nature MILESTONES

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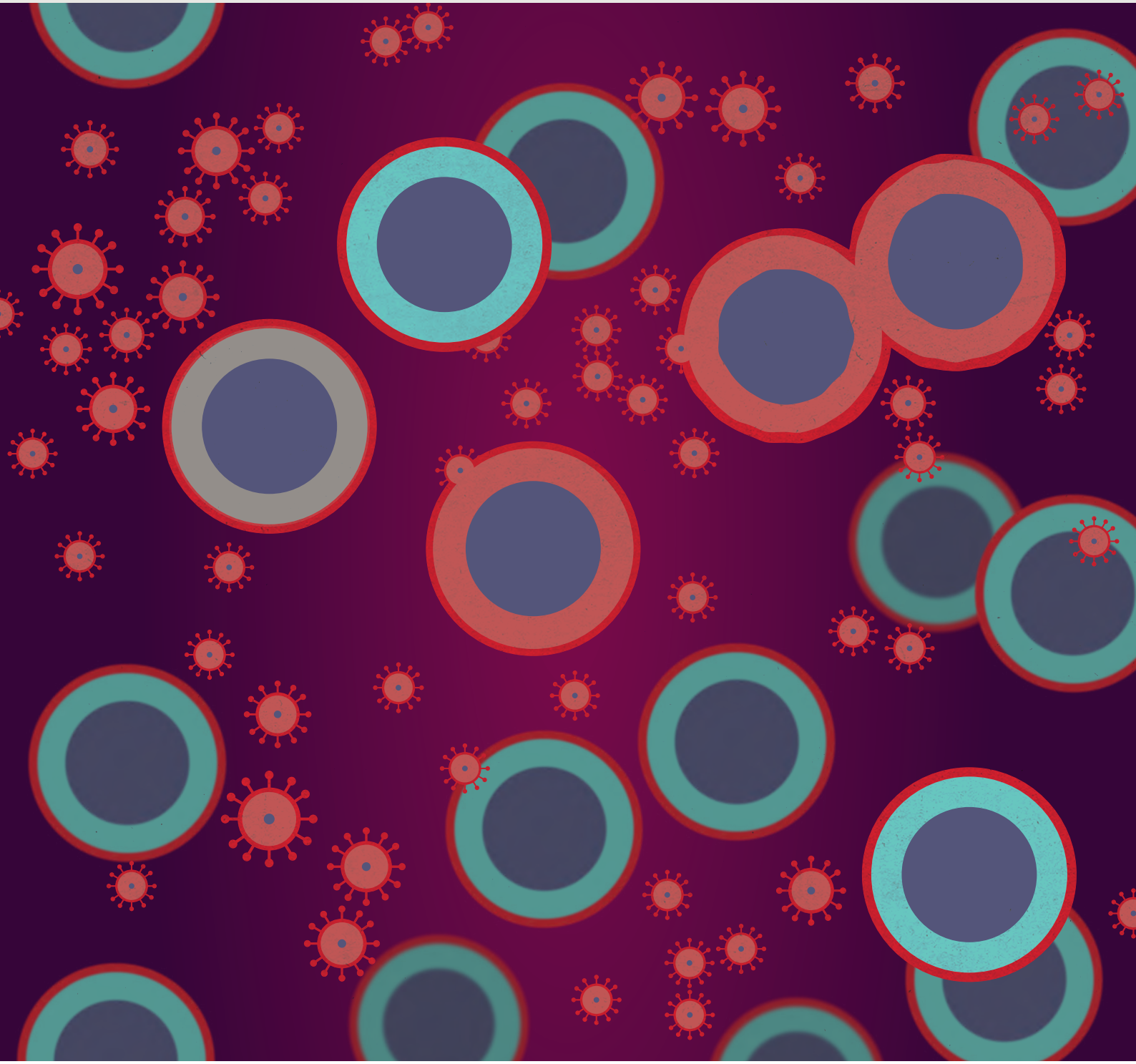
# HIV research



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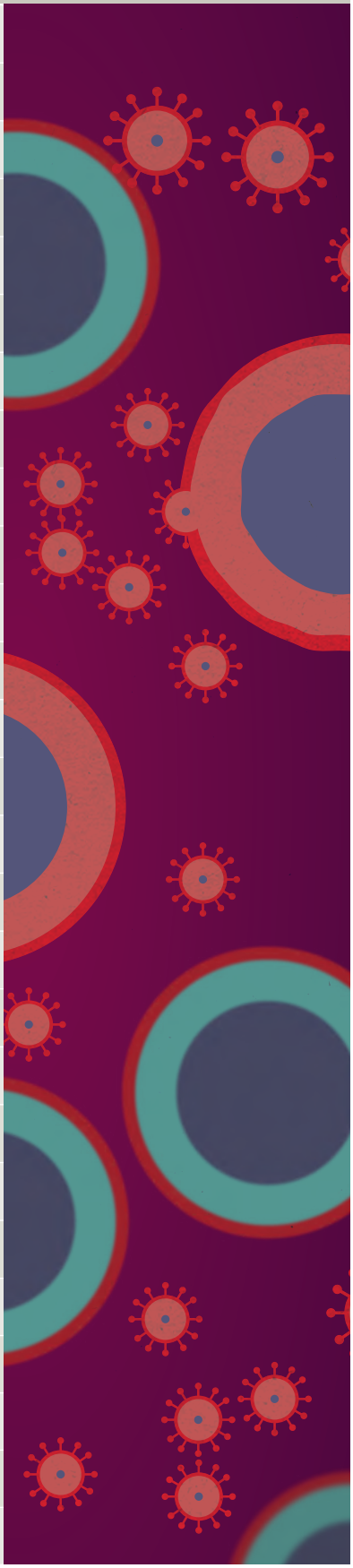
# TIMELINE—MILESTONES IN HIV RESEARCH

| | |
|---|---|
| 1981 | First report of AIDS cases (Milestone 1) |
| 1983 | Discovery of HIV-1 (Milestone 2) |
| 1984 | CD4 is the receptor for HIV-1 (Milestone 3) |
| 1984 | AIDS is a pandemic disease (Milestone 4) |
| 1985 | Complete HIV-1 sequence is described (Milestone 5) |
| 1985 | Clinical test to detect HIV-1 |
| 1986 | Discovery of HIV-1-related viruses in nonhuman primates (Milestone 6) |
| 1987 | First antiretroviral drug approved |
| 1989 | Origins of HIV (Milestone 7) |
| 1989 | Structure of HIV-1 protease (Milestone 8) |
| 1990 | HIV-1 envelope vaccine protects chimpanzees from infection (Milestone 9) |
| 1990 | Immune activation is prognostic of AIDS progression (Milestone 10) |
| 1991 | In-host variation of SIV leads to evasion of antibodies |
| 1993 | HIV-1 replicates at all stages of infection (Milestone 11) |
| 1994 | Antiretroviral treatment reduces maternal–infant transmission of HIV-1 |
| 1994 | $CD8^+$ T cells control virus levels (Milestone 12) |
| 1995 | HIV-1 dynamics drive $CD4^+$ T cell turnover (Milestone 13) |
| 1995 | First HIV protease inhibitor approved: key to combination antiretroviral therapy (Milestone 14) |
| 1996 | Identification of CCR5 and CXCR4 as HIV-1 co-receptors (Milestone 15) |
| 1997 | Latent integrated HIV-1 forms a stable, inducible viral reservoir (Milestone 16) |
| 2002 | Identification of host-encoded HIV restriction factors (Milestone 17) |
| 2008 | Nobel prize for the discovery of HIV |
| 2009 | The Berlin patient (Milestone 18) |
| 2009 | Vaccination against HIV-1 may reduce risk of infection |
| 2009 | Advancing broadly neutralizing antibodies (Milestone 19) |
| 2011 | Antiretroviral treatment as prevention (Milestone 20) |
| 2015 | START trial shows benefit of early antiretroviral treatment (Milestone 21) |

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MILESTONE 1

# The levee breaks—initial reports of AIDS

In June of 1981, an astute team of clinicians led by Michael Gottlieb reported an unusual occurrence in the *Morbidity and Mortality Weekly Report*, a publication led by the US Centers for Disease Control and Prevention (CDC). Five apparently healthy individuals in Los Angeles had contracted *Pneumocystis* pneumonia (PCP), a serious infection that is normally limited to individuals who are severely immunosuppressed. At the time of the report, two of the patients had died. These were the first published incidences of an unrecognized deadly illness, which later became known as AIDS.

Notably, the patients in this first report were all men who have sex with men, and laboratory testing of isolates from three of the men showed defects in cellular immunity, including abnormally low T cell counts and decreased in vitro lymphocyte proliferative responses. These were the first hints of the emergence of a disease causing cellular immune dysfunction that is spread by sexual contact.

A second report was published in July of 1981 by a team led by Alvin Friedman-Kien, documenting 26 patients, all men who have sex with men from New York City or California, with Kaposi sarcoma (KS), PCP or other opportunistic infections. KS is a rare malignant neoplasm that manifests with skin lesions and is associated with immunosuppression. These initial reports alerted the CDC to the potential presence of a new disease, and in response, the CDC formed a taskforce to undertake surveillance and laboratory testing of KS, PCP and other serious opportunistic infections, particularly in men who have sex with men.

The CDC Task Force published their initial report in *The New England Journal of Medicine* in January of 1982, documenting 159 patients with KS, PCP and other opportunistic infections, which often occurred together in the same patient. Importantly, the mortality rate was very high and the number of patients was increasing over time, providing further evidence of a deadly new infectious disease.

As the year 1982 progressed, more reports trickled in. In May, Donna Mildvan and colleagues documented 57 patients, again all men who have sex with men, in Atlanta, New York and San Francisco, with unexplained, persistent, generalized lymphadenopathy. A large proportion (~70%) also had other generalized symptoms, such as fatigue, fever and weight loss. Importantly, biopsy samples of 43 of these patients showed evidence of reactive hyperplasia and depressed numbers of $CD4^+$ helper T cells, suggesting cellular immune dysfunction. Generalized lymphadenopathy is associated with many different infections; however, the cause of lymphadenopathy and immune suppression in these patients was unclear.

By September of 1982, enough evidence had been gathered for the CDC to issue an update on a new illness, which they termed AIDS. They provided an official case definition for AIDS, which was described as a disease associated with defects in cellular immunity and the occurrence of serious opportunistic infections or malignant neoplasms, or non-specific symptoms such as fever, weight loss and generalized, persistent lymphadenopathy, in persons with no known etiology.

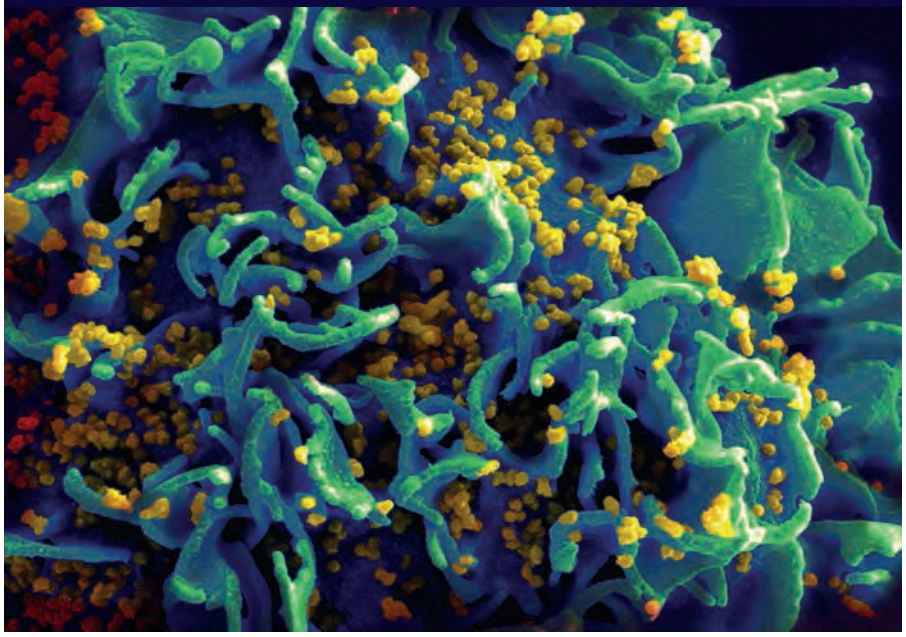
As time progressed, patients with AIDS began to be recognized in various populations, including individuals of Haitian descent who were living in the US, patients with hemophilia A who had received blood-derived products, children of parents with AIDS, intravenous drug users, recipients of blood transfusions and sexual partners of patients with AIDS (MILESTONE 4). These reports hinted at the sexual, blood-borne and vertical transmission routes of an infectious agent. Consequently, in March of 1983, in conjunction with the US Food and Drug Administration (FDA) and the US National Institutes of Health, the CDC released a series of recommendations to prevent the spread of AIDS. These included a recommendation that sexual contact with individuals with AIDS be avoided and advised groups at higher risk of contracting AIDS to temporarily refrain from blood donation. However, the fight to stop the spread of AIDS would be futile unless the infectious agent responsible for this deadly disease could be identified.

