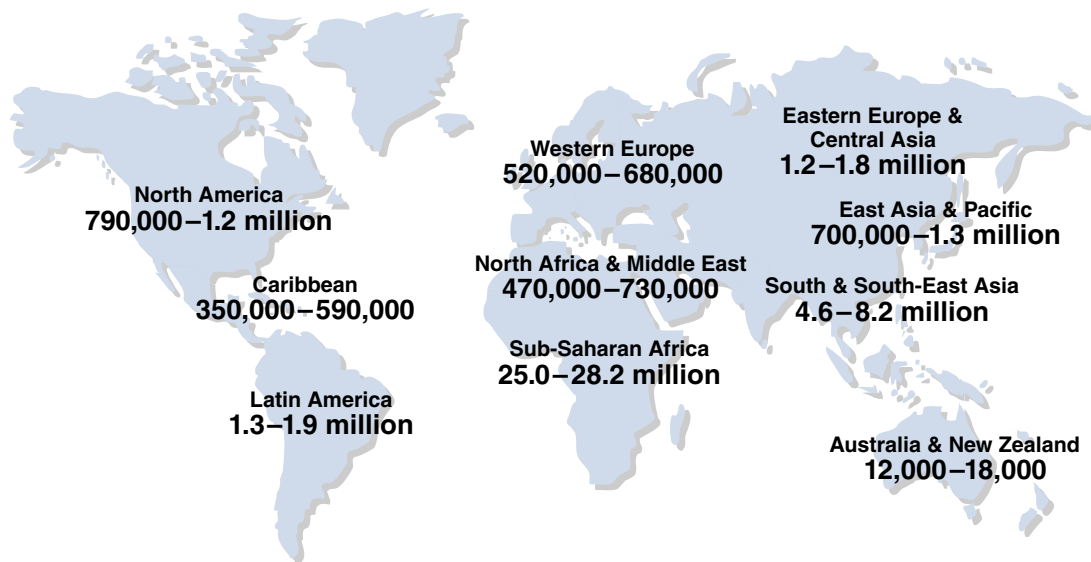


Moving Basic HIV Discoveries toward New Antiviral Therapies

The Gladstone Connection



The HIV/AIDS pandemic is global in reach. The most rapid spread is now occurring in developing nations.

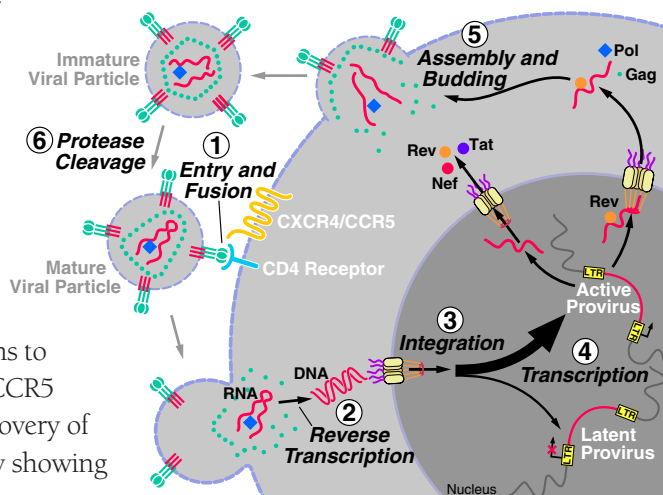
Worldwide, the AIDS epidemic continues to expand at an alarming pace. More than 40 million people are now infected with HIV, the virus that causes AIDS. Every day, 14,000 people are newly infected, and 8,000 people die as a result of AIDS. HIV targets mainly young adults in the prime of life, and it is spreading most rapidly in developing parts of the world where resources are the most limited.

In the 20 years since the discovery of the virus, basic science advances, coupled with the translation of these discoveries into new therapies, have transformed HIV disease from an acute, invariably lethal infection into a chronic, treatable disease. Using the tools of molecular biology and biochemistry, scientists at the Gladstone Institute of Virology and Immunology (GIVI) continue to play key roles in advancing our understanding of how the virus grows and how it cripples the immune system.

