Investigating targets for a new class of anti-HIV drugs

Despite effective combination therapies that can suppress viral infection, there is an urgent need for the discovery of a new class of anti-HIV drugs as the virus is increasingly developing resistance to current treatments. **Dr Debnath** and his team have focused on the structure of the binding site between the virus and host cells that is critical for the establishment of infection. They have therefore been working on small inhibitory molecules targeted at this site and have identified several promising candidates suitable for further drug development leading to a new class of anti-HIV therapeutics.

Q&A

What made you take the move into studying the gp120-CD4 binding site in the search for new antiviral drugs?
The 1998 discovery by Dr Peter Kwong of a cavity in the gp120, termed Phe43 cavity, in the X-ray structure of CD4-gp120-17b provided the initial motivation. It prompted us to study this site in early 2000 as a target for novel anti-HIV-1 drug discovery because it was a novel target and no one, as far as we know, had attempted to target that site using small molecule inhibitors. In addition, there is no drug approved yet that targets this binding site.

How important has collaborative work been for furthering your discoveries in the field?
We realised that the discovery of drugs using a structure-based approach is a complex multi-disciplinary undertaking and collaboration with leaders in this field is key to any success. All our major discoveries so far have been the result of successful collaborations with Dr Peter Kwong's group in structural biology at the Vaccine Research Center, NIH and Dr Andrea Altieri's group in medicinal chemistry at Edasa Scientific, Russia.

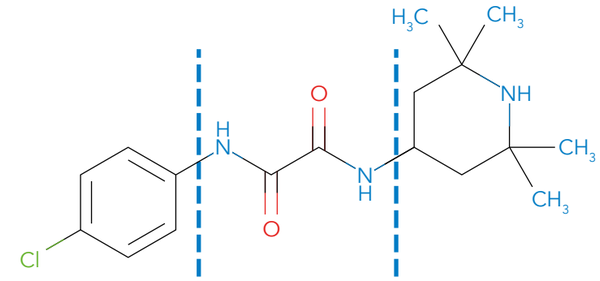
How far has research following your discovery of a broad-spectrum inhibitory drug progressed towards developing an available treatment for HIV-1 patients?
Admittedly, there is a lot more work to do before this class of drugs will be available to treat HIV-1 patients. However, we are hopeful that with continued financial support we will be able to achieve our goal.

What avenues of your research could hold the most promise in developing a cure for HIV-1 infection?
A successful discovery of a combination of latency reversing agents (LRAs) that can efficiently help expose HIV-1 surface proteins on latently infected cells, along with an effective design of antibody drug conjugate, may help in achieving a functional cure for HIV-1 infection.

How do you see your research progressing over the following years?
We expect to start early clinical studies of our gp120 targeted drugs in the next 4–5 years.

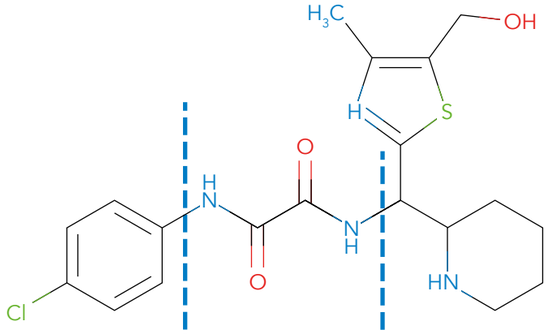
Dr Asim K Debnath has been conducting research on antiviral drug design for the past two decades and is head of research at the Laboratory of Molecular Modeling and Drug Design in the Lindsley F Kimball Research Institute, New York Blood Center. Using state-of-the-art drug design methods, his team's main goal is to identify new antiviral drugs effective against viral cell entry and assembly of human immunodeficiency virus type 1 (HIV-1). The laboratory's current projects are funded by the National Institute of Health (NIH).

HIV-1 is the virus responsible for the AIDS pandemic. The infection is global with a similar rate of infection in several major US cities to some sub-Saharan African countries. Currently, viral replication can be controlled for extended periods of time using drugs that target viral enzymes (reverse transcriptase, protease and integrase) and viral entry (gp41 and cellular receptor CCR5), but there is no known cure. Strains of HIV-1 are increasingly developing resistance to drugs used in combination therapies; there is a serious need for new antivirals in order to combat the growing threat posed by these resistant infections. For the next generation of antivirals to stand the best chance of combating the virus's rapidly evolving resistance, it is important that they do not target similar viral components as existing drugs.



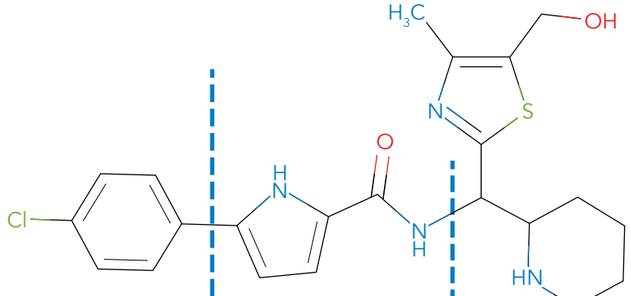
NBD-556

Agonist



NBD-09027

Partial Agonist



NBD-11021

Antagonist

EVADING IMMUNE DEFENCES TO MEDIATE CELL ENTRY

Viral entry into a cell begins when the HIV-1 envelope glycoprotein, gp120, interacts with a receptor on the surface of host immune cells, known as CD4. In the face of limited funding, Debnath and his dedicated collaborators have been carrying out pioneering research to identify small inhibitory drug molecules capable of disrupting binding of the virus to the CD4 receptor.