Shimona Starling,
*Nature Reviews Disease Primers*

**ORIGINAL ARTICLES** Centers for Disease Control and Prevention. *Pneumocystis* pneumonia—Los Angeles. *MMWR Morb. Mortal Wkly. Rep.* **30**, 1–3 (1981) | Centers for Disease Control and Prevention. Kaposi's sarcoma and *Pneumocystis* pneumonia among homosexual men—New York City and alifornia. *MMWR Morb. Mortal Wkly. Rep.* **30**, 305–308 (1981) | Task Force on Kaposi's Sarcoma and Opportunistic Infections. Epidemiological aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. *N. Engl. J. Med.* **306**, 248–252 (1982) | Centers for Disease Control and Prevention. Persistent, generalized lymphadenopathy among homosexual males. *MMWR Morb. Mortal Wkly. Rep.* **31**, 249–251 (1982) | Centers for Disease Control and Prevention. Update on acquired immune deficiency syndrome (AIDS)—United States. *MMWR Morb. Mortal Wkly. Rep.* **31**, 507–508, 513–514 (1982) | Centers for Disease Control and Prevention. Prevention of acquired immune deficiency syndrome (AIDS): report of inter-agency recommendations. *MMWR Morb. Mortal Wkly. Rep.* **32**, 101–103 (1983)

MILESTONE 2

# The discovery of HIV-1



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AIDS was first diagnosed in 1981 (MILESTONE 1) and, on the basis of epidemiological evidence, was soon speculated to be caused by an infectious agent. Around the same time, the first human retroviruses were discovered in T cells of leukemia patients and were associated with abnormal T cell replication. T cell function and numbers (MILESTONE 10, 13) are also affected in AIDS patients, and scientists hypothesized that a related retrovirus, preferentially infecting T cells, could be the infectious agent underlying AIDS.

Techniques developed during the work with animal and human retroviruses were essential to test this hypothesis and in isolating the putative retrovirus from AIDS patients. Scientists had already optimized in vitro culture conditions for long-term propagation of human T cells and relatively high retrovirus replication. They had developed sensitive techniques to detect reverse transcriptase, an enzyme essential and specific for retroviruses, and were able to identify retroviral particles by electron microscopy. Without this knowledge of retroviruses and essential techniques for their characterization, the discovery of HIV-1 would arguably have been much delayed.

In 1983, Luc Montagnier's team at the Pasteur Institute in Paris discovered HIV-1. Using the established techniques, they cultured T cells from a lymph node biopsy from a 33-year-old homosexual French patient with symptoms that can precede AIDS (subsequently called pre-AIDS), such as lymphadenopathy. Reverse transcriptase activity in the supernatant of this culture and the morphology of virions showed that they had isolated a retrovirus. They were able to infect T cells from a healthy donor, but attempts to infect other cell types, including B cells and fibroblasts, failed. The group concluded that this patient at risk for AIDS was infected with a T cell–tropic retrovirus, but an association with AIDS remained tentative at this point. In 2008, Luc Montagnier and Françoise Barré-Sinoussi from his team were awarded the Nobel Prize for the isolation and characterization of HIV-1.

> “The group concluded that this patient at risk for AIDS was infected with a T cell–tropic retrovirus, but an association with AIDS remained tentative”

In 1984, Robert Gallo's team at the National Cancer Institute in Bethesda, Maryland, isolated HIV-1 from a larger group of patients and suggested causative involvement of the virus in AIDS. They isolated the virus from 48 individuals, including patients with symptoms of pre-AIDS and patients with AIDS, mothers of juveniles with AIDS and one healthy male homosexual. Overall, they isolated HIV-1 in approximately 47% of patients with pre-AIDS or AIDS, but in none of 115 heterosexual individuals with no known risk for AIDS. In the same year, Gallo's group made another important contribution to the field that allowed production of virus in higher quantities, facilitating further studies. After testing several human cell lines, they identified a T cell line that was permissive for HIV-1 and allowed long-term propagation of patient isolates.

A third team of scientists from the University of California, San Francisco, and the California Department of Health Services in Berkeley further strengthened the link between AIDS and HIV-1. Using similar techniques as the other groups, they detected HIV-1 in 22 of 45 AIDS patients and antibodies to HIV-1 in 86 AIDS patients tested, as well as in a high percentage of homosexual men. Their isolates were antigenically and structurally related to the first isolate described by Montagnier's group.

In less than two years, at least three groups had isolated and characterized HIV-1, showing an association of HIV-1 with AIDS and suggesting a causal link. Each group initially gave the virus a different name, based on the symptoms of patients from whom the virus was isolated or on similarities to known viruses. At the time, HIV-1 was called lymphadenopathy-associated virus, human T cell leukemia virus type III and AIDS-associated retrovirus, in addition to other names. In 1986, a group of scientists suggested the name HIV-1, which is how we know the virus today.

Sonja Schmid,
*Nature Communications*

**ORIGINAL ARTICLES** Poiesz, B. J. et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **77**, 7415–7419 (1980) | Barré-Sinoussi, F. et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**, 868–871 (1983) | Gallo, R. C. et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **224**, 500–503 (1984) | Levy, J. A. et al. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* **225**, 840–842 (1984)
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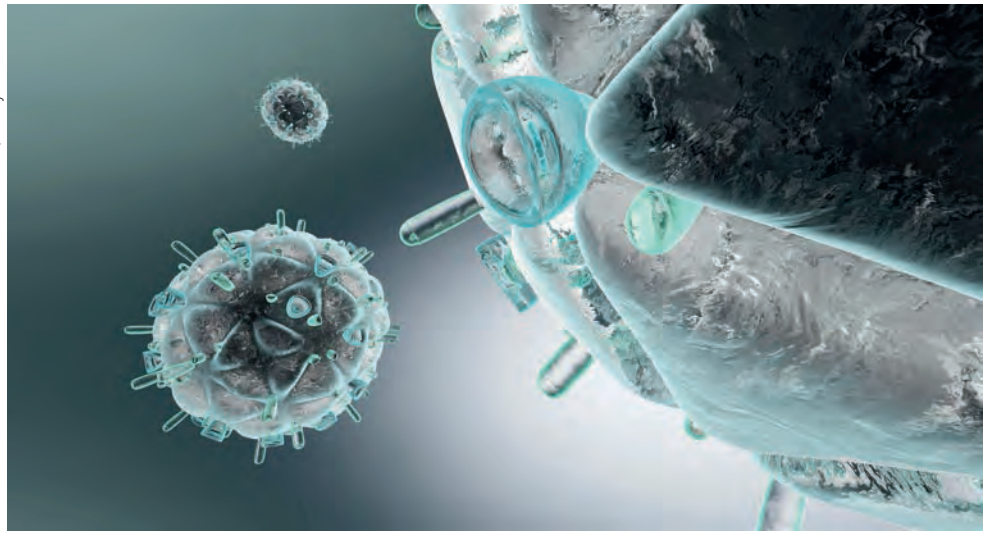

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MILESTONE 3

# CD4 opens the door

Like an uninvited visitor tricking the host to gain entry, viruses induce 'door-opening' cellular processes like endocytosis by engaging the host cell's surface proteins. Although not intended by the host cell to function as viral entry receptors, these proteins are subverted into a gateway role by the virus.

CD4 was originally described in 1979 by Ellis Reinherz in Stuart Schlossman's lab as helper T lymphocyte antigen. Four years later, Luc Montagnier's group reported that HIV-1 was contained within the CD4$^+$ T cell fraction isolated from a patient with HIV-1 and selectively infected and depleted CD4$^+$ T cells in healthy-donor-derived lymphocyte cultures. The authors postulated HIV-1 tropism for CD4$^+$ T cells and speculated that the virus mediates CD4$^+$ T cell loss in AIDS. They also noted normal CD4$^+$ T cell numbers in one HIV-1-positive individual, raising the question of what factors constitute the in vivo determinants of HIV-1cytopathology, a puzzle that took decades to solve.

In 1984, two seminal studies showed that CD4 is critical for HIV-1 entry. In the absence of HIV-1-specific reagents, Robin Weiss and colleagues postulated CD4's role as an HIV-1 receptor using two proxy readouts of HIV-1 entry: multinucleated syncytia formation and cell lysis by vesicular stomatitis virus (VSV) virions bearing HIV-1 envelope proteins. This approach was a fortuitous choice, as native HIV-1 entry requires co-receptors unknown at that time (MILESTONE 15). CD4-specific antibodies blocked HIV-1-induced syncytia formation in cell lines. In parallel, Montagnier's team reported that anti-CD4 antibodies blocked HIV-1 replication in primary T cells. The *CD4* gene was cloned in 1985 by Richard Axel and colleagues, who then introduced recombinant *CD4* cDNA into HIV-1-resistant CD4-negative cells, conferring susceptibility to virus infection. Together with later characterization of CD4 binding to the HIV-1 envelope glycoprotein gp120, this research led the way to the development of HIV-1 entry-targeting therapies, elucidation of CD4's role in HIV-1 pathogenesis and characterization of HIV-1 cellular reservoirs.

In the clinic, CD4$^+$ T cell depletion was recognized as a hallmark of AIDS from the outset (MILESTONE 13), and a CD4$^+$ T cell count of less than 200 cells per microliter of blood is now part of the defining features of AIDS. Although HIV-1 seropositivity was instrumental in determining HIV-1 status, CD4$^+$ T cell count remained unrivalled as a quantitative measure of pathology in the era preceding tests for viral RNA load. During that time, CD4$^+$ T cell count was the key prognostic factor for immune function loss and an essential biomarker of therapeutic efficacy. To this day, CD4$^+$ T cell count informs disease staging and therapeutic choices.

> “CD4 is critical for HIV-1 entry”

Yet, reliance on CD4 for clinical decisions historically had drawbacks. Until blood immunophenotyping standardization in the 1990s, results varied considerably across medical centers. Along with natural variation in CD4$^+$ T cell counts across individuals and age groups, this contributed to diagnostic inconsistencies. Moreover, the decade-long lag between HIV-1 acquisition and the decline to very low levels of CD4$^+$ T cells in blood posed another major challenge to understanding AIDS epidemiology and the link to HIV-1. Finally, antiretroviral therapy was initially indicated only once blood CD4$^+$ T cell counts declined, as the virus was considered dormant until then (MILESTONE 16, 21).

This view was overturned by reports of acute CD4$^+$ T cell depletion in the gut. In fact, activated memory CD4$^+$ T cells, which are particularly permissive to HIV-1 replication and abundant in the intestine, are destroyed within days of infection in all tissues and in the circulation (MILESTONE 11). Loss of memory CD4$^+$ T cells impairs replenishment of the mature T cell pool, thus contributing to systemic CD4$^+$ T cell depletion (MILESTONE 13).

These studies illustrate the importance of research into viral entry receptors. CD4 opens the door to HIV-1, but it has also unlocked insights leading to disease control and prevention (MILESTONE 9, 20, 21).

Tanya Bondar,
*Nature Communications*

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MILESTONE 4

# Realizing the extent of the AIDS epidemic

The first documented AIDS cases occurred in men who have sex with men and intravenous drug users who lived in US metropolitan areas. Other affected groups, including people who had received blood products and Haitians, were identified soon after. During 1983 to 1986, it became apparent that AIDS was not a localized epidemic, either in the populations or regions that it affected, but instead was a pandemic that spread around the globe and throughout different groups of society.

A series of papers, starting with a report by Clumeck et al. in 1983 that described acquired immunodeficiency in five African men who had emigrated to Belgium, began to point toward the prevalence of HIV-1 infection in Africa. The same authors followed up with a more detailed report of 23 previously healthy African AIDS patients hospitalized in Belgium, nine of whom were women. Importantly, none of these patients had any of the previously identified risk factors and the study therefore indicated heterosexual transmission.

Further reports reinforced the widespread occurrence of AIDS in Africa. In 1984, a key study by Piot et al. described a cohort of 38 patients in Zaire with a roughly even distribution of cases between men and women. This led the authors to proclaim heterosexual transmission as a "new epidemiological setting for this worldwide disease." In 1985, Serwadda et al. reported the identification of 71 AIDS patients in Uganda, although the authors called the syndrome "slim disease," as diarrhea and weight loss were the clinically dominant features. Similarly to the above study, almost half of the patients were women and, overall, most cases occurred in a rural, heterosexual population. Notably, the authors were able to detect antibodies to HIV-1 (or HTLV-III, as it was called at the time) in almost 90% of the patients. In 1986, Kreiss et al. reported that the AIDS epidemic had spread extensively among urban prostitutes in Nairobi, Kenya. HIV-1 and AIDS in Africa were definitively on the map.

Besides sexual transmission and transmission through blood, the occurrence of immunodeficiency in children indicated that HIV-1 can also be transmitted from mother to child. In 1983, Rubinstein et al. and Oleske et al. described acquired immunodeficiency in seven and eight children, respectively, who were born to mothers with symptoms of immunodeficiency themselves and/or risk factors such as intravenous drug use. As the children did not have direct sexual or blood exposure themselves, mother-to-child transmission of the disease was assumed. This is in contrast to AIDS in children with hemophilia, who were infected through contaminated blood products.

> "it became apparent that AIDS was not a localized epidemic, either in the populations or regions that it affected, but instead was a pandemic that spread around the globe and throughout different groups of society"

Later findings pointed toward the modes of mother-to-child transmission, occurring via blood during birth and possibly via breast-feeding. In 1985, Ziegler et al. reported a case in which a mother received a transfusion to treat blood loss after birth. The blood donor was diagnosed with AIDS 13 months later. Both the mother and baby were then shown to have antibodies against HTLV-III and, as there was no other exposure, the authors speculated that the baby was infected through breast milk.

