Emerging Drug Targets For HIV Therapy: A Review

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Abstract: The viral enzymes reverse transcriptase and protease are the current targets for antiretroviral therapy (ART). The use of a combination of inhibitors targeting these enzymes can reduce viral load for a prolonged period and delay disease progression. However, complications of ART, including the emergence of viruses resistant to current drugs, are driving the development of new antiretroviral agents targeting not only the reverse transcriptase and protease enzymes but novel targets as well. CCR5 and Integrase inhibitors have recently been approved for use in HIV treatment. The drug targets, which are under investigation, include CCR5 and CXCR4, maturation inhibitors, gene therapy, viral envelope protein (env). Novel virus that targets HIV-1-infected cells and Human deoxyhypusine synthase (DHS) are discussed as novel targets for HIV therapy. A multifaceted approach to ART, using combinations of inhibitors that target different steps of the viral life cycle, has the best potential for long-term control of HIV infection. This review outlines the key drug targets and steps where pharmacologic intervention can have a favorable therapeutic benefit.

Keywords: HIV, Anti-Retroviral Therapy, DHS.

Introduction

Current targets for antiretroviral therapy (ART) include the viral enzymes reverse transcriptase and protease. The use of a combination of inhibitors reduce viral load for a prolonged period and delay disease progression. The existence of viruses resistant to current antiretroviral, and transmission of drug-resistant strains, complication and toxicity of current regimen are driving the development of new antiretroviral agents targeting not only the reverse transcriptase and protease enzymes but novel targets as well.

HIV entry into susceptible cells, the integration of HIV DNA into the host cell genome, virion maturation etc. are currently promising emerging targets for ART as shown in figure 1. Inhibitors of novel targets are most likely to be fully active against resistant viruses and present new treatment options for individuals with multi drug resistant viruses. Therefore, inhibitors against new targets will complement and diversify current treatment options, and may act additively or synergistically with other inhibitors.

1. HIV-1 entry inhibitors

The entry of HIV-1 into target cells is a multistep process requiring several conformational rearrangements and highly specific interactions between the Env protein and cellular receptors. Identification and characterisation of the HIV-1 envelope proteins and determination of the process of viral entry has enabled design of specific inhibitory agents (Table 1.2). There are essentially four steps of viral entry, attachment, CD4 binding, co-receptor binding, and membrane fusion. All the steps of virus entry are relevant targets for anti-HIV intervention.
Figure 1. The life cycle of HIV presents several targets for inhibition.

1.1 Attachment inhibitors
HIV attachment inhibitors are agents that interact with the virus or the target cell to block virion absorption/attachment or env-receptor interactions. Attachment inhibitors can be subdivided into those with nonspecific or specific modes of action. Nonspecific inhibitors target basic regions of gp120 to block cell surface binding and co-receptor interactions (e.g., Cyanovirin-N, dextran sulfate and heparin). The CD4 cell surface molecule serves as the primary receptor for HIV infection and inhibitor that target the CD4 receptor, inhibit viral entry into the cells. Inhibitors directly targeting CD4 binding include compounds that down-modulate CD4, ligands to CD4 and agents that bind to Env to prevent Env-CD4 binding or functional Env-CD4 interactions. Compounds that specifically down modulate CD4 from the cell surface include cyclotriazadisulfonamide (CADA) analogues. CADA (Figure 2) down-regulate CD4 expression at the post translational level. They have synergistic effect in their anti-HIV activity when combined with NRTIs, NNRTIs, protease inhibitors, fusion inhibitor and chemokine co-receptor CXCR4 antagonist.

1.2 Co-receptor binding inhibitors
Env interacts with a co-receptor molecule, typically CCR5 and/or CXCR4 after HIV attachment to the cell surface and CD4 binding, to mediate infection. Both CCR5 and CXCR4 are potential targets for antiretroviral therapy. CXCR4 is the coreceptor for HIV-1 strains that infect T-cells (T-tropic or X4 strains) and CCR5 is the coreceptor for HIV-1 strains that infect macrophages (M-tropic or R5 strains). Inhibitors of co-receptor interactions are a second broad category of entry inhibitors that include proteins, small molecules and antibodies. These co-receptor-binding inhibitors interact either with CCR5, CXCR4 or Env to prevent functional Env-co-receptor interactions.
TAK 779 (Takeda Chemical Industries, Ltd) was the first identified small molecule CCR5 antagonist. Maraviroc and Vicriviroc are currently in clinical trial (Phase III) and have been well tolerated and exhibit potent antiviral activity in HIV-infected patients. Plerixafor was the first recognized molecule as CXCR4 antagonist that potently blocks infection of X4 viruses. Development of plerixafor for HIV therapy halted because of unfavorable pharmacokinetic properties, negligible bioavailability, and cardiac side effects.  