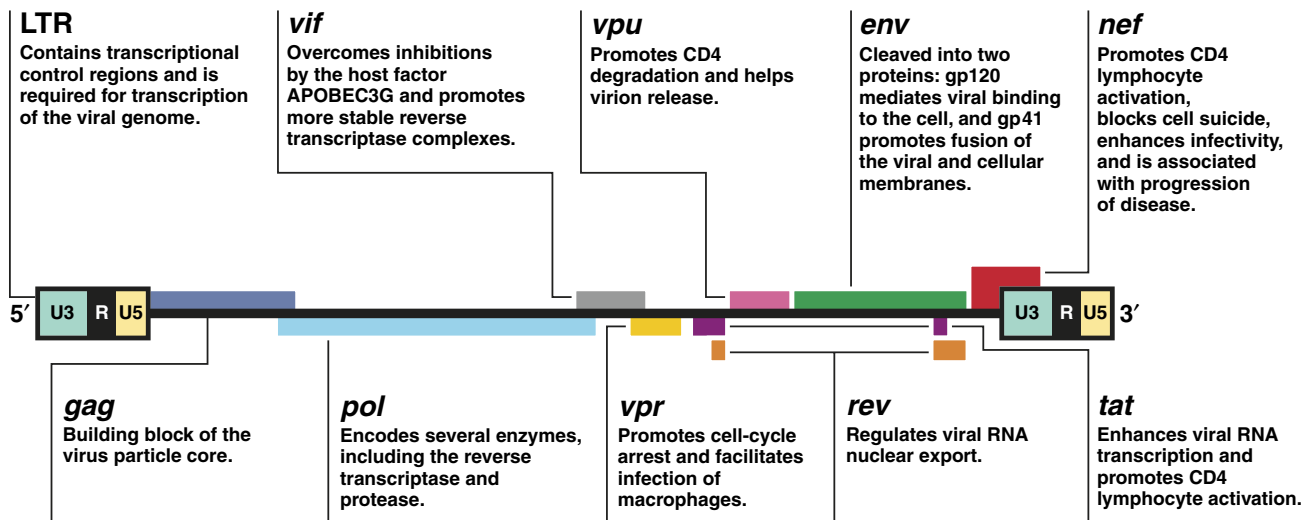
The HIV life cycle can be broken down into several important events, each of which offers exciting therapeutic possibilities.

Gaining a Foothold

In the very first step of infection, HIV binds to CD4 receptors on the surface of T cells and to one of two “coreceptors,” CCR5 and CXCR4. GIVI investigator Dr. Mark Goldsmith made important initial contributions to characterizing the molecular interplay of HIV with the CCR5 and CXCR4 coreceptors. Only seven years after the discovery of CCR5 as an HIV coreceptor, inhibitors of CCR5 are now showing



Steps in the HIV life cycle.



The ~9-kilobase genome of the HIV provirus contains 9 genes whose major functions are summarized here.

singular promise in early tests in patients. The next events involve a viral protein called gp41 that causes the virus and host cell membranes to fuse. By elucidating the molecular gymnastics of fusion, scientists were able to develop an HIV fusion inhibitor (enfuvirtide, or T20) that is now in clinical use.

To aid in the search for new and even better fusion inhibitors, the GIVI laboratory of Dr. Warner Greene developed a novel virus-based fusion assay that can be used for high-throughput screening.

For HIV to replicate, its RNA genome must be converted into a DNA version, a reaction mediated by an HIV enzyme called reverse transcriptase (step 2). Many of the currently approved anti-HIV drugs inhibit this enzyme. Unfortunately, resistance to these drugs develops in some patients. Dr. Robert Grant and his colleagues have shown that these resistant viruses are frequently crippled in their ability to replicate. Rather than discarding these drug combinations as ineffective, such therapies are now being continued, as long as declines in immune function do not intervene.

Into the Nucleus and Beyond

The newly copied DNA must next be imported into the nucleus. Dr. Greene's laboratory has defined the nuclear import properties of the HIV protein Vpr. They further showed that Vpr compromises the nuclear envelope by inducing herniations that often rupture and allow the nuclear and cytoplasmic contents to mix, which may underlie the additional ability of Vpr to induce cell-cycle arrest.

Once in the nucleus, the DNA copy is integrated into the host chromosome (step 3). The laboratory of Dr. Eric Verdin is studying how the HIV integrase protein helps in selecting the site of integration of HIV into the host chromosomes. Site selection is important because some regions of the genome do not favor efficient viral replication. After a sustained effort by many scientists, inhibitors of HIV integrase have been identified and are being evaluated for therapeutic effectiveness.

Once integrated into a host chromosome, the viral DNA is copied by the host transcriptional machinery into new viral messenger RNAs (step 4). This process is accelerated by an HIV protein called Tat. Dr. Melanie Ott and her colleagues have found that Tat is modified by the attachment of small acetyl groups. These critical modifications are performed by cellular enzymes that are potential future therapeutic targets.

High-level production of HIV RNA converts the host cell into a virus factory. Newly produced proteins modify the cellular environment to favor HIV growth. One of the most important is Nef. Dr. Greene's laboratory has shown

that this HIV protein delays the programmed cell death of the infected host cell, thus allowing more virus to be produced. These mechanistic insights provide hope that Nef may soon be therapeutically targeted.

Once all of the viral proteins are synthesized and the new genomic viral RNA is transcribed, these parts are assembled into new virions (step 5). The new virions then bud out to infect other cells. Budding subverts the actions of host proteins that normally mediate the trafficking of intracellular transport vesicles. It will be difficult to interfere with this step, since the host proteins involved are so important for the health of the cell.

To be fully infective, newly budded virions must undergo a maturation process involving sequential cleavage of the HIV polyproteins Gag and Gag-Pol by HIV protease (step 6). Inhibitors of HIV protease have shown great success in patients, most often in combination with reverse transcriptase inhibitors. Indeed, the combination of these two classes of antiviral drugs heralded an era of more effective HIV treatment. However, protease inhibitors remain very expensive and produce many unwanted side effects.