To evade the immune response of a host cell, the envelope glycoproteins gp120 and gp41 undergo structural modifications. These allow them to avoid detection and mediate viral entry into the cell. When a viral particle binds to a host cell, gp120 interacts with the CD4 receptor on the host cell surface, resulting in conformational changes that expose particular sites of gp120. The newly exposed region then interacts with other cellular receptors which leads to the gp120-gp41 complex becoming destabilised. Once this has occurred, gp41 undergoes another structural change, initiating entry of the HIV-1 virus particle into the cell. These steps are crucial for the virus to establish infection. Due to this, the early stages of the gp120-CD4 binding process provide an ideal novel target for the development of therapeutic interventions.

> **For the first time, they had discovered a new drug with the desired broad spectrum anti-HIV-1 characteristics**

SEMINAL DISCOVERY

In their search to find new drugs that effectively target gp120, Debnath and his team carried out a targeted screen that led to the discovery of two small inhibitory molecules, NBD-556 and NBD-557. Research by another team, sparked by this finding, showed that they have a remarkable ability to mimic the CD4 receptor. This finding ignited research in the field attempting to develop inhibitors targeted at gp120, the first time drug development had focused on this target.

Debnath and his group opted to take a structure-based design approach to see if they could modify this type of small molecule to produce an effective viral entry antagonist. To enable the researchers to precisely apply this approach to further develop NBD-556, in collaboration with Dr Peter Kwong at the Vaccine Research Center, NIH, they employed X-ray crystallography to uncover the structure of the NBD-556 gp120 binding complex. This revealed that NBD-556 binds to a region known as the Phe43 cavity, where during gp120-CD4 binding two similar interactions take place that are critical for viral cell entry. The NBD-556 and gp120 complex was found to possess one of these interactions but lack the other, with the amino acid Arg368gp120.

NBD-556 and its analogues were also found to act as viral entry agonists, which results in increased viral cell entry, not suitable for an inhibitory drug. However, with these aspects of the molecule elucidated, they set out to improve upon the inhibitory molecule they had discovered. They suspected that it may be essential to gain the interaction they had found missing between NBD-556 and Arg368gp120, as well as needing to preserve other key interactions and develop an antagonist.

STRUCTURE-BASED DRUG DESIGN

The team synthesised a series of compounds based on NBD-556 with different scaffolds of basic molecular groups and tested them for anti-HIV-1 activity. These molecules were found to enhance anti-HIV-1 activity, but other tests showed that the molecules still retained the agonist properties of NBD-566.

Excitingly, among these new molecules they found one that showed reduced agonist properties, a partial agonist.

This motivated the researchers to further investigate this molecule and led them to determine the X-ray crystal structure of the partial agonist bound to gp120. Their results showed that within the new molecule a basic nitrogen atom resided only 4.4 Angstroms away from Arg368gp120. However, this was not quite close enough for interaction to occur and further modification was required.

DISCOVERING A NEW CLASS OF HIV-1 DRUG TREATMENTS

The team succeeded in transforming the full viral entry agonist NBD-556 to a full viral entry antagonist, NBD-11021, and demonstrated that this molecule exhibits anti-HIV activity against a diverse range of viral strains. For the first time, they had discovered a new drug with the desired broad spectrum anti-HIV-1 characteristics, targeting the Phe43 cavity of gp120. NBD-11021 was found to bind to gp120 and block gp120-CD4 interaction, prevent cell-to-cell fusion and block cell-associated transmission of HIV-1.

The molecule was also discovered to target HIV-1 transcriptase, the enzyme the virus employs to manufacture complementary DNA, though with lower potency. This could potentially be another mechanism of action through which the molecule may be effective against HIV-1 infection.

PAVING THE WAY FOR NOVEL THERAPEUTICS

They are now working to further develop more potent entry inhibitors from NBD-11021, with a higher selectivity index for pre-clinical studies. Their pioneering research also paves the way for further development of other molecules in the NBD series for clinical application. Another project they are working on is attempting to discover the best combination of drugs known as latency reversing agents (LRAs), which will facilitate to eradicate HIV-1 from virus reservoirs. If an effective combination can be found to totally eradicate the virus from reservoirs by antibody drug conjugates (ADC) and the immune system, this will aid the development of a functional HIV-1 cure. They are also working on identifying new agents capable of inactivating pathogens in blood products.

Dr Debnath hopes that his team's contributions thus far, along with their future research, will pave the way for discovery of a new class of anti-HIV-1 drugs targeted at the gp120-CD4 binding site. Their novel approach to drug development has opened up several avenues for further research to develop such therapeutics, which are urgently needed to deliver new drugs.

Detail

RESEARCH OBJECTIVES

Dr Asim K Debnath and his team focus on the development of novel anti-HIV drugs. Their work focuses on the binding site that allows the virus to enter host cells. It is hoped that their work will lead to a new drug that will help combat the increasing resistance of HIV-1 to current modes of treatment.

FUNDING

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COLLABORATORS

- Dr Francesca Curreli, Assistant Member in Dr Debnath's lab
- Dr Peter Kwong, Chief, Structural Biology Section, Vaccine Research Center, NIAID, NIH
- Dr Young Do Kwon, Vaccine Research Center, NIAID, NIH
- Dr Andrea Altieri, Chemist, Edasa Scientific, Russia, and his team

BIO

Dr Asim K Debnath received his PhD in Medicinal Chemistry in 1987 from Jadavpur University, Kolkata, India and completed his postdoctoral study with Prof. Corwin Hansch at Pomona College, California, USA. He is a Member (equivalent to Professor) and Head of the Laboratory of Molecule Modeling & Drug Design at the Lindsley F Kimball Research Institute of the New York Blood Center. Dr Debnath has been active in antiviral drug design research for the last 20 years. Dr Debnath has published more than 97 peer-reviewed research papers and he has 14 US issued patents.

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