment of gp120 to either or both CCR5, CXCR4 co-receptors; and approximation and fusion of target cell and viral membranes by conformational changes in the bridging trans-membrane protein gp41, due to cross linking of two helical regions (HR1 and HR2) of gp41.

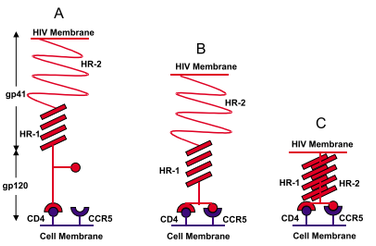
Compounds inhibiting each of these processes are at varying stages of development. All three targets have promising agents, with demonstrated ARV activity with mechanisms of action as shown in Fig. IB. While there is considerable clinical experience with the fusion inhibitor (T-20), which was registered for use in the USA and Europe in 2003, attachment inhibitors are at a very much earlier stage of development. Inhibitors of the human CCR5 co-receptor are unique among ARVs because their targets are encoded by human rather than the more mutation-prone HIV-genome.

CD4 ATTACHMENT INHIBITORS

PRO 542 is fusion protein (CD4-IgG2 tetramer), which binds to gp120, inhibits gp120/CD4 attachment, and has broad *in vitro* antiviral activity independent of co-receptor usage or resistance to existing classes of ARVs.[1] The incorporation of human IgG2 heavy chain increases the half-life and minimises potential for immunogenicity of the protein. PRO 542 is able to neutralise primary viral isolates *in vivo*. A phase I study of HIV-infected adults using single doses of 25 mg/kg of PRO 542 demonstrated acute reductions in viral load, which continued for 4 - 6 weeks.[2] A small 4-week monotherapy paediatric study of weekly infusions of 10 mg/kg demonstrated a decline in viral load of > 0.7 $\log_{10}$ copies/ml , which was sustained for 14 days post-therapy, in 4 of 6 subjects.

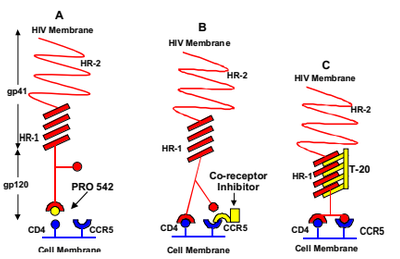
CHEMOKINE RECEPTOR INHIBITORS

HIV binds initially to its target cell via the gp120/CD4 interaction but also requires the presence of human membrane CCR5 or CXCR4 co-receptors. CCR5 is the co-receptor utilised by the most commonly transmitted macrophage-tropic HIV strains, which predominate in early HIV-infection. It has been recognised that individuals with a △32-CCR5 homozygous phenotype lack a functional CCR5 receptor and are resistant to HIV infection. It is postulated that effective CCR5 inhibition would be able to reproduce this cellular resistance to HIV infection. The assays used to determine HIV co-receptor tropism are complex and expensive and currently lack the sensitivity to identify minority populations of CXCR4 using virus. However, these assays have been utilised in cross-sectional studies of receptor tropism to show increasing use of the CXCR4 with disease progression. CXR4 tropic viruses are usually associated with increased CD4 cell destruction. *In vitro* and *in vivo* data to date indicate that CCR5 inhibition does not lead to change in viral tropism, but



A. Initial contact between viral extra-membrane protein gp120 and target cell CD4 receptor.
B. HIV gp120 binding to human co-receptor CCR5 or CXCR4.
C. Helical region 1 (HR-1) binds to helical region 2 (HR-2) of viral transmembrane protein gp41, leading to conformation change and apposition of viral and cell membranes prior to fusion.

Fig. IA. Interactions between viral gp120 and cellular receptors leading to conformational changes in transmembrane protein gp41 and fusion of HIV and cell membranes.



A. Attachment inhibitor e.g. PRO 542 links to gp120 and inhibits binding to CD4 receptor.
B. CCR5 co-receptor inhibitor e.g. UK 427,857, SCH-D or GSK-873140 prevent binding of gp120 to co-receptor.
C. Fusion Inhibitor e.g. T-20 binds to HR-1 and prevents cross-linkage to HR-2.

Fig. 1B. Mechanisms of action of three classes of entry inhibitors.

can result in expansion of pre-existing minority species of dual-tropic (CCR5/CXCR4) and monotropic (CXCR4) subpopulations. Evolution of HIV co-receptor binding can also occur in absence of CXCR4 variants.