The laboratory of Dr. Cheryl Stoddart has evaluated potential antiviral effects of a betulinic acid derivative that blocks Gag cleavage. This derivative can be produced in a one-step chemical modification of betulinic acid, a component of tree bark. Propelled in part by Dr. Stoddart's studies, this compound has already entered human clinical trials.

Regulating the Immune System

HIV infection ultimately kills cells of the immune system that protect us from foreign invaders, including bacteria, fungi, and viruses. Even if growth of the virus is successfully controlled, infected individuals lack an

effective immune system to protect them from infections. Is there hope for resurrecting an effective immune response? Dr. Laura Napolitano is looking for new ways to reconstitute the immune responses of HIV-infected patients. One possibility is to use human growth hormone to stimulate "regrowth" of the key immune cells lost during HIV infection.

Dr. Napolitano's work complements work in two other Gladstone laboratories. Dr. Mike McCune focuses on understanding the growth dynamics of CD4 T cells and how HIV influences this process. His work led to the discovery that CD4 T-cell declines involve both destruction and diminished production of these cells by the thymus. Dr. Douglas Nixon studies the role of cytotoxic and regulatory T cells, two types of immune cells that are essential for inducing and regulating the immune response against HIV. This work could lead to fundamentally new approaches for modulating that response.

Therapy as Prevention

The Gladstone/UCSF Laboratory of Clinical Virology, directed by Dr. Grant, has been at the forefront of clinical research that aims to determine the optimal use of existing and new antiretroviral drugs. The use of antiviral drugs to prevent infection after accidental exposure of healthcare workers was pioneered in San Francisco. Dr. Grant's laboratory was instrumental in demonstrating the safety

HIV Therapy Timeline

- 1981** First cases of new immune deficiency.
- 1983** HIV isolated.
- 1984** First diagnostic test available for HIV infection.
- 1987** First FDA-approved therapy against HIV, a reverse transcriptase inhibitor called zidovudine.
- 1994** Zidovudine recommended for pregnant women to reduce perinatal transmission.
- 1995** First protease inhibitor available. Highly active antiretroviral therapy (HAART) is tested in patients.
- 1996** AIDS-related deaths decline in the US by more than 40%, largely in response to HAART.
- 1997** Introduction of viral load assays to measure viral burden in patients.
First cases of resistance to HAART and recognition of complications linked to therapy.
- 2000** Identification of latent reservoir as a barrier to HIV eradication.
- 2003** Introduction of a new class of drugs, known as fusion inhibitors.

of post-exposure prophylaxis. Dr. Grant is providing leadership for the next generation of trials to evaluate the effectiveness of antiviral drugs for preventing HIV transmission. In the absence of an effective vaccine, a prevention drug might play a pivotal role in turning back the HIV/AIDS epidemic.

What's on the Horizon?

HIV replication also relies on several small proteins that are under intensive investigation at Gladstone. One singularly exciting study focuses on Vif, a viral protein that is required for HIV to replicate effectively in some cell types. Dr. Greene's laboratory was the first to identify the mechanism by which Vif neutralizes a potent innate antiviral factor called APOBEC3G. His group has created a new cell line that can be used to screen for small molecules that inhibit the action of Vif. Most HIV virologists agree that Vif is the most exciting new target to emerge since the discovery of the HIV coreceptors.

Currently available multidrug therapies usually reduce the viral load to undetectable levels. Initially, hope emerged that patients might actually be completely cured after sufficient treatment. Unfortunately, when medications were withdrawn from patients treated effectively for several years, viral production rebounded within 2–4 weeks. Subsequent studies revealed that HIV persists in long-lived memory CD4 T lymphocytes. These latently infected cells carry the HIV genome in their DNA, but no viral RNA or proteins are made. These cells are indistinguishable from uninfected cells. However, when appropriately stimulated, the HIV provirus springs into action, and infectious virus is produced.

Dr. Verdin's laboratory has used a novel approach to obtain T-cell lines enriched in latently infected cells. In collaborative studies, Drs. Greene and Verdin recently used these cell lines to identify a key role for the transcription factor NF- κ B as an important activator of latent HIV proviruses. Dr. Verdin's laboratory is also using these cell lines to screen for small molecules that reactivate latent HIV. Once identified, these drugs could be used to "purge" the body of latently infected cells. If successful, this therapy might lead to the eradication of HIV infection in patients—the ultimate goal of HIV medicine.

“The urgency of the global HIV epidemic demands our continued efforts to rapidly translate basic science advances into new anti-HIV therapies and to ensure the availability of these novel treatments to all people in need throughout the world. GIVI scientists will continue to answer this call.”

— Dr. Warner C. Greene, Director
Gladstone Institute of Virology and Immunology

Gladstone's Commitment

Much has been accomplished in the 20 years since HIV was identified as the causal agent for AIDS. HIV infection has been transformed from a uniformly fatal disease to a chronic viral infection that can be managed with drugs. Despite these successes, much work remains. By integrating our molecular studies of HIV virology and a detailed understanding of the immune response to HIV infection, scientists at the Gladstone Institute of Virology and Immunology are attacking one of the greatest healthcare challenges in history.