Together, these early epidemiological studies were crucial in establishing the true extent of the HIV-1/AIDS pandemic and the routes of transmission. The studies in African cohorts also foreshadowed the devastating effects HIV infection would have on entire countries and societies on the continent. Finally, recognizing the risk and modes of mother-to-child transmission set the stage for some of the most successful interventions to limit the further spread of HIV infection (MILESTONE 20).

Ursula Hofer,
*Nature Reviews Microbiology*


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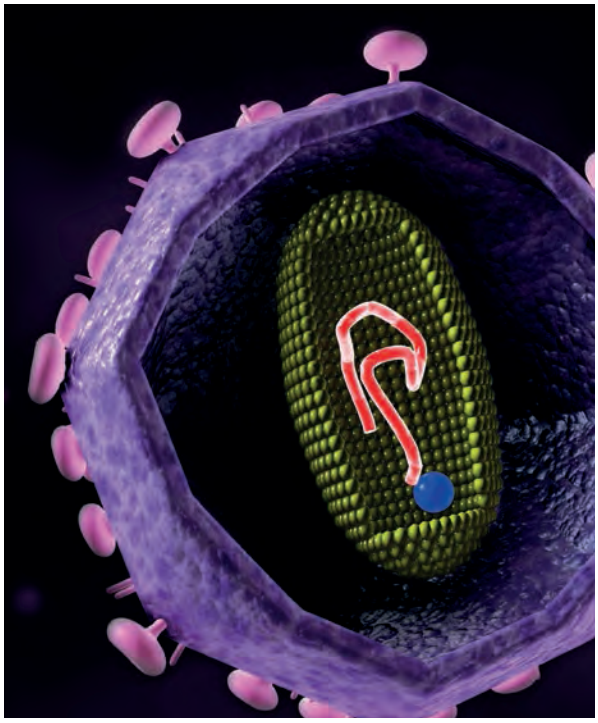
**ORIGINAL ARTICLES** Clumeck, N. et al. Acquired immune deficiency syndrome in Black Africans. *Lancet* **1**, 642 (1983) | Clumeck, N. et al. Acquired immunodeficiency syndrome in African patients. *N. Engl. J. Med.* **310**, 492–497 (1984) | Piot, P. et al. Acquired immunodeficiency syndrome in a heterosexual population in Zaire. *Lancet* **2**, 65–69 (1984) | Serwadda, D. et al. Slim disease: a new disease in Uganda and its association with HTLV-III infection. *Lancet* **2**, 849–852 (1985) | Kreiss, J. K. et al. AIDS infection in Nairobi prostitutes. *N. Engl. J. Med.* **314**, 414–418 (1986) | Rubinstein, A. et al. Acquired immunodeficiency with reversed T4/T8 ratios in infants born to promiscuous and drug-addicted mothers. *J. Am. Med. Assoc.* **249**, 2350–2356 (1983) | Oleske, J. et al. Immune deficiency syndrome in children. *J. Am. Med Assoc.* **249**, 2345–2349 (1983) | Ziegler, J. B., Cooper, D. A., Johnson, R. O. & Gold, J. Postnatal transmission of AIDS-associated retrovirus from mother to infant. *Lancet* **1**, 896–899 (1985)

MILESTONE 5

# From sequence to proteins



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1985 marked the year when the full nucleotide sequence of HIV-1 was reported by three groups, a development that was instrumental to further understanding of the genetics and molecular biology of the virus. Ratner et al., Sanchez-Pescador et al. and Wain-Hobson et al. were the first to describe the full DNA sequence and genome organization of viral isolates. The complete sequences (>9,000 kb in length) were derived from proviral DNA and circular unintegrated viral DNA, and they encompassed the long terminal repeats (LTRs), which have crucial roles in the regulation of transcription of viral genes and integration. It is now established that the viral genome encodes the capsid proteins (Gag), viral enzymes (Pol) and the envelope glycoprotein (Env), as well as six additional open reading frames. By determining the locations and sizes of the viral open reading frames, it was revealed that the fundamental genetic structure is similar to that of other retroviruses, but that HIV-1 not only has distinctive genetic complexity but also encodes genes with features not previously recognized in biology.

> “Understanding the genetic structure of the virus led to new insights into the regulation of viral gene expression and RNA export”

Understanding the genetic structure of the virus led to new insights into the regulation of viral gene expression and RNA export. An important discovery following rapidly on the heels of the full sequence information was the finding that HIV-1 encodes a trans-acting factor, termed Tat, which was shown to be vital for the transactivation of viral gene expression from the 5′ LTR. During the following years, several studies revealed the mechanism of Tat-mediated transactivation: early during infection, low levels of viral transcripts are generated, which are subsequently spliced and translated to make Tat. Tat binds to an RNA stem-loop structure, the trans-activation response element (TAR), a regulatory element located downstream of the transcriptional initiation site at the 5′ end of nascent viral transcripts. Following binding to TAR, Tat recruits the positive transcription elongation factor b (P-TEFb), a host factor that comprises cyclin-dependent kinase 9 (CDK9) and cyclin T1 as well as other elongation factors. Cyclin T1 binds directly to Tat and CDK9 phosphorylates the C-terminal domain of RNA polymerase II, thus promoting efficient transcriptional elongation. Smaller fully spliced messages, such as those encoding Tat, are exported readily from the nucleus to the cytoplasm and are translated, whereas unspliced and incompletely spliced mRNAs require the action of Rev (regulator of expression of virion proteins), a regulatory HIV-1 protein that is also expressed during the early phase of infection.

The mechanism of Rev-dependent export of HIV-1 mRNA species became apparent in the late 1980s. Rev induces the sequence-specific nuclear export of late-phase HIV-1 mRNA species and promotes the cytoplasmic expression of HIV-1 mRNAs that encode viral accessory and structural proteins, including Gag and Env. The initial step in this pathway involves binding of Rev to the Rev-response element (RRE; a stem-loop structure that is present in intron-retaining viral mRNAs) in a highly cooperative manner. An important finding was that RRE-bound Rev forms a complex with cellular nuclear export factor CRM1 through its nuclear export signal. This interaction enables CRM1 to transport the mRNA–Rev complex into the cytoplasm for ensuing translation.

The description of the full nucleotide sequence enabled remarkable discoveries that revealed how gene expression in HIV-1 is controlled by the HIV-1 RNA-binding proteins Tat and Rev and how the virus hijacks the core molecular machinery of the host during viral replication, using mechanisms that were unprecedented at that time. The sequencing work also set the stage for further discoveries regarding the origins and diversity of the virus (MILESTONE 7).

Andrea Du Toit,
*Nature Reviews Microbiology*

**ORIGINAL ARTICLES** Ratner, L. et al. Complete nucleotide sequence of the AIDS virus, HTLV-III. *Nature.* **313**, 277–284 (1985) | Sanchez-Pescador, R. et al. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). *Science* **227**, 484–492 (1985) | Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. Nucleotide sequence of the AIDS virus, LAV. *Cell* **40**, 9–17 (1985)

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MILESTONE 6

# Monkey insights

In 1985, following fast on the heels of the identification of HIV-1, researchers at Harvard and at the New England Regional Primate Research Center isolated virus from captive rhesus macaques in the center's colony that displayed symptoms of an immunodeficiency syndrome similar to AIDS in humans. Like HIV-1, the isolated virus—originally named STLV-III and now called simian immunodeficiency virus, or SIV—was tropic for $CD4^+$ T cells, budded from cells as viral particles and killed infected cells in culture. The findings, coupled with those of a companion study by the same researchers that demonstrated that antibodies in AIDS patients recognized SIV proteins, provided some of the first direct evidence that HIV-1 has a primate ancestor (MILESTONE 7).

Within months, these researchers formally demonstrated that the virus they had isolated from macaques (and now called $SIV_{mac}$) caused immunodeficiency in infected animals. By recapitulating in macaques symptoms seen in humans—including wasting, opportunistic infections and $CD4^+$ T cell depletion—the study provided experimental proof of concept that a virus causes AIDS.

> "By recapitulating in macaques symptoms seen in humans… the study provided experimental proof of concept that a virus causes AIDS"

Soon after, distinct SIVs were identified that infect other nonhuman primates, including chimpanzees, African green monkeys, sooty mangabeys, gorillas and other macaque species, lending insights into the origins of HIV-1 in humans. Not all SIVs are pathogenic in their natural primate hosts, however, so the ability of $SIV_{mac}$ to induce an AIDS-like illness in rhesus macaques made it a vital experimental tool to model HIV-1 infection and pathogenesis in animals.

Yet, studies later concluded that $SIV_{mac}$, unlike other SIVs, is not found in the wild. Instead, it is believed to have been generated as a function of experimentally inoculating captive rhesus macaques with SIV-infected tissue from sooty mangabeys. $SIV_{smm}$, which infects sooty mangabeys, is also likely the ancestor of HIV-2, a human retrovirus related to both SIV and HIV-1 (MILESTONE 7).

In spite of its lab-based origins, and some differences in disease progression in macaques as compared with humans, $SIV_{mac}$ has become a cornerstone of HIV-1 research. But antigenic differences between the envelope (Env) glycoproteins of SIV and HIV-1, which allow the virus to bind and enter $CD4^+$ T cells, preclude the direct testing of human vaccines targeting HIV-1 Env in monkeys. In 1996, the development of chimeric simian-human immunodeficiency viruses (SHIVs) that caused AIDS-like disease in rhesus and pigtailed macaques overcame this hurdle. Whereas earlier SHIVs had not induced disease, serial passaging of SHIVs through macaques resulted in highly virulent viruses that caused rapid $CD4^+$ T cell loss and death of infected animals. The chimeric viruses incorporate the genes encoding Env and other regulatory factors from HIV-1 within an SIV backbone, thereby enabling the testing of vaccines and drugs in monkeys that target HIV-1 Env and block its role in viral infection and disease, which was previously not feasible.

These early and invaluable efforts characterizing SIV and AIDS in monkeys not only helped inform subsequent studies of HIV pathogenesis and treatment, but also shed light on the cross-species transmission patterns that gave rise to HIV-1 and related viruses.

Alison Farrell,
*Nature Medicine*

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MILESTONE 7

# Origin story

> HIV-1 was circulating cryptically in Africa for generations

AIDS was first described as a new disease in 1981 (MILESTONE 1), and the causative lentivirus that came to be known as HIV was identified a couple of years later (MILESTONE 2). However, the origins of HIV and the reasons for its explosive appearance and rapid transformation into a global epidemic remained a mystery.

By the mid-1980s, evidence had accumulated that AIDS had been present in Central Africa considerably earlier than the first descriptions of the disease in the West. Therefore, the suspicion was that the HIV pandemic might have arisen from a related virus circulating in an animal reservoir in sub-Saharan Africa—but in which species?

The inklings of an answer began to emerge from two West African patients presenting with an antigenically distinct AIDS-causing virus that subsequently came to be known as HIV-2 (MILESTONE 6). Genetically distant from HIV-1, HIV-2 was instead shown to be closely related to a lentivirus infecting an African monkey—the sooty mangabey. Subsequent studies confirmed that lentiviruses are endemic to many species of African monkeys and some apes—and these have collectively become known as simian immunodeficiency viruses (SIVs). Sooty mangabeys in particular were found to be naturally infected with a lentivirus known as $SIV_{smm}$ (with the subscript indicating the name of the infected species), and genetic evidence showed that this virus was transmitted to humans, generating the HIV-2 epidemic.

HIV-2 is usually less pathogenic than HIV-1 and is far less common and widespread, being largely confined to a few West African countries. However, the AIDS pandemic that has led to more than 30 million deaths worldwide is overwhelmingly driven by HIV-1, and in particular the M group of HIV-1 strains. A key advance in nailing down the origin of HIV-1 was made in 1989 by Martine Peeters and colleagues. The study identified an SIV in wild chimpanzees ($SIV_{cpz}$) that was serologically identical to HIV-1 but not HIV-2. Soon after, work by Simon Wain-Hobson's group confirmed that $SIV_{cpz}$ had a very similar genetic organization to HIV-1. Evidence was therefore stacking that $SIV_{cpz}$ was the likely source of the HIV-1 pandemic, but the timing, and locale of its origin were unclear.

An important clue to the timing of the epidemic was found in 1998 by David Ho's group, who managed to amplify and partially sequence HIV-1 from a 1959 plasma sample—to date, the oldest known definitive case of HIV-1 infection. This pinpointed the infection to the area around what is now Kinshasa in the Democratic Republic of Congo. Indeed, it's now known that this region is where most of the early diversification of M group HIV-1 has occurred. Another critical insight into the origins of the HIV-1 epidemic was made by Beatrice Hahn and colleagues in a 1999 paper. Studying wild-caught chimpanzees, this study combined $SIV_{cpz}$ sequencing with mitochondrial DNA analysis to identify the infected chimpanzee subspecies. Used together, this information showed that two chimpanzee subspecies, *Pan troglodytes troglodytes* and *Pan troglodytes schweinfurthii*, hosted divergent SIV lineages—$SIV_{cpzPtt}$ and $SIV_{cpzPts}$, respectively. Strikingly, all HIV-1 strains known at the time, including those in the pandemic M group, were closely related to $SIV_{cpzPtt}$, strongly suggesting that *P. t. troglodytes* served as the origin of the HIV-1 pandemic. This was subsequently confirmed by identifying natural *P. t. troglodytes* reservoirs of the HIV-1 precursor in southeastern Cameroon. Why $SIV_{cpzPts}$ has been unsuccessful at jumping species into humans remains unknown but may be related to this virus's inability to overcome restriction factors in potential human hosts (MILESTONE 17). How $SIV_{smm}$ and $SIV_{cpzPtt}$ made the leap into humans is not entirely clear, but it's likely to have occurred via the unsafe consumption and preparation of bushmeat.