UK-427,857 is a CCR5 inhibitor which has shown dose-related antiviral activity in 80 ART-naïve individuals with predominately CCR5 tropic virus with doses of up to 300 mg bid, which were well tolerated. Doses of ≥ 100 mg bid and qd resulted in viral load reductions of ≥ 1 $\log_{10}$ when given as short-term monotherapy. Dosing with food did not have a significant effect on the antiviral efficacy. The viral load did not rebound immediately upon cessation of the drug, indicating that a proportion of receptors remain blocked for some time.[3] Receptor-binding modelling studies indicate that a dose of 100 mg bd or equivalent daily dose will be necessary for long-term treatment.[4] Recent studies have shown that resistance to UK-427,857 probably emerges slowly, but is not always associated with loss of use of the CCR5 co-receptor.[5]

SCH-D is a piperazine-based CCR5 antagonist unrelated to SCH-C, from which it has taken over as a lead compound. SCH-D has greater *in vitro* and *in vivo* potency than SCH-C, which has been withdrawn from further clinical development. When SCH-D binds to the CCR5 receptor, there is a conformation change that prevents HIV gp120 binding. SCH-D does not induce CYP3A4 and has an elimination half-life of 24 hours. In a randomised study of 48 patients receiving 10, 25 or 50 mg SCH-D twice daily or placebo, the maximum viral load reduction occurred in the 50 mg group: by day 15, viral load had fallen by a mean of 1.5 $\log_{10}$, compared with 1 $\log_{10}$ in the 10 mg group and no change from baseline in the placebo group.[6] The proportions achieving a viral load reduction greater than 1.5 $\log_{10}$ were similar in the 25 and 50 mg groups (46% v. 45%). There was only one adverse event possibly related to the drug (fever).

GSK-873140 is a spirodiketopiperizine CCR5 antagonist with potent *in vitro* activity. In HIV-infected individuals with CD4 cell counts > 200 µl and predominately CCR5 tropic virus at baseline, a dose-related antiviral effect was shown. After 10 days, mean viral load decline varied between –0.4 $\log_{10}$ with 200 mg od to –1.6 $\log_{10}$ with 600 mg bd. The commonest adverse events were gastrointestinal.[7]

AMD-070 is an investigational CXCR4 receptor inhibitor. *In vitro* it prevented CXCR4 monotropic and CCR5/CXCR4 bitropic HIV strains from infecting peripheral blood mononuclear cells (PBMCs). When combined with a CCR5 receptor inhibitor, no viral replication of any of the viral strains tested occurred in PBMCs.[8]

FUSION INHIBITORS

T-20, or enfurvitide, is the first fusion inhibitor approved by the FDA for treatment of HIV infection in patients failing existing therapies.[9] T-20 protein is a linear 36 amino acid corresponding to a sequence within HR2, and acts extracellularly at nanomolar concentrations. T-20 binds to HRI, thereby inhibiting its interaction with its natural ligand (gp41 HR2) necessary for the contraction of gp41 and drawing together of the viral and target membranes. Mutations in the HR1 region of gp41 can result in resistance to T-20; however there is no evidence of cross-resistance to other ARVs including the newer fusion inhibitors under development. HIV-1 isolates resistant to NRTIs, NNRTIs and PIs are susceptible to T-20 *in vitro*. The peptide is not an inducer of CYP3A4 and no clinically significant pharmacological interactions have been reported with other ARVs. The usual dosage of T-20 is 90 mg (1 ml) administered by subcutaneous injection twice daily, and T-20 should be augmented with other potent drugs. The formulation is a lyophilised powder, which is dissolved in sterile water before use and should be refrigerated and used within 24 hours. Irritation at the injection site is the commonest adverse event and hypersensitivity reactions are rare (< 1%). An increase in bacterial pneumonia was observed in trial patients receiving T-20 compared with those receiving placebo, but the causation was uncertain. T-20 has a role in salvage therapy of heavily pretreated individuals, but its manufacture is complex, requiring multiple steps, and it is likely to remain a costly drug.

T-1249 is a second-generation fusion inhibitor, which has greater *in vitro* potency than T-20 and has activity against viral strains resistant to T-20. The drug is a 39-amino acid peptide from a hybrid of sequences of HIV-1, HIV-2 and SIV peptides, which is administered as a subcutaneous injection once or twice daily. The optimal dose for T-1249 has not been determined. Adverse events include irritation at the injection site, hypersensitivity and neutropenia. Monotherapy studies in heavily pretreated patients demonstrated a 1.3 $\log_{10}$ decline in viral load after 14 days with a 25 mg twice-daily dose. In a 10-day study of 54 individuals with resistance to T-20 switching to T-1249 at a dose of 192 mg intramuscularly daily, 67%