1.3 Glycoprotein 120 inhibitors

All gp120 inhibitors, whether synthetic or of natural origin, are assumed to exert their anti-HIV activity by shielding-off the positively-charged sites in the V3 loop of the viral envelope gp120 which is necessary for virus attachment to the cell surface heparin sulfate proteoglycans, a primary binding site, before a more specific binding occurs to the CD4 receptor and to co-receptors. Cyanovirin-N blocks both CD4-dependent and independent binding of soluble gp120 to target cells, and dissociates bound gp120 from target cells. The attachment of the HIV surface glycoprotein gp120 to CD4 cells can be blocked by recombinant antibody CD4-immunoglobulin G2 (CD4-IgG2, PRO 542).

Viral entry can also be blocked by linear synthetic peptides corresponding to the central 15–21 amino acid sequence of the gp120 V3 loop, such as the R15K peptide. These V3-derived peptides inhibit the interaction of gp120 with the host cell surface glycosphingolipids22. A new target cell surface protein disulfide isomerase (PDI) is attached to the primary receptor CD4 close to the gp120-binding site. PDI reduces gp120 disulfide bonds, which triggers the major conformational changes in gp120 and gp41 required for virus entry. A novel molecule BMS 806 (Figure 3) binds to a gp120 site rich in disulfide bonds and this binding might protect gp120 disulfide bonds from reduction and finally viral entry to cell.5

Figure 3. Structure of BMS 806

Table 1. HIV entry inhibitors2

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Clinical trial status</th>
<th>Target</th>
<th>Company/Developer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enfuvertide (Fuzeon, T20)</td>
<td>Approved</td>
<td>gp41</td>
<td>Trimeris/Roche</td>
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<tr>
<td>Maraviroc (Selzentry)</td>
<td>Approved</td>
<td>CCR5</td>
<td>Pfizer</td>
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<tr>
<td>Vicriviroc</td>
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<td>Phase II (2006)</td>
<td>CCR5</td>
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<td>Progenics</td>
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<tr>
<td>Ibalizumab TNX-355</td>
<td>Phase II (2006)</td>
<td>CD4</td>
<td>Tanox/Biogen TaiMed Biologics</td>
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</table>
2. Maturation inhibitors

The viral-encoded protease is present in the immature virus particles and is responsible for the cleavage of the Gag and Gag-Pol polyproteins into individual protein components. This cleavage step, known as maturation step, is essential for the HIV-1 particles to become infectious. If this step is not followed, the produced virions are noninfectious. The Gag polyprotein is the main structural component of retroviral particles since it contains the structural sequences for the matrix (p17, MA), capsid (p24, CA), nucleocapsid(p7, NC), and p6 proteins and two spacer peptides, spacer peptide 1 (SP1) and spacer peptide 2 (SP2). Upon maturation, p24 is released from the matrix protein and condenses into a conical capsid that encases and stabilizes the ribonucleoprotein complex. Maturation inhibitors are a new class of antiretroviral agents. Currently, PA-457 is the only compound within this class. Unlike conventional PIs that act at the enzyme active site, PA 457 appears to act on the substrate of protease. Very specifically preventing cleavage between the junction of capsid (CA) and spacer peptide 1 (SP1), thus blocking conversion of the HIV capsid precursor, CA-SP1 (p25) to the mature capsid protein (p24). 1,6

3. Transcription inhibitors

The HIV transactivator protein Tat is essential for viral replication. Tat is responsible for transcription of the HIV-1 LTR and transcription of HIV genomic RNA. In the absence of Tat the transcription of HIV mRNAs can be initiated but cannot be efficiently elongated to produce full-length viral RNA genome. The Tat-mediated transactivation is a particularly attractive target for the development of new antiretroviral drug therapies because Tat is required not only for viral gene expression during the exponential growth, but also for the activation of the integrated proviral genomes that give drug resistant strains of HIV-1. 7,8

4. Human deoxyhypusine synthase (DHS) inhibitors

HIV-1 replication depends on the viral regulatory protein Rev and hypusine containing protein eIF-5A. eIF-5A is a critical cofactor of the HIV-1 Rev regulatory protein. Inhibition of hypusine formation in eIF-5A by interfering with DHS activity serves as a new target for antiretroviral therapy. Hypusine is required for the biological activity of the protein. eIF-5A is the only known cellular protein that contains the amino acid hypusine. The hypusine formation is highly specific and is spermidine-dependent posttranslational reaction that is catalyzed by two enzymes. In the first step, the aminobutyl moiety of spermidine is transferred to the ε-NH2 group of a single lysine residue at position 50 within the human 154-amino acid eIF-5A protein.