More recent molecular timing and modeling studies have pushed back the earliest appearance of M group HIV-1 to around the beginning of the twentieth century. This would suggest that HIV-1 was circulating cryptically in Africa for generations before factors such as urbanization and mass movement of people propelled it onto the global stage.

Zoltan Fehervari,
*Nature Immunology*

Credit: Getty Images

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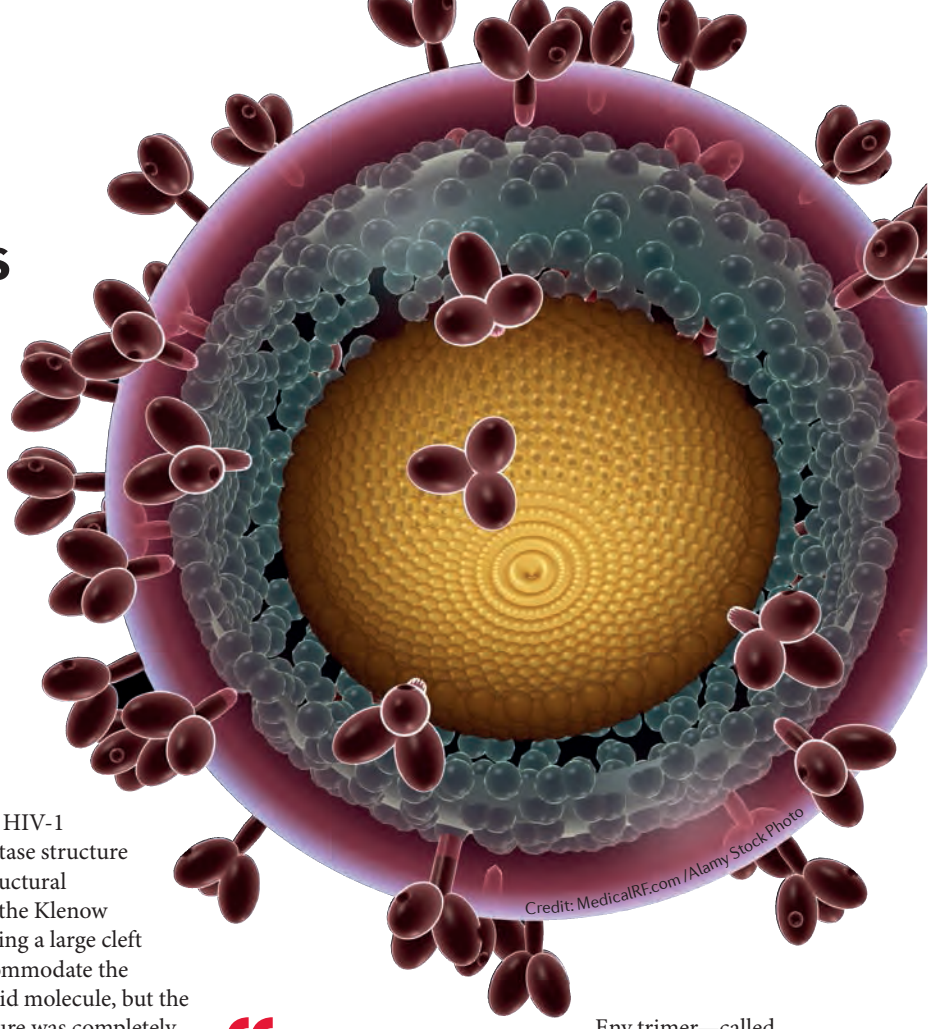
MILESTONE 8

# Structural insights into HIV proteins




Soon after the discovery of HIV, intense efforts focused on developing drugs to tackle the virus. Early targets were the HIV-1 protease and reverse transcriptase, and drug development was greatly assisted by breakthroughs in solving the structures of these viral proteins.

The first HIV-1 protein to yield a high-resolution structure was the HIV-1 protease. Scientists at Merck Sharp and Dohme Research Laboratories published the first structure in 1989, using recombinant protease expressed in bacteria, which revealed essential features of the catalytic apparatus. Soon after, using a chemical synthesis approach to obtain enough protein for crystallization and some modeling based on the Rous sarcoma virus protease structure, scientists at NCI-Frederick obtained a 2.8-Ångstrom structure of the HIV-1 protease in which all 99 amino acids could be located. Shortly afterward, the first co-crystal structure of its complex with an inhibitor was determined, paving the way for rapid drug development and approval of the first protease inhibitor for HIV-1 therapy six years later in 1995 (MILESTONE 14).

The first drug to treat HIV, however, was approved in 1987, and it targeted the viral reverse transcriptase. Although this drug, azidothymidine (AZT), was rather poor, many of the drugs used today to treat HIV infection target this enzyme: nucleoside reverse-transcriptase inhibitors (NRTIs) become incorporated into viral DNA by the action of reverse transcriptase and block viral RNA synthesis, whereas non-nucleoside reverse-transcriptase inhibitors (NNRTIs) inhibit the enzyme by direct binding.

In 1992, Thomas Steitz and colleagues provided the first high-resolution structural glimpse of how an NNRTI, nevirapine, interacted with the HIV-1 reverse transcriptase enzyme. Before this time, the mechanism by which nevirapine worked was unknown, and furthermore, the only polymerase for which there was structural information was the Klenow fragment from *Escherichia coli* polymerase. The HIV-1 reverse transcriptase structure showed some structural similarities with the Klenow fragment, including a large cleft sufficient to accommodate the RNA–DNA hybrid molecule, but the rest of the structure was completely different. The structure also revealed where nevirapine bound, suggesting potential mechanisms by which the drug inhibits reverse transcriptase as well as revealing the location of known resistance-conferring mutations. This paper and related structural work from Edward Arnold and colleagues published around the same time, as well as structures that soon followed, set the stage for the design of more effective reverse transcriptase inhibitors—drugs that remain mainstays of treatment today.

The HIV-1 envelope protein, Env, which mediates fusion of the virus with the cell membrane, has also been a focus of intense interest—mainly for vaccine design (MILESTONE 9). But this conformationally plastic protein proved difficult to study. Crystal structures of the postfusion conformation of gp41 were solved by the groups of Peter Kim and Don Wiley in 1997 and that of the CD4-bound conformation of gp120 was solved by the group of Wayne Hendrickson in 1998. In 2002, John Moore and colleagues reported a stabilized version of the Env trimer—called SOSIP—in which the gp120 and gp41 subunits were held together with an engineered disulfide bond. But it took another decade to obtain high-resolution structures of this SOSIP trimer—eventually determined simultaneously through crystallography and cryo-EM, by the groups of Andrew Ward and Ian Wilson. The information obtained from these structures and others that have followed is hoped to aid the design of an effective vaccine.

> “drug development was greatly assisted by breakthroughs in solving the structures of these viral proteins”

Clare Thomas, *Nature*

**ORIGINAL ARTICLES** Navia, M. A. et al. Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature* **337**, 615–620 (1989) | Wlodawer, A. et al. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. *Science* **245**, 616–621 (1989) | Swain, A. L. et al. X-ray crystallographic structure of a complex between a synthetic protease of human immunodeficiency virus 1 and a substrate-based hydroxyethyamine inhibitor. *Proc. Natl. Acad. Sci. USA* **87**, 8805–8809 (1990) | Arnold, E. et al. Structure of HIV-1 reverse transcriptase/DNA complex at 7 Å resolution showing active site locations. *Nature* **357**, 85–89 (1992) | Kohlstaedt, L. A., Wang, J., Friedman, J. M., Rice, P. A. & Steitz, T. A. Crystal structure at 3.5 Å resolution of HIV-1 reverse transc riptase complexed with an inhibitor. *Science* **256**, 1783–1790 (1992) | Chan, D. C., Fass, D., Berger, J. M. & Kim, P. S. Core structure of gp41 from the HIV envelope glycoprotein. *Cell* **89**, 263–273 (1997) | Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J. & Wiley, D. C. Atomic structure of the ectodomain from HIV-1 gp41. *Nature* **387**, 426–430 (1997) | Kwong, P. D. et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**, 648–659 (1998) | Sanders, R. W. et al. Stabilization of the soluble, cleaved trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J. Virol.* **76**, 8875–8889 (2002) | Lyumkis, D. et al. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. *Science* **342**, 1484–1490 (2013) | Julien, J. et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science* **342**, 1477–1483 (2013)

MILESTONE 9

# HIV vaccines: gp120 and beyond

Identification of the cell receptors that serve as a gateway for HIV, first reported in the 1980s, opened the door to the development of immunization protocols based on the viral envelope proteins with which they interact. In a breakthrough study reported in 1990 by Philip Berman and colleagues, a vaccine based on the HIV glycoprotein gp120, which binds CD4 and chemokine receptors on target cells, protected chimpanzees from HIV-1 infection. The study showed that vaccination with a single recombinant viral protein (in the context of an aluminum hydroxide adjuvant) was sufficient to elicit a protective immune response to HIV in a nonhuman primate, without requiring attenuated viral particles or complexes of multiple viral proteins.

Berman and colleagues tested two vaccine formulations containing distinct HIV-1 proteins: two chimpanzees were immunized against recombinant gp120 and two were immunized with a formulation containing recombinant gp160. The animals received three immunizations and were then challenged with the IIIb isolate of HIV-1.

Whereas chimpanzees immunized with the gp160 vaccine as well as a control animal showed evidence of HIV infection, the researchers found no signs of infection in the animals inoculated with the recombinant gp120 formulation. Infection in the control and gp160-immunized animals was further confirmed by PCR, and viable HIV-1 could only be recovered from these chimpanzees, and not from the two animals immunized against gp120. Levels of virus-neutralizing antibodies on the day of challenge were higher in the protected animals than in the unprotected ones, suggesting a possible key role for them in preventing infection.

The study provided the first evidence of protection against HIV infection by vaccination in an animal model and highlighted the potential of the HIV-1 envelope protein as a candidate vaccine target. The work would eventually lead to tests of recombinant-gp120-based vaccines in humans.

But whether a single-target strategy for vaccination could be successfully translated to humans and elicit a persistent protective response against heterologous viruses remained unclear at the time, particularly when taking into consideration the broad diversity of HIV-1 variants infecting human populations and the speed and efficacy of viral immune escape. A further consideration is that, unlike humans, HIV-infected chimpanzees do not develop AIDS, so protection against disease development induced by vaccination with gp120 could not be assessed by the Berman study. Nevertheless, the strategy was taken further in multiple efforts in nonhuman primates and was eventually tested in large clinical trials (e.g., the ALVAC-HIV and AIDSVAX B/E formulations). The human studies showed that gp120- or gp160-based vaccines could elicit HIV-specific cellular and humoral responses in vaccinated individuals, but the protection rates reported were low.

> “first evidence of protection against HIV infection by vaccination in an animal model”

More detailed understanding of the molecular structures of HIV envelope proteins and host factors such as CD4 and CCR5, as well as the viral epitopes recognized by broadly neutralizing antibodies, is now enabling the design of targeted vaccine strategies using defined immunogens and the testing of passive immunization in humans using monoclonal antibodies that bind gp120 and neutralize the virus. Novel adjuvants, viral vectors and delivery methods, as well as approaches that aim to elicit responses to conserved regions of viral proteins or to diverse HIV-1 subtypes, are also in development and will generate important clinical insights in the near future.

Given that HIV can integrate into the host genome during its replication cycle, it can persist as a long-lived reservoir of latent virus that may be invisible to the immune system (MILESTONE 16). Therapeutic vaccines that aim to eradicate existing infection have been tested, but without success, and new strategies—such as gene and genome editing, checkpoint blockade and latency modulation—will be needed in conjunction with novel vaccine modalities to both prevent and eliminate infection.

João Duarte,
*Nature Biomedical Engineering*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**ORIGINAL ARTICLE** Berman, P. W. et al. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. *Nature* **345**, 622–625 (1990)
**FURTHER READING** Escolano, A., Dosenovic, P. & Nussenzweig, M. C. Progress toward active or passive HIV-1 vaccination. *J. Exp. Med.* **214**, 3–16 (2017) | Barouch, D. H. & Picker, L. J. Novel vaccine vectors for HIV-1. *Nat. Rev. Microbiol.* **12**, 765–771 (2014)

MILESTONE 10

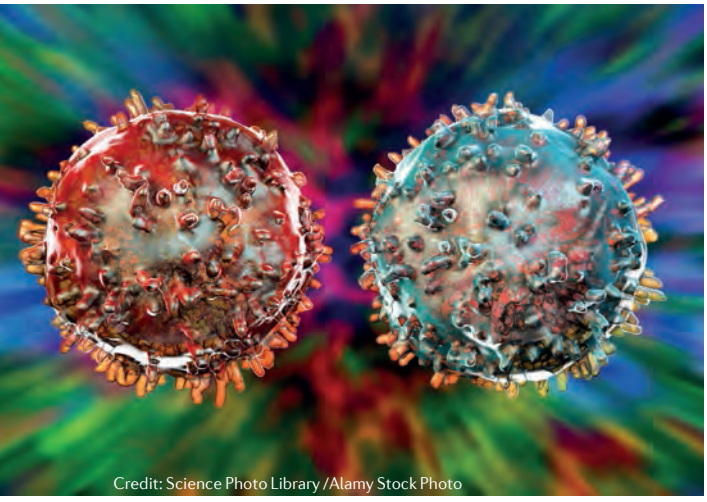
# Immune activation linked to pathogenesis

After the description of CD4 as a receptor for HIV-1 (MILESTONE 3), it was generally assumed that infection of $CD4^+$ T cells by HIV-1 would drive loss of these cells and subsequent development of AIDS. However, it gradually became apparent that chronic immune activation, rather than immunodeficiency alone, was a feature of progressive HIV-1 disease.

