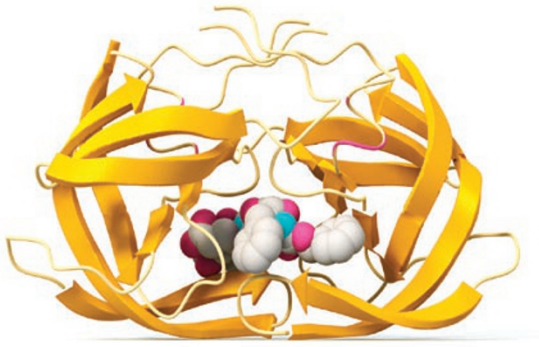
The outlook for a cure

There is a formidable arsenal of drugs available to treat HIV. **Virginia Hughes** finds that, for the first time in years, there is also renewed hope of a cure.



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Protease inhibitor drugs (spheres) bind to a viral enzyme (yellow) and prevent HIV particles from maturing.

Not long ago, you would have been hard-pressed to find an HIV researcher who would utter the word 'cure'.

HIV has a remarkable ability to resist antiviral drugs and hide in the body, so the idea of eradicating the virus seemed impossible. Suggesting otherwise, researchers feared, could create false hope and complacency.

In the past few years, however, there are increasingly loud whispers about a cure for HIV. The year 2007 saw the clinical debut of integrase inhibitors, which prevent HIV from inserting into the host genome. The following year, a bone-marrow transplant eliminated the virus from the body of an infected German man (see sidebar, Interfering with genes). Last year, breakthroughs in cell-culture techniques allowed researchers to screen for drugs that can lure HIV from its hiding places.

"I was very pessimistic five years ago, but we had to try," says Warner Greene, director of the Gladstone Institute of Virology and Immunology in San Francisco. "And as we've tried, I've become much more optimistic that we might be able to achieve a drug-free remission."

His optimism is understandably tinged with caution, however, as scientists promising eradication were proven wrong once before.

In the summer of 1996, researchers attending the eleventh international AIDS meeting in Vancouver trumpeted data showing that certain combinations of antiretroviral drugs can suppress HIV to undetectable levels in the blood.

The buzz grew over the course of the following year. In May 1997, David Ho's group at the Aaron Diamond AIDS Research Institute in New York reported in *Nature* that HIV levels in eight people dropped by two orders of magnitude within ten days of receiving a particular three-drug combination and were undetectable within eight weeks[1].

Using a mathematical model, the researchers estimated that, barring any complications, the drugs could eradicate the virus from an infected person in less than three years.

If that sounded too good to be true, it was. In the same issue of the journal, Bob Siliciano's group from Johns Hopkins University inspected the small number of dormant immune cells that harbour HIV. Because these cells do not replicate, they are impervious to treatment. Once activated, however, his team found that these cells can start pumping out the virus into the blood and lymph nodes[2].

Two other groups described these so-called latent reservoirs in 1997, and two years later Siliciano's team estimated that it would take about 60 years for drugs to flush out HIV from these stores[3]. "It was a real blow, I think, to people who were interested in eradication," Siliciano says.

The field instead shifted focus to preventing resistance by using combinations of three or more drugs — dubbed highly active antiretroviral therapy (HAART) — and to decreasing the side effects of treatment.

These efforts were hugely successful: there are 32 approved HIV drugs, and at least a dozen more in the pipeline (Table 1), which, together, can suppress the virus for decades.

"Now that HAART works so well," Siliciano says, "we're turning to the next step: can we actually cure anybody?"

"It's marvellous that now we have very effective inhibitors for all the three HIV enzymes — it will be very hard for the virus to escape them all."

In the pipeline

Like most viruses, HIV hijacks its host's cellular machinery to replicate. HIV is a member of a particularly nefarious family of viruses, however, which insert their DNA into the host genome. When the host cell replicates, its daughters make more virus.

Antiretroviral drugs target different stages of this replication cycle, limiting the viral load and allowing immune cells a chance to clear out infected cells. The most widely used drugs block the enzymes that HIV needs to infect new cells. The standard regimen includes two drugs that block reverse transcriptase, which HIV needs to convert its RNA genome into DNA, and one protease inhibitor that prevents viral particles from maturing.

In 2007, Merck released raltegravir. This was the first drug to target HIV's integrase enzyme, which stitches the viral DNA into the host genome.

Clinicians welcomed drug cocktails spiked with raltegravir after large clinical trials showed they trounce drug-resistant strains of HIV, and suppress virus levels significantly faster than do standard combinations. Several other integrase inhibitors are in the pipeline, and early data suggest that they are better than raltegravir.

"The number of papers on HIV integrase inhibitors has just boomed over the last two years," notes Yves Pommier, chief of the Laboratory of Molecular Pharmacology at the US National Cancer Institute. "It's marvellous that now we have very effective inhibitors for all the three HIV enzymes — it will be very hard for the virus to escape them all."

Other HIV proteins might also make good targets. In 2002, Michael Malim and colleagues fired up the field with the discovery that one of these proteins, Vif, degrades a human enzyme, APOBEC3G, which evolved eons ago to damage viral DNA[4]. Without Vif, APOBEC3G would block HIV replication.

That basic research is starting to pay off, and many scientists are hunting for drugs that block Vif. In 2008, Greene's group at the Gladstone Institute launched a collaboration with Gilead Sciences to find Vif inhibitors.

Another tactic is to target the host cell, rather than the virus. HIV primarily attacks CD4 T cells, which normally help the immune system fend off invaders. A handful of new compounds block CCR5, which is a receptor on the surface of CD4 cells that HIV must bind in order to penetrate the cell.

In 2007, Pfizer debuted the first CCR5 blocker, maraviroc, which is effective at keeping

Interfering with genes

In 2008, doctors in Germany made an astounding announcement: they had wiped out HIV from a middle-aged man when they gave him a bone-marrow transplant to treat his leukaemia.

The doctors had replaced the bone marrow with that of a donor who carried a genetic variation that disrupts the function of the CCR5 receptor in T cells. When this molecular doorway is broken, HIV cannot enter host cells. To this day, despite having stopped all antiretroviral therapy, the man has no detectable HIV in his blood.

Bone-marrow transplants are not a practical option for treating HIV infection: upwards of one in three people who receive them die, and they cost at least US$150,000. Still, the case offers hope for scientists using gene therapy to tinker with *CCR5*, and perhaps other genes involved in HIV replication.

For example, RNA interference, which is a method of silencing genes, has been shown to prevent the virus from replicating and mutating in cultured T cells. Researchers have also launched several clinical trials using methods to modify the *CCR5* gene in host cells.

Carl June and his colleagues at the University of Pennsylvania are harvesting T cells from HIV-infected individuals and disrupting the *CCR5* gene using zinc fingers — protein components that recognize specific DNA sequences and turn genes on or off. Preliminary results from the 18-person trial are expected in March 2011.

At a conference in January this year, June reported that the gene therapy has allowed one participant who is off antiretroviral treatment to maintain undetectable levels of virus for two weeks longer than is typical.

Last year, the first randomized and placebo-controlled test of a gene therapy for HIV showed that a technique that uses ribozymes — RNAs that cut other kinds of RNA — to disrupt HIV genes is safe, but does not reduce viral load.

Although these methods have not yet been proven effective, the potential benefits have encouraged researchers.

"Drugs have to be taken every day, but gene therapy could do the same thing in a one-time treatment," notes Ben Berkhout, professor of virology at the University of Amsterdam. –*V.H.*

Table 1 | New HIV drugs

Drug	Type	Manufacturer	Status
Rilpivirine	NNRTI	Tibotec	Phase III
UK-453061	NNRTI	ViiV Healthcare	Phase II
IDX889	NNRTI	ViiV Healthcare	Phase II
Apricitabine	NRTI	Avexa	Phase III
Amdoxovir	NRTI	RFS Pharma	Phase II
Maraviroc	CCR5 antagonist	ViiV Healthcare	Approved in 2007; marketed as Selzentry/Celsentri
Vicriviroc	CCR5 antagonist	Schering-Plough	Phase III
PF-232798	CCR5 antagonist	ViiV Healthcare	Phase II
PRO 140	CCR5 inhibitor (monoclonal antibody)	Progenics Pharmaceuticals	Phase II
Raltegravir	INI	Merck & Co., Inc.	Approved in 2007; marketed as Isentress
Elvitegravir	INI	Gilead Sciences	Phase III
GSK1348572	INI	ViiV Healthcare/Shionogi	Phase II
GSK1265744