![Figure 4. Schematic representation of the pathway of eIF-5A biosynthesis and inhibition of hypusine formation in eIF-5A.](image)
This reaction is catalyzed by DHS as shown in Figure 4. The resulting eIF-5A intermediate is further modified by deoxyhypusine hydroxylase, leading generation of the active form of eIF-5A. The most potent inhibitors of DHS are structural derivatives of spermidine. Recently a novel DHS inhibitor identified by high-throughput screening is guanylylhydrozone CNI-1493 as displayed in Figure 5 below.

5. Novel virus that targets HIV-1-infected cells

Vesicular stomatitis virus (VSV) has a high level expression vector that is capable of incorporating foreign proteins like HIV-1 receptor CD4 and a co-receptor, CXCR4 into the viral envelope. Construction of recombinant VSV has been carried out by incorporating different kind of genes leading to generation of VSV-CD4, VSVΔG-CD4, and VSVΔG-CC4 virus. These viruses were unable to infect normal cells but did infect, propagate on, and kill cells that were first infected with HIV-1 and therefore had the HIV membrane fusion protein on their surface. Killing of HIV-1-infected cells controlled HIV infection in a T cell line and reduced titers of infectious HIV-1 in the culture by as much as 104-fold. Cells infected with HIV-1 have the gp120/41 envelope protein on their surface. The gp120/41 molecules present on the cell surface and bind to CD4 and CXCR4 of the virus surface and promote fusion of the viral and cell membranes.

These are some of the reasons that make it novel for destruction of HIV-1 virus. First VSVΔG-CC4 is specifically targets to cells already infected with HIV-1, the VSVΔG-CC4 infection level would be expected to parallel HIV infection and thus decline with HIV clearance. Second, VSVΔG-CC4 encodes only human proteins in its envelope proteins and thus would not induce production of neutralizing antibodies. The internal VSV proteins would be expected to induce cytotoxic T cell responses, but such responses might help in clearing HIV infection by rapidly killing cells infected with HIV-1 and VSVΔG-CC4.

6. Vaccines

Both cellular and humoral immunity are necessary for controlling and eradicating infection at an early stage or for preventing disease progression. Initially a vaccine based on viral structural protein (Env) was developed but this did not show promising results because of the complex structure of Env, its high variability, and the difficulty of generating broadly reactive high-titer neutralizing antibody (nAb). Vaccines based on various combinations of the structural proteins Env, Gag and Pol were tried out but these were partially protective. The HIV genome encodes two small regulatory proteins Tat and Rev and four accessory proteins, Nef, Vif, Vpr and Vpu. Later vaccines based on nonstructural proteins were tried that give good results.

Tat and Rev are early products and are essential for productive infection and virus replication. Tat is crucial for viral replication, cell-to-cell virus transmission and pathogenesis. Tat has been recently proposed as a novel vaccine target on the basis of its early expression and immune-modulating properties. Active native Tat or inactivated Tat, respectively, have been evaluated in animal models and humans, to block viral infection at a very early stage and prevent progression to AIDS. Vaccination with Tat peptides has also been evaluated and found safe and immunogenic in several preclinical trials.

Figure 5. Structure of human deoxyhypusine synthase (DHS) inhibitor CNI-1493
Like Tat, Rev alone as vaccine antigen has been used for therapeutic vaccination. HIV-1 infected individuals were safe and immunogenic, in both HAART-treated and HAART-naive individuals. Vaccines based on multiple nonstructural HIV proteins have been tried to induce broad immunity to multiple viral targets. Tat-Rev combined vaccines protected macaques against systemic infection with SIVmac32H or SHIV-BX08, respectively. Nonstructural vaccine targets have proven to be safe and immunogenic in preclinical and clinical trials. Promising efficacy data have been obtained in nonhuman primates, where immunity to nonstructural viral proteins provided long-lasting protection against challenge with pathogenic SIV or SHIV strains.11

Recently dendritic cell based vaccine developed and tested in chronically HIV-1 infected patient. This lead to suppression of plasma viral load by 80 in 112 days and 90 in one year. It is interesting that monocyte derived DCs from hiv-1 infected person are functionally intact and stimulate memory T-cells.12

Conclusion
A significant number of human immunodeficiency virus (HIV) infections have become resistant to antiretroviral treatment, which means that there is a paramount need for novel drug targets to defeat the virus. There are currently 25 drugs belonging to 6 different inhibitor classes approved for the treatment of human immunodeficiency virus (HIV) infection. However, new anti-HIV agents are still needed to confront the emergence of drug resistance and various adverse effects associated with long-term use of antiretroviral therapy. New classes of anti-HIV drugs and new drugs in existing classes represent the best hope for people infected with HIV, especially those who have exhausted current therapies. The described drug targets represent some of the most noted examples of recent scientific breakthroughs that are opening unexplored avenues to novel anti-HIV target discovery and validation, and should feed the antiretroviral drug development pipeline in the near future.

References