The very first description of AIDS (MILESTONE 1) had reported increased levels of the surface activation marker T10 (later called CD38) on T cells from the peripheral blood of patients. Other studies in the early 1980s by scientists such as Janis Giorgi, John Fahey and Anthony Fauci showed that HIV-1 infection is associated with the upregulation of activation markers on $CD8^+$ T cells and B cells.

An important insight came in 1990, when a study by Fahey, Giorgi and co-workers showed that increased expression of immune activation markers was linked to disease progression in patients with HIV-1. They found that higher serum levels of neopterin (a metabolite of interferon-γ-activated macrophages) and the major histocompatibility complex (MHC) class I component β2-microglobulin were almost as powerful as $CD4^+$ T cell counts in predicting AIDS progression in HIV-1-infected patients. A later study from the laboratory of Giorgi reported that higher expression of CD38 on $CD8^+$ T cells was a better predictive marker for the development of clinical AIDS than $CD4^+$ T cell counts. These discoveries facilitated the development of a rapid, inexpensive test to predict whether a patient would progress to AIDS.

> “AIDS is not simply caused by the absence of an immune response, but is characterized by the presence of a chronic dysfunctional immune response”



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Moreover, they suggested that chronic activation of the immune system (as opposed to direct destruction of $CD4^+$ T cells by the virus) was linked to HIV pathogenesis. This idea was further strengthened by the realization that SIV viruses do not generally induce the development of AIDS-like diseases in their natural primate hosts, even though the viruses infect $CD4^+$ T cells and establish chronic infections. A key study by Guido Silvestri and colleagues in 2003 reported that T cell populations are essentially normal in sooty mangabey monkeys that are chronically infected with non-pathogenic SIV, despite high levels of viral replication in $CD4^+$ T cells. Notably, the monkeys had low levels of immune activation. These findings gave credence to a growing school of thought that an aberrant chronic immune response has a major role in the pathogenesis of AIDS.

Another crucial piece of evidence came from the findings of the SMART study group in 2006. This clinical trial showed that intermittent (as opposed to continuous) antiretroviral therapy (ART) in patients infected with HIV caused increased death from non-AIDS-related causes, secondary to immune activation.

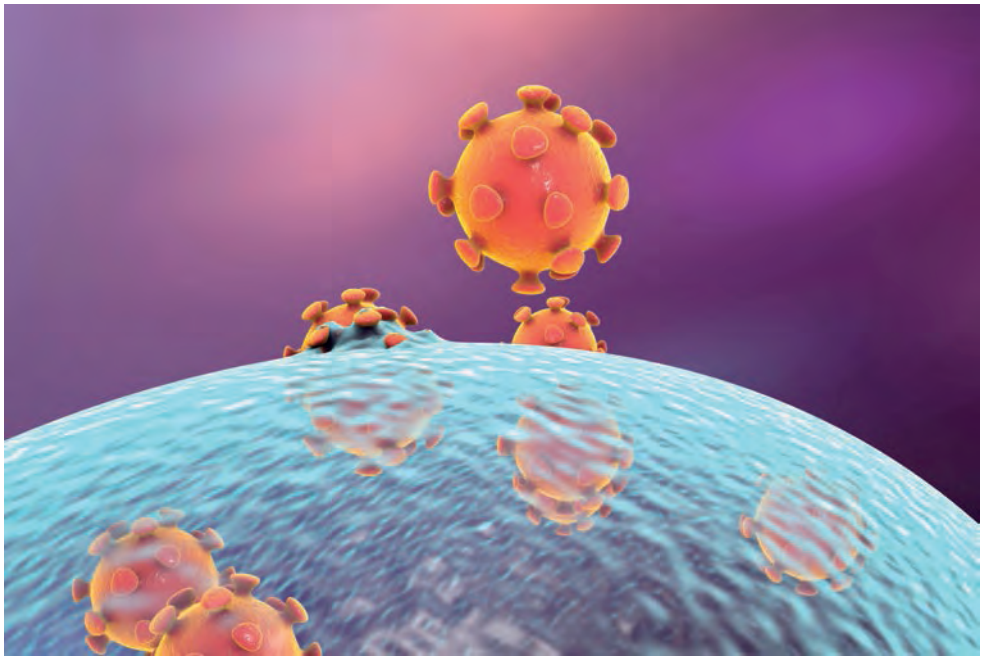
Although by the mid-1990s it had become clear that chronic immune activation was a hallmark of progressive HIV infection, the mechanistic basis of this was still not understood. In 2006, Jason Brenchley et al. reported that microbial translocation from the gastrointestinal tract occurs in HIV-infected individuals and results in increased circulating levels of lipopolysaccharide (LPS). Similar findings were made in rhesus macaques infected with pathogenic SIV. Importantly, increased levels of LPS in the blood circulation correlated with higher activation of both the innate and adaptive immune systems. This suggested that loss of mucosal barrier function (most likely as a result of acute $CD4^+$ T cell depletion) leads to systemic immune activation.

However, even today, it is still not clear exactly how chronic immune activation occurs during pathogenic HIV infections. Other proposed mechanisms include immune responses to the virus and opportunistic infections, or loss of specific $CD4^+$ T cell subsets that are important for immune homeostasis. Importantly, recent work has also indicated that markers of systemic inflammation, particularly plasma levels of IL-6, are in fact better predictors of clinical outcome than levels of T cell activation.

In summary, through time, it has become clear that AIDS is not simply caused by the absence of an immune response, but is characterized by the presence of a chronic dysfunctional immune response, which drives disease progression by causing tissue damage and organ failure.

Yvonne Bordon,
*Nature Reviews Immunology*

**ORIGINAL ARTICLES** Fahey, J. L. et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N. Engl. J. Med.* **322**, 166–172 (1990) | Liu, Z. et al. Elevated CD38 antigen expression on $CD8^+$ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than $CD4^+$ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **16**, 83–92 (1997) | Silvestri, G. et al. Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia. *Immunity* **18**, 441–452 (2003) | Strategies for Management of Antiretroviral Therapy (SMART) Study Group. $CD4^+$ count–guided interruption of antiretroviral treatment. *N. Engl. J. Med.* **355**, 2283–2296 (2006) | Brenchley, J. M. et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* **12**, 1365–1371 (2006) | Hunt, P. W. et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. *J. Infect. Dis.* **210**, 1228–1238 (2014) | Tenorio, A. R. et al. Soluble markers of inflammation and coagulation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. *J. Infect. Dis.* **210**, 1248–1259 (2014) | Grund, B. et al. Relevance of IL-6 and D-dimer for serious non-AIDS morbidity and death among HIV-positive adults on suppressive antiretroviral therapy *PLoS One* **11**, e0155100 (2016)



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MILESTONE 11

# Revealing the 'hidden' virus

By the 1990s, it was generally understood that HIV replicates vigorously on initial infection and then seems to 'hide' for many years, causing few clinical symptoms until AIDS develops. Researchers had struggled to detect the virus during the clinically latent phase, assuming it lay dormant. However, with improved sensitivity of the methods used to detect HIV RNA, this assumption did not stand. A series of three papers published in 1993 concluded that HIV actually replicates continuously and is present throughout lymphoid tissues over the course of disease.

By using a PCR method—known as quantitative competitive PCR (QC-PCR)—that has more stringent internal controls and greater sensitivity, Piatak et al. were able to accurately quantify levels of HIV RNA in the blood of patients at all stages of infection. They revealed ranges of viral RNA copy number of 100 to ~22,000,000 copies per milliliter of plasma and proved this approach to be much more sensitive and more reproducible than the standard PCR approaches and methods of the time that measured circulating HIV p24 antigen and culturable virus. Significantly, the 235-fold decrease in virus levels that can occur following initial infection was only detectable by QC-PCR and not by measuring p24 antigen or culturing virus. The authors were able to correlate HIV RNA levels with clinical stage of disease and $CD4^+$ T cell counts, and they inferred, as others had suspected, that higher levels of circulating virus and failure to effectively control viral replication after initial infection are associated with a negative prognosis.

Importantly for the time, Piatak et al. showed that QC-PCR provided a more sensitive means to evaluate the impact of new therapeutic interventions (MILESTONE 13, 14) on viral load. Finally, their observations in patients receiving antiviral treatment and after discontinuing treatment hinted at the dynamic nature and high levels of ongoing viral replication and the role of this replication in the pathogenesis of HIV infection and AIDS.

The active nature of infection was confirmed by two other groups, who published their findings in *Nature* at the same time. Pantaleo et al. analyzed viral burden and levels of viral replication in the blood and lymphoid tissues of the same individuals at various stages of HIV disease. They observed for the first time a striking dichotomy in viral burden and replication between the blood and lymphoid tissues in early-stage disease. Even in early stages of disease, when HIV burden in the blood is low or absent, infected cells preferentially accumulate and actively replicate in lymphoid tissues. As had been previously described, closer examination confirmed that HIV particles, probably as part of immune complexes, are trapped by the villous processes of the follicular dendritic cells that surround lymphocytes in lymphoid tissues. As disease progresses, lymph node architecture is disrupted and virus-trapping capabilities are lost, which the authors assumed results in increased levels of virus in the blood at late stages of disease.

> "HIV actually replicates continuously and is present throughout lymphoid tissues over the course of disease"

Similarly, Embretson et al. revealed a large pool of infected $CD4^+$ lymphocytes and macrophages throughout the lymphoid system of patients with early to late stages of infection. Extracellular virus associated with follicular dendritic cells was also detected, which the authors suggested may be transmitted to lymphocytes migrating through lymphoid follicles. They calculated that the reservoir of infected cells is large enough to account for immune depletion in AIDS and thereby represented a new challenge and new target for therapeutic interventions.

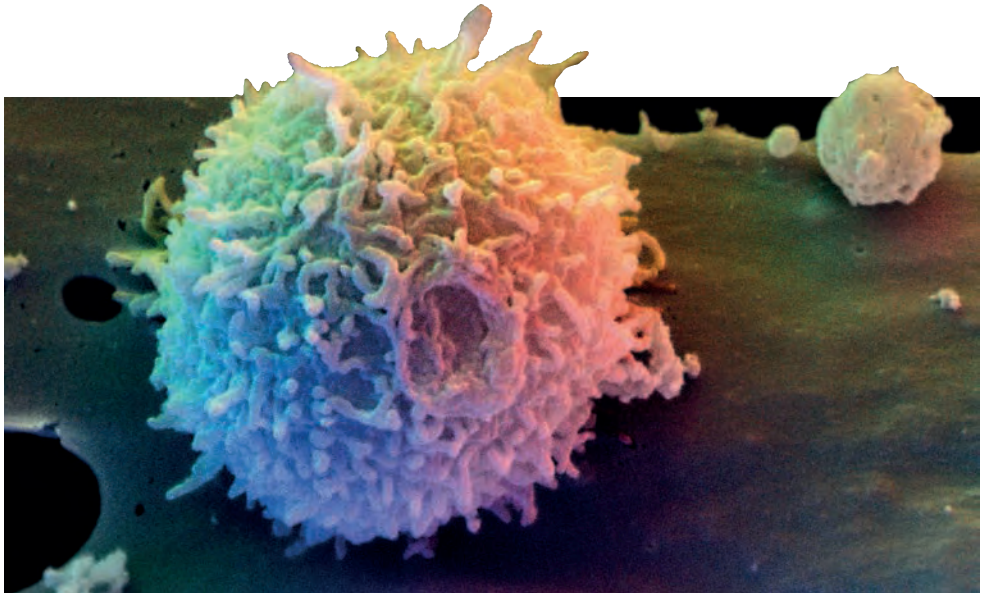
Finally, the impression that HIV disease was not active had led to a misguided strategy to only begin antiretroviral therapy once clinical symptoms appeared or when there was a marked decline in $CD4^+$ T cell count. The knowledge of its continually active state represented a major conceptual change and paved the way for better strategies of intervention (MILESTONE 20, 21).

Lucy Bird,
*Nature Reviews Immunology*

**ORIGINAL ARTICLES** Piatak, M. Jr et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* **259**, 1749–1754 (1993) | Pantaleo, G. et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* **362**, 355–358 (1993) | Embretson, J. et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* **362**, 359–362 (1993)

MILESTONE 12

# CD8s crave control



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Understanding how the human immune system responds to HIV-1 infection is critical to inform the development of protective vaccines. In 1987, Bruce Walker and colleagues reported that HIV-1-infected individuals had circulating T cells that recognized and killed target cells expressing HIV-1 proteins in vitro. But whether cytotoxic T lymphocytes (CTLs) could thereby control virus levels in humans was initially unclear.

To address the influence of CTLs on the virus—and vice versa—Rodney Phillips and colleagues analyzed the longitudinal responses of CTLs specific for the HIV-1 Gag protein in three HIV-infected individuals. The researchers showed fluctuations over time in terms of the specific epitopes recognized and the dominance of CTL specificities. They further found mutations arising in or near T cell epitopes in Gag and the failure of some CTLs to recognize altered epitopes. Their results suggested that amino acid variation in viral epitopes abolishes recognition by CTLs, which may confer a survival advantage to the virus by enabling immune escape.

So, could viral control be achieved by CTLs in HIV-1 infection? In 1994, two studies in humans and a further study in monkeys suggested that $CD8^+$ T cells do not simply play a bystander role in HIV infection, but instead are temporally linked to the control of viremia during the acute phase of infection.

Analyzing five early-stage HIV-1-infected individuals, Richard Koup and colleagues found $CD8^+$ T cell responses to HIV-1 Gag, Pol and Env proteins, including at time points when no neutralizing antibody response to autologous virus was yet detectable. Four of the five patients had measurable CTL responses at early time points after symptom presentation, while the one patient lacking this early response failed to control virus levels.

In a separate group of five patients, Persephone Borrow and colleagues showed that individuals who cleared infectious virus from the plasma within the first few weeks of symptom onset, and maintained virus control for more than a year, had strong $CD8^+$ T cell responses to HIV-1 gp160, Gag and Tat proteins. Patients lacking a strong HIV-1-specific CTL response had reduced ability to clear the virus and sustain control of virus levels. All of the patients lacked HIV-1-specific antibodies at the time of symptom presentation, although they later seroconverted. However, the timing of induction of antibodies after observable $CD8^+$ T cell responses suggested, in both studies, that CTLs are the initial responding cells that exert control over the virus during early infection.

Extending these findings, Jörn Schmitz and colleagues provided causal evidence in rhesus macaques that $CD8^+$ T cells restrict SIV infection. Following virus inoculation in monkeys (and similarly to the trajectory of HIV-1 infection in humans), levels of SIV peak and then rapidly decline by 21 days after infection. When researchers depleted $CD8^+$ T cells during primary acute SIV infection, the animals failed to show this rapid decline in virus levels. In one animal with transient depletion of $CD8^+$ T cells, recovery of SIV-specific CTL responses was coincident with restored control of virus in blood, while in one animal that failed to regain $CD8^+$ T cells viremia was never controlled. The findings provided definitive evidence that $CD8^+$ T cells constrain viremia in the SIV model of HIV infection.

> “$CD8^+$ T cells constrain viremia”

Genetic evidence supporting the critical importance of $CD8^+$ T cells in modulating HIV disease came from a genome-wide association study of HIV-1 controllers—HIV-1-infected individuals who control viral replication long term in the absence of antiretroviral therapy—and HIV-1 progressors. The only single-nucleotide polymorphisms of genome-wide significance that associated with HIV-1 control mapped to the major histocompatibility complex genes, and specifically to a region focused around the class I human leukocyte antigen genes, which restrict $CD8^+$ T cell responses. Collectively, these and other studies demonstrate the crucial role of $CD8^+$ T cells in controlling the outcome of HIV-1 infection—by exerting epitope-targeted immune pressure, CTLs can both constrain HIV-1 and force its evolution, causing loss of viral fitness or enabling viral outgrowth and disease progression.

Alison Farrell, *Nature Medicine*

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MILESTONE 13

# A tap and drain: sinking CD4$^+$ T cells

The hallmark of HIV-1 infection is a progressive reduction in CD4$^+$ T cells, which leads to a general decline in immune function and is the primary factor responsible for the clinical course of disease. Although the discovery of CD4 as a receptor for HIV-1 in the 1980s (MILESTONE 3) could help explain the susceptibility of CD4$^+$ T cells to infection, the mechanisms responsible for their decline remained elusive. In 1995, two seminal studies by the groups of George Shaw and David Ho published in *Nature* provided important insights on the dynamics and pathophysiology of HIV-1 infection, including pivotal observations concerning CD4$^+$ T cell decline.

The advent of new quantitative assays for measuring HIV-1 RNA concentrations (viral load) and experimental drugs that could potently inhibit HIV-1 replication enabled both groups to perform experiments in which the rates of CD4$^+$ T cell and viral turnover could be extrapolated from measurements of changes in plasma viral load and CD4$^+$ T cell counts following antiviral therapy. In both studies, abrupt inhibition of viral replication led to a substantial rise in CD4$^+$ T cell numbers and revealed a scenario in which continuous and highly productive viral replication drove rapid turnover of CD4$^+$ T cells.

The initial stage of HIV-1 infection is followed by an asymptomatic period that can last for years before disease progresses and results in the development of AIDS. Given that this asymptomatic period is accompanied by relatively stable levels of CD4$^+$ T cells and viral load, loss of these cells was initially thought to involve a gradual process of destruction. However, these new findings challenged this view, supporting a model of accelerated CD4$^+$ T cell destruction, which Ho and colleagues likened to a 'tap-and-drain' scenario.

> the idea of drug resistance and immune escape fueled the search for effective antiviral strategies

In this analogy, the destruction of CD4$^+$ T cells (the drain) is counterbalanced by homeostatic production of T cells (the tap) during the asymptomatic period; however, once production of T cells becomes exhausted, this balance is disrupted, resulting in eventual loss of CD4$^+$ T cells (emptying of the sink) and AIDS. Although the mechanisms underlying this imbalance were an area of debate and later evidence pointed to the existence of other (potentially non-mutually exclusive) models of CD4$^+$ T cell depletion (MILESTONE 10), the findings nonetheless had important clinical implications.

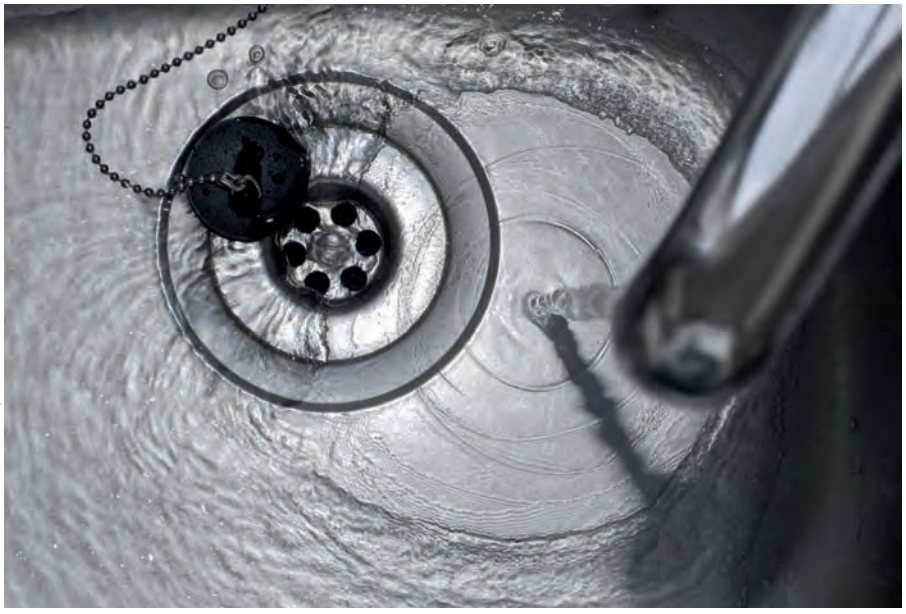
In both studies, evolving resistance to the antiviral drug led to a rise in viral load and a concurrent decrease in CD4$^+$ T cell numbers to pretreatment levels. The previously unrecognized regenerative capacity of CD4$^+$ T cells in HIV-1 infection along with the idea of drug resistance and immune escape fueled the search for effective antiviral strategies.

The findings also raised questions about the utility of CD4$^+$ T cell count as a predictor of long-term survival (at the time, it was the main surrogate marker of disease progression). Just one year later, John Mellors and colleagues linked viral load and HIV prognosis in a paper published in *Science*. By measuring plasma HIV-1 RNA concentrations in a cohort of 180 HIV-seropositive men who were followed for >10 years, they reported that plasma viral load (irrespective of duration of infection) was a better predictor of patient outcome (that is, progression to AIDS or death) than number of CD4$^+$ T cells.

Thomas Quinn and colleagues later revealed that viral load was also a risk factor for viral transmission. Of the factors they measured (including various behavioral and biological risk factors) in a study of 415 couples who were followed for up to 30 months, viral load was the best predictor. Indeed, measurements of viral load are now routinely used for the clinical assessment and monitoring of patients infected with HIV-1, alongside CD4$^+$ T cell count.

These findings spurred the development of antiviral therapies and therapeutic strategies (such as combination therapies) to reduce viral load in individuals infected with HIV-1, with the aim of improving long-term patient outcomes and potentially preventing viral transmission (MILESTONE 14, 20, 21).

Jessica McHugh,
*Nature Reviews Rheumatology*



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MILESTONE 14

# Protease inhibitors give wings to combination therapy

The first antiretroviral therapies (ARTs) for people with HIV were nucleoside reverse-transcriptase inhibitors (NRTIs), but these drugs were only partially effective. The addition of an orally administered protease inhibitor, the first of which was approved in 1995, reduced HIV plasma concentrations and increased $CD4^+$ cell counts to levels that enabled patients to have fairly normal life expectancies. This combination—two nucleoside analogs and a protease inhibitor—is now the cornerstone of ART.

The HIV genome encodes a long polypeptide that must be cleaved into functional proteins by the HIV protease. Following virus uncoating and reverse transcription of the RNA genome, a polypeptide is produced that contains all viral gene products, including the structural proteins and enzymes. The HIV protease then cleaves this polypeptide into its constituent viral proteins. Inhibiting the activity of the protease is therefore an attractive means to prevent virion production.

The first protease inhibitors were peptidomimetic molecules designed to look like the peptide linkages in the precursor polypeptide and therefore compete with the substrate. However, like most peptidomimetic proteins, the early protease inhibitors had poor pharmacokinetic properties, namely, low oral absorption and rapid elimination. Key medicinal chemistry–led structural changes improved these properties. For example, substituting a pyridine with the less electron-rich thiazole to produce ritonavir improved both metabolic stability and aqueous solubility. This molecule was also more potent in animal studies than its parent, predominantly because it also had a lower inhibitory constant ($K_i$).

These drugs wowed the community in early clinical trials. The addition of a protease inhibitor to two NRTIs approximately halved the number of patients whose disease progressed to AIDS or death. In 90% of patients taking the three-drug combination, the number of HIV RNA particles in the blood went from >20,000 particles per milliliter to <500 in 24 weeks.

The first protease inhibitor to be approved by the US Food and Drug Administration (FDA) was saquinavir, in December 1995, a mere 97 days after the FDA received its marketing application. Within months, two other protease inhibitors, ritonavir and indinavir, were also approved. There are currently ten FDA-approved protease inhibitors on the market for HIV.

The remarkable results from the clinical trials of this first wave of protease inhibitors also highlighted important aspects of the biology of HIV infection. First, the clearance rate of virus was independent of initial viral loads and suggested that, on average, half of plasma virions turn over every two days. Second, the number of $CD4^+$ cells destroyed and replenished each day was close to the total number of infected cells. The potential to generate viral diversity (and resulting drug-resistant clones) is therefore substantial, arguing for early initiation of ART.

ART has changed the course of HIV. In the US, mortality among patients with advanced HIV infection declined from 29.4 per 100 person-years in 1995 to 8.8 once ART including a protease inhibitor became the standard of care. In geographic locations with high rates of HIV infection, ART has also changed economics and demographics. Places with high rates of infection, such as Swaziland, saw a 10- to 15-year decrease in life expectancy during the peak of HIV deaths. In the neighboring rural KwaZulu-Natal region of South Africa, where an estimated 29% of adults are HIV positive, adult life expectancy (the mean age to which a 15-year-old could expect to live) increased from 49 to 61 years in the ~10 years after government programs for HIV treatment were initiated. Because many people with HIV were dying during their most economically productive years, the knock-on societal and economic effects are substantial.

Since 1995, new protease inhibitors and combinations with improved dosing have become available, but the protease inhibitors developed in the mid-1990s changed the course of the disease and formed the foundations of ART.

Megan Cully,
*Nature Reviews Drug Discovery*


Credit: PhotoDisc/GettyImages

> “The addition of a protease inhibitor […] halved the number of patients whose disease progressed to AIDS or death”

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MILESTONE 15

# Con-fusin' co-receptors

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Following the discovery of CD4 as the main receptor for HIV-1 in the mid-1980s (MILESTONE 3), it became clear that expression of a *CD4* transgene rendered human cells, but not mouse cells, permissive for infection with HIV-1. There was also a growing awareness that different HIV-1 isolates have different tropisms in vitro for the infection of different human CD4$^+$ cell types. Macrophage-tropic virus strains (which infect primary macrophages and T cells, but not immortalized T cell lines) predominate during the asymptomatic phase of infection, whereas T cell–tropic strains (which infect primary T cells and T cell lines, but not primary macrophages) become more common during progression to AIDS. The viral envelope protein Env, a ligand for CD4, was known to be the main viral determinant of this cell tropism. Together, these observations led to the suggestion that additional human-specific receptors for Env are required for infection, the expression of which determines cell tropism.

In May 1996, Berger and colleagues identified, in an unbiased manner, the first of these co-receptors for HIV-1. They developed a method to study Env–receptor-mediated cell fusion by expressing a phage T7 polymerase in a CD4$^+$ mouse cell line and a reporter gene linked to the T7 promoter in a second, Env-expressing mouse cell line. Expression of the reporter would occur only in the cytoplasm of fused cells. By screening a cDNA plasmid library from HeLa cells for cofactors that would enable fusion of these nonhuman cells, they cloned a G-protein-coupled receptor of unknown ligand and function, but with the greatest homology to the receptor for the chemokine CXCL8. This cofactor was named 'fusin' in the original paper and was renamed later that year as CXCR4 when its ligand was identified as CXCL12. Importantly, fusin was shown to enable entry mediated by Env from T cell–tropic HIV-1 but not macrophage-tropic HIV-1, which led to a race to identify the second cofactor for macrophage tropism.

That the T cell–tropic factor fusin had homology to an α-chemokine receptor fit well with an observation made the previous year by Cocchi et al. that the β-chemokines RANTES (CCL5), MIP-1α (CCL3) and MIP-1β (CCL4) produced by CD8$^+$ T cells inhibit infection with macrophage-tropic HIV-1. Thus, it seemed likely that a β-chemokine receptor was the cofactor for infection of macrophages.

Five papers published within eight days of each other in June 1996 identified CCR5 as the second co-receptor for HIV-1. Another study by Berger's group, using the same fusion assay that had identified fusin, described the role of CCR5 in macrophage infection. Deng et al. showed that CD4 and CCR5 function cooperatively in mouse cells to permit membrane fusion with macrophage-tropic HIV-1. Similarly, Choe et al. described that macrophage-tropic HIV-1 uses CCR5, as well as CCR3, to facilitate infection. In keeping with the switch in viral tropism that accompanies pathogenesis in vivo, Dragic et al. identified CCR5 as a second co-receptor for macrophage-tropic HIV-1 in primary CD4$^+$ T cells, and Doranz et al. showed that a dual-tropic 'intermediate' HIV-1 isolate used both fusin and CCR5.

> "additional human-specific receptors for Env are required for infection, the expression of which determines cell tropism"

Discovery of CXCR4 and CCR5 as co-receptors provided an explanation for the long-standing puzzle of Env-related differences in HIV-1 tropism and opened up the possibility of developing new antiretroviral drug therapies to block infection. Soon thereafter it was recgonized that individual differences in the expression or activity of these co-receptors could underlie susceptibility to infection and disease progression, and this was confirmed by three papers published later in 1996. Liu et al., Samson et al. and Dean et al. described a 32-base-pair deletion in the coding region of *CCR5* that was variously shown to protect homozygotes from infection, partially protect heterozygotes from infection and delay disease progression in heterozygotes. The lack of an obvious phenotype associated with the mutation, together with the later description of the Berlin patient (MILESTONE 18), gave hope that pharmacological or genetic targeting of CCR5 could be a safe and effective therapeutic approach.

Kirsty Minton,
*Nature Reviews Immunology*

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MILESTONE 16

# Latent inducible HIV-1 in T cells

The advent of combination therapy for HIV treatment in the mid-1990s had a huge impact on patient morbidity and mortality (MILESTONE 14). Together, reverse-transcriptase inhibitors and newly developed protease inhibitors caused plasma virus to fall to undetectable levels within 2–4 months. It was presumed that integration of HIV-1 DNA into the host genome could enable persistence of the virus, and indeed, a 1995 paper by Chun et al. had detected integrated proviruses in some infected patients. These proviruses were found in resting CD4$^+$ T cells, which did not produce virus unless activated. But in 1995 it was not clear how much of an obstacle this potential latent reservoir of integrated proviruses would be to ultimate eradication of the virus by the new combination therapy.

A 1997 paper from the group of David Ho described two phases of viral decline in infected patients treated with combination therapy: a first phase in which virus levels dropped by around 99% within the first two weeks of treatment and a second phase of slower decline, which the authors concluded was driven by loss of long-lived infected cells. Extrapolating from these decay characteristics, they predicted that around 2–3 years of effective treatment would be required to eradicate HIV-1, perhaps longer if the virus persisted in sanctuary sites. Sadly, this estimate proved too optimistic.

Papers from the groups of Robert Siliciano and Douglas Richman, published in 1997, began to characterize and quantify the latent reservoir of HIV-1 virus in patients. Chun et al. provided the first snapshot of the latent reservoir of virus, in a group of 14 asymptomatic HIV-1-infected donors. Looking in the lymph nodes, the authors found that around 0.5% of resting CD4$^+$ T cells harbored HIV-1 DNA, but that less than 0.05% of resting cells contained integrated provirus. The relatively small size of this latent reservoir was a surprise, but the authors cautioned against underestimating its importance, due to the long survival of memory CD4$^+$ T cells. In work published later that year, Finzi et al. looked at 22 patients successfully treated with drugs for up to 30 months and reported the sobering finding that, despite apparently complete suppression of virus replication, highly purified resting CD4$^+$ T cells from each of these individuals could be induced to make replicating virus. Furthermore, levels of inducible replication-competent virus did not decline with increasing time on therapy and the inducible viruses had not acquired mutations conferring drug resistance, suggesting that they were derived from long-lived cells that were infected before the initiation of therapy. Similar findings were also reported in the same issue of *Science* by Wong et al. and in *PNAS* by Chun et al.

> “the latent reservoir of HIV-1 is a formidable obstacle to eradication of the virus”

Today, we know that the latent reservoir of HIV-1 is a formidable obstacle to eradication of the virus. We know that the reservoir is maintained at least in part by clonal expansion of CD4$^+$ T cells containing integrated provirus and that it is hard to measure, as the vast majority of integrated proviruses are defective. The reservoir also extends beyond quiescent cells in the blood and lymph nodes, to sanctuary sites such as the brain that are impenetrable to antiretroviral drugs. Nevertheless, achieving at least a functional cure of HIV infection remains a hotly pursued goal and there is intense interest in learning more about the HIV reservoir and how to prevent the virus from rebounding so that medication might be stopped.

Clare Thomas, *Nature*

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MILESTONE 17

# A battle between HIV and host

Numerous positively acting cellular factors and pathways support HIV replication, but in the 1990s evidence emerged that suggested that cells express dominantly acting factors that suppress HIV replication. For instance, HIV replication is affected by the animal origin of target cells and requirements for the viral accessory genes *vif* and *vpu* vary significantly between human cell lines. These observations hinted that primate cells express antiviral proteins (termed restriction factors) that block infection. The existence of restriction factors has important implications for understanding viral replication and pathogenesis, host range and HIV evolution, and for developing animal models.

In 2002, Sheehy et al. reported the isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein—the first identification of an HIV restriction factor. In this initial report, a subtracted cDNA screen using cells that were permissive and non-permissive to infection by *vif*-deficient HIV-1 identified the human *APOBEC3G* gene as responsible for suppression of *vif*-deficient HIV-1 infection. Subsequent studies determined that APOBEC3G can restrict HIV-1 by incorporating itself into nascent virions through its RNA-binding activity and subsequently hypermutating newly synthesized viral DNA through its cytidine deaminase activity, leading to a catastrophically altered nucleotide sequence. It was also found that Vif–APOBEC3G binding leads to degradation of the restricting factor, enabling HIV-1 to circumvent this intrinsic immune response.

This initial study represents an important milestone in HIV/AIDS research, as it revealed an integral part of the host's first line of defense against HIV and suggested that further HIV restriction factors might exist.

APOBEC3 proteins do not alone account for the observed infection restriction phenotype in non-permissive cells. Since the discovery of APOBEC3G, numerous restriction factors that target diverse components of HIV-1, HIV-2 and SIV during various stages of their life cycles have been identified. For instance, identification of the monkey TRIM5α and TRIMCyp proteins in 2004 that restrict HIV through interactions with the viral capsid revealed that this class of molecules is responsible for the majority of restriction phenomena in mammalian cells following virus entry. Later, the discovery of tetherin (also known as BST2), a transmembrane protein, revealed a crucial function of the lentiviral *vpu* gene. In the absence of *vpu* expression, tetherin physically tethers nascent virions to the surface of infected cells and the virions are subsequently internalized into endosomes, thus preventing onward transmission.

Similarly, an important function of the HIV-2 (and SIV) Vpx accessory protein was elucidated through the discovery of SAMHD1, a deoxynucleotide triphosphohydrolase that limits reverse transcription of incoming viral RNA genomes: the viral Vpx protein induces ubiquitin–proteasome-dependent degradation of SAMHD1.

More recently, further restriction factors with distinct or unknown mechanisms of antiviral activity have been implicated as having a role in the outcome of initial HIV infections (for example, Mx2, SERINC3 and SERINC5, and ZAP), highlighting the important and complex involvement of restriction factors in the life cycle of HIV and in the evolutionary battle between host and virus. Indeed, a major raison d'être for lentiviral accessory proteins is to remove or displace host antiviral proteins.

During this evolutionary battle, humans have not emerged as the victors, as HIV-related illnesses cause thousands of deaths each year. HIV has an extraordinary degree of genetic plasticity that has enabled the virus to adapt to new host proteins when crossing species barriers and during the evolutionary arms race. Despite HIV's ability to evade host restriction factors, the discovery of these factors and understanding of how they interface with viral accessory proteins provide remarkable insight into the evolution of HIV. Their discovery also provides targets for new antivirals and valuable knowledge in the development of primate animal models for HIV/AIDS, which may lead to HIV losing the battle.

> “The existence of restriction factors has important implications for understanding viral replication and pathogenesis, host range and HIV evolution, and for developing animal models”

Ashley York,
*Nature Reviews Microbiology*

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Timothy Ray Brown—the only person known to have been cured of HIV. Credit: Bloomberg/Rob Waters via Getty Images

MILESTONE 18

# Editing a cure

Timothy Ray Brown—also known as the 'Berlin patient'—is the only person ever to be cured of HIV. His story and the research that followed are intimately linked to the discovery, back in 1996, that homozygosity for a CCR5 allele with a 32-base-pair deletion (delta32/delta32) renders some individuals resistant to infection despite exposure through sexual intercourse with HIV-positive partners (MILESTONE 15).

Brown was diagnosed with HIV in 1995 and a decade later underwent allogeneic hematopoietic stem cell transplantation (HSCT) for relapsed acute myeloid leukemia. But the transplant, performed by Gero Hütter of Charité–Universitätsmedizin Berlin, had a twist. Hütter used peripheral blood stem cells from a human leukocyte antigen (HLA)-identical donor that harbored the *CCR5* delta32 allele.

The procedure led to complete remission of the cancer and, remarkably, despite the long-lived viral reservoir (MILESTONE 11, 16) being expected to lead to HIV rebound and disease progression during the process of immune reconstitution, no active virus and no viral RNA or proviral DNA could be detected in the blood, bone marrow or rectal mucosa, even almost two years after transplantation and interruption of antiretroviral therapy (ART).

Although at the time of transplant Brown also carried HIV variants tropic for the CXCR4 chemokine receptor, Hütter reasoned that their numbers might have been too low to allow reseeding of the new immune system. A follow-up study showed that Brown's long-lived $CD4^+$ HIV target cells had been successfully replaced with donor-derived cells. Thus, the *CCR5* deletion in donor stem cells and their ability to engraft—killing Brown's infected cells—may have together contributed to the functional cure of HIV.

Yet, a similar approach subsequently performed in the so-called 'Boston patients' proved the latent reservoir of HIV to be far more resilient than previously thought. Timothy Henrich and Daniel Kuritzkes at Brigham and Women's Hospital in Boston gave HSCT to two HIV-infected men diagnosed with lymphoma, but used donor cells with wild-type *CCR5*. In this approach, despite a significant reduction in the size of the reservoir following transplant, both patients experienced rebound viremia shortly after cessation of ART.

Still, these landmark studies demonstrated the critical role that CCR5 has in maintaining HIV-1 infection and prompted further research into gene-based therapies to target HIV.

Although genome editing had been proven efficient in rendering T cells refractory to HIV infection, it was not until years later, in 2014, that a team led by Pablo Tebas and Carl June at the University of Pennsylvania in Philadelphia showed this could be done in infected individuals. The clinical trial enrolled 12 patients who were infused with autologous $CD4^+$ T cells modified at the *CCR5* locus by zinc-finger nucleases (ZFNs). The patients who stopped ART after cell transfusion exhibited slow viral rebound and proliferation of the modified T cells, indicating enhanced control of the virus. Moreover, one study participant with no viral rebound during ART interruption was found to harbor a single mutated copy of *CCR5* and after transfusion a large proportion of this patient's T cells were resistant to HIV.

Together, these findings demonstrated that gene-targeting approaches could provide a safer and more practical approach than the risky and restrictive HSCT and opened the door for ZFNs and other gene-editing methods, such as TALENs and CRISPR, to be exploited in the targeting of latently infected cells.

Although many challenges remain, these groundbreaking discoveries, together with those on early ART initiation (MILESTONE 20, 21), broadly neutralizing antibodies (MILESTONE 19) and new-generation latency-reversing agents, are paving the way toward the development of a broad-scale and safe strategy for the complete eradication of HIV or its control in the absence of lifelong therapy.

Javier Martinez-Vesga,
*Nature Communications*

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MILESTONE 19

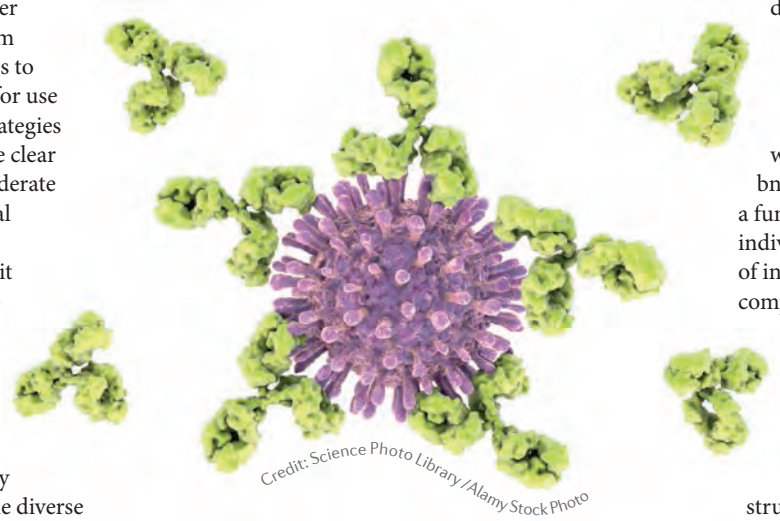
# Needles in a haystack: the quest for bnAbs

HIV induces antibody responses in infected individuals, but only a few of these individuals manage to produce antibodies that are capable of viral neutralization—and even fewer produce antibodies that can neutralize different strains of HIV. First attempts to find such broadly neutralizing antibodies (bnAbs) date back to the early 1990s, when phage libraries were used to identify, isolate and amplify antibodies from asymptomatic individuals with HIV-1 infection. Further antibodies were isolated from hybridomas. However, hopes to translate these early bnAbs for use in passive immunization strategies were dashed when it became clear that they displayed only moderate breadth and potency for viral neutralization.

Breakthroughs had to wait for almost 20 years and were eventually facilitated by the development of single-cell antibody cloning, advances in screening methods and a better understanding of structurally conserved epitopes across the diverse circulating strains of HIV-1.

In 2009, Dennis Burton and co-workers used a systematic approach to search for bnAbs in the sera of 1,800 HIV-1-infected individuals. This was followed by a high-throughput neutralization screen of activated memory B cells from one individual. The effort proved worth it—they identified two bnAbs (PG9 and PG16) with remarkable potency and breadth, neutralizing 73% and 79% of viruses tested, respectively. Interestingly, the two antibodies targeted a previously undescribed epitope of the HIV-1 envelope (Env) protein, which is located within conserved regions of the variable loops of the gp120 subunit of Env.

The discovery of these antibodies was followed in 2010 by a report by Gary Nabel, John Mascola and co-workers that described the rational design of probes for the targeted identification of bnAbs. At this time, a conserved site on gp120, which facilitates binding of the virus to the host receptor CD4, had been identified as a common target for naturally occurring bnAbs. With new insights into Env structure and using computer-assisted protein design, antigenically resurfaced glycoproteins were designed that specifically bound to neutralizing antibodies. These were used to screen sera for the presence of bnAbs, and then to screen for probe-specific memory B cells. These efforts led to the identification of two antibodies, VRC01 and VRC02, that neutralize over 90% of all major circulating HIV-1 strains.



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An accompanying paper showed that VRC01 had undergone extensive affinity maturation, resulting in an antibody that partially mimics the interaction of CD4 with gp120. A slight shift in binding allows it to overcome the glycan and conformational masking that diminish the neutralization capacity of other antibodies.

> “combinations of bnAbs provide durable control…”

Since then, many more bnAbs have been identified and the first clinical studies have been initiated. VRC01 and 3BNC117, a bnAb that targets a similar site, first entered the clinic in 2015–2016. They suppressed viral titers in HIV-infected individuals for 6–10 weeks, before viral rebound due to escape mutants. Much longer viral suppression was achieved in recent combination trials. In patients undergoing treatment interruption from antiretroviral therapy (ART), three doses of 3BNC117 and 10-1074, a bnAb that targets a different site in Env, achieved a median of 21 weeks of complete viral suppression before viral rebound. Encouragingly, no viral escape mutants were detected. This indicates that combinations of bnAbs provide durable control in the absence of ART and therefore provide an alternative, less toxic treatment.

Preferable to passive treatment would be a vaccine that elicits bnAbs. This could allow for a functional cure of infected individuals and protect those at risk of infection. Given the rarity and complexity of bnAbs (they typically contain 40–100 somatic mutations and unusual structural features), making such a vaccine is exceptionally challenging. However, insights into their development, structure and function, as well as the immunological mechanisms that make their generation such a rare event, have led to the design of promising vaccination strategies in animal models.

Alexandra Flemming,
*Nature Reviews Immunology*

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MILESTONE 20

# Antiretrovirals for prevention

Since the first reports identifying patients with AIDS in 1981 (MILESTONE 1) and the discovery of HIV as the etiological agent in 1983 (MILESTONE 2), a major goal has been to find and develop effective anti-HIV therapeutic options. This yielded results in 1987 with the approval of the first antiretroviral therapy, azidothymidine (AZT), only a year after it was first administered and shown to reduce HIV viral load in patients. Further antiretroviral drugs targeting other aspects of the retroviral lifecycle were discovered and resulted in combinations of drug classes associated with successful and sustained prevention of AIDS progression (MILESTONE 14).

The advent and widespread use of combinatorial antiretroviral therapy resulted in successful virological control in HIV-infected patients. However, this did not address another key tenet of HIV medicine: the prevention of viral transmission. For most viruses, lower viral loads are known to minimize the chance of transmission, and it is on the basis of this that the initial premise of antiretrovirals for prevention came to fruition. Proof of concept for antiretrovirals as prevention was first shown in 1994, where administration of AZT reduced mother-to-child transmission of HIV. Since this first clinical demonstration, antiretrovirals as prevention have gathered momentum, which has resulted in pivotal strides forward.

On the basis of these initial findings showing reduced rates of transmission by minimizing viral loads in HIV-seropositive patients, two key studies demonstrated a crucial role for such applications of early antiretroviral intervention. Initiation of early antiretroviral therapy and reduction in viral load in confirmed HIV-positive patients was shown to successfully reduce sexual transmission in serodiscordant couples, establishing the potential benefits of early commencement of therapy and the associated reduction in HIV transmission.

Complementary approaches utilizing antiretroviral therapy in HIV-seronegative persons have also shown efficacy in terms of preventing infection in at-risk populations. Two large-scale, randomized, double-blind, placebo-controlled studies paved the way toward the advent of pre-exposure prophylaxis (PrEP). The iPrEx trial assessed the administration of the anti-retrovirals emtricitabine plus tenofovir disoproxil fumarate (Truvada) in 2,499 high-risk HIV-negative men or transgender women who have sex with men. The Partners PrEP trial evaluated administration of Truvada or tenofovir disoproxil fumarate monotherapy among 4,758 HIV-serodiscordant heterosexual couples. PrEP administration to seronegative individuals in both settings was estimated as conferring greater than 90% protection from HIV infection in persons with good adherence to the PrEP regimen.

> “these pivotal studies… firmly establish the importance of antiretrovirals for preventing HIV transmission”

Consequently, in 2012, the US Food and Drug Administration approved the use of Truvada as PrEP for HIV on the basis of the initial data from these studies, which established that its use was well tolerated, safe and effective in reducing HIV transmission in high-risk individuals. Although the implementation of antiretrovirals for prevention in both HIV-positive and HIV-negative persons is still in its relative infancy and the further impact of such interventions remains to be fully determined, collectively these pivotal studies transform the risk of HIV transmission and firmly establish the importance of antiretrovirals for prevention as a potential game changer in preventing HIV transmission and turning the tides of the HIV epidemic.

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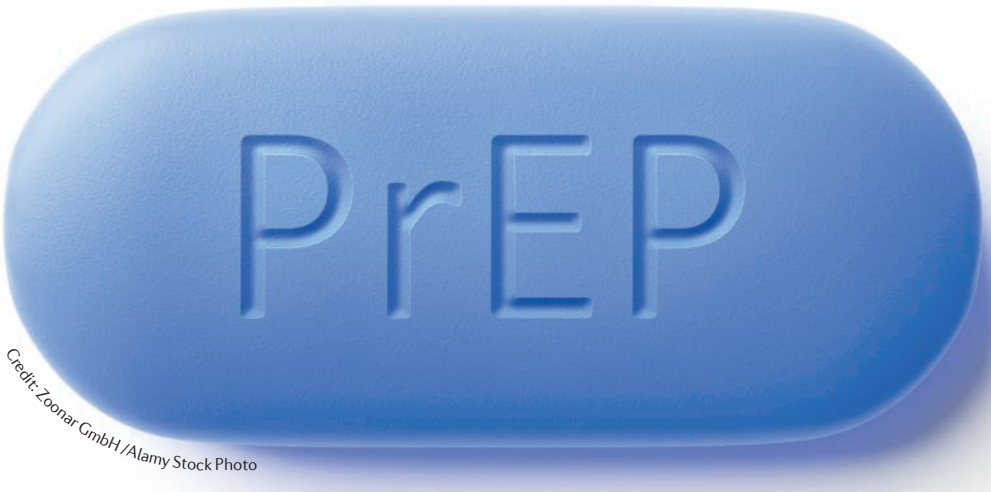


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MILESTONE 21

# STARTing treatment immediately

In August 2015, the INSIGHT START study group published the results of the START trial investigating the timing of initiation of antiretroviral therapy (ART) in patients with asymptomatic HIV infection. The trial results showed that immediately initiating ART in adults who were positive for HIV and had CD4$^+$ T cell counts of >500 cells/mm$^3$ was more beneficial than deferring treatment until their CD4$^+$ T cell counts dropped to ≤350 cells/mm$^3$.

For years, when to start ART had been a topic of debate, and initially patients at a high risk of developing AIDS (those with low CD4$^+$ T cell counts of ≤200 cells/mm$^3$) were prioritized for treatment. However, evidence emerged of the benefits of initiating treatment early. In 2009, the results of a cohort study suggested that initiation of ART before CD4$^+$ T cell counts fell below 351 cells/mm$^3$ or 500 cells/mm$^3$ improved survival. Interim analysis of HPTN 052 study data in 2011 showed that starting ART in patients with CD4$^+$ T cell counts between 350 and 550 cells/mm$^3$ before symptoms manifested or CD4$^+$ T cell counts were ≤250 cells/mm$^3$ reduced the rate of HIV transmission and clinical events.

The case of the 'Mississippi baby' also provided some evidence for the benefit of early ART initiation. This baby was given ART 30 hours after being born to an HIV-positive woman and tested positive for HIV herself. Treatment was continued until she was 18 months old, and she had undetectable levels of HIV between the ages of 29 days and 3 years, 10 months. Although HIV became detectable after treatment was stopped, these observations suggested that starting ART early is beneficial in controlling HIV. As new data such as these became available, the cutoff for starting ART progressively increased, eventually reaching ≤500 CD4$^+$ T cells/mm$^3$.

In this context, the START study aimed to assess the benefits and risks of immediately initiating or deferring ART. This study was conducted at 215 centers in 35 countries, included 4,685 patients with CD4$^+$ T cell counts of >500 cells/mm$^3$ and the mean follow-up duration was three years. Patients in the immediate-treatment arm received ART straight away, whereas those in the deferred-treatment arm were not given ART until their CD4$^+$ T cell counts decreased to ≤350 cells/mm$^3$. Overall, 41 events (including death, AIDS and serious non-AIDS events) occurred in the immediate-treatment group compared with 86 events in the deferred-treatment group—a 57% reduction. Furthermore, no increase in the rate of adverse events was observed in the immediate-treatment group.

The original completion date for the START study was 2018. However, after the interim analysis in May 2015, the data and safety monitoring board decided that the study question had been answered and recommended that all patients in the deferred-treatment arm be offered ART.

The results of the START study contributed to the World Health Organization (WHO) issuing a recommendation to treat all patients as soon as possible after diagnosis (the treat-all policy, also known as test and treat) in September 2015. The results are also cited as supporting evidence in the WHO's consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, which were published in 2016. These guidelines contain the new recommendation that "ART should be initiated in all adults living with HIV, regardless of WHO clinical stage and at any CD4$^+$ cell count."

> "The results of the START study contributed to the World Health Organization (WHO) issuing a recommendation to treat all patients as soon as possible after diagnosis"

Starting ART early can preserve intestinal lymphoid structures and dendritic cell maturation pathways in the gut. Early initiation of treatment can also help to maintain HIV-1-reactive memory B cells in the gut and follicular T helper cells. Furthermore, early ART reduces the size of the HIV reservoir in the long term compared with deferring treatment.

In 2017, analysis of self-assessed quality-of-life (QOL) outcomes from patients involved in the START study showed that immediate ART resulted in better outcomes than deferred ART after a mean follow-up duration of three years. QOL outcomes were improved for those receiving immediate treatment regardless of demographic or clinical subgroup. Later the same year, the WHO published analysis of the adoption and implementation status of the treat-all policy. As of November 2017, adoption rates were promising, with 70% of low- to middle-income countries and 89% of countries in the Fast-Track strategy for ending AIDS signing up to the treat-all policy. These adoption rates support the 90–90–90 targets for ending the AIDS epidemic by 2020.

Louise Stone,
*Nature Reviews Urology*

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