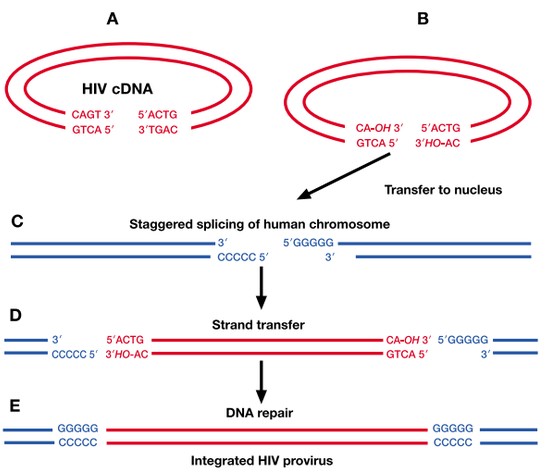
achieved a 1 $\log_{10}$ drop in viral load.[10] Those who had been on T-20 for less than 48 weeks had a better response than those who had had more than 48 weeks' therapy. T-1249 development was discontinued in January 2005 because of formulation problems.

INTEGRASE INHIBITORS

Integration of a DNA copy of the viral genome into the host cell chromosome is necessary for latent HIV-infection to be established. The process is catalysed by integrase (IN), a 288 amino acid molecule encoded by the 3'-end of the *pol* gene. Integration is a multi-stepped process (Fig. 2) including formation of an DNA-IN complex by IN recognition of specific sequences in the long terminal repeat of viral retro-transcribed DNA; 3' processing by removal of the terminal GT nucleotides from 3'-end; translocation of the DNA-IN complex from the cytoplasm through the nuclear pore; strand transfer into the host DNA by joining of the 3'-end of double-strand viral DNA to the 5'-end of host DNA strands at a staggered cleavage 5 nucleotides apart; and finally repair of the junctions by host cellular DNA repair machinery. The end result of these processes is a full-length cDNA insertion of the viral genome, flanked by two identical 5-nucleotide sequences of host DNA. IN has remained an elusive target of drug development, for although a number of compounds have been shown to inhibit this enzyme *in vitro* many of them have shown high toxicity. Recently IN inhibitors have been identified which act by inhibiting either DNA binding or strand transfer.

S-1360 is a diketo acid (DKA) derivative displaying anti-HIV activity at micro-molar concentrations. The DKAs represent the first class of IN inhibitors specifically inhibiting the DNA strand transfer step of the integration process (INSTI). Multiple passage of virus in presence of S-1360 resulted in selection of strains resistant to S-1360 associated with the sequential acquisition of mutations of the integrase gene.[11] S-1360 is orally bioavailable and was generally well tolerated in a phase I study of single doses up to 2 000 mg and twice-daily dosing up to 2 000 mg for 11 days, in healthy volunteers.

V-165, a dipyrimidine derivative, is an 'IN-DNA binding inhibitor' (INBI) which constitutes a second class of integrase inhibitor. Studies of the mechanism of action of V-165 indicate inhibition of the formation of the IN-DNA complex. DKA-resistant HIV strains remained sensitive to *in vitro* inhibition by V-165.



A. Formation of integrase-DNA (IN-DNA) complex by IN binding to long terminal repeat (LTR) region of viral DNA.
B. IN 3 end processing of LTR and transport of IN-DNA complex through nuclear pore.
C. IN staggered splicing at random site in human chromosome.
D. IN strand transfer by joining of 3 LTR with 5 end of spliced human DNA.
E. DNA repair by nuclear enzymes; removal of 5 terminal viral AC nucleotides and repair of spliced chromosome DNA. Result: integrated provirus flanked by identical five nucleotide sequences.

Fig. 2. Steps required for HIV DNA integration into the human genome.

SUMMARY

ARV therapy remains a rapidly evolving field, which has paralleled the expanding knowledge of the HIV life cycle and greater understanding of the complex interactions between host and pathogen. The hold on development of T-1240 illustrates that even very promising lead candidates can be lost at later stages of the development pipeline. However, the increasing number of new compounds and the development of completely new targets improves our ability to counter the high mutability of HIV and develop new antiviral strategies. Attachment inhibition, for example, may offer a logical target for aborting HIV infection after exposure. T-20, the first of the new class of entry inhibitors, already has an established role in salvage therapy. Inhibition of gp120/CCR5 binding is particularly exciting because the target is the first to be encoded by the host rather than the viral genome. The ability to pharmacologically reproduce the △32-CCR5 homozygous phenotype may also eventually give us a modality that can be used in early HIV infection, when CCR5 variants are predominant. Combinations of CCR5 and CXCR4 inhibitors may be needed to have activity later in HIV disease progression and the use of combinations of two classes of IN inhibitors would increase the genetic barrier required to overcome inhibitors of this third HIV enzyme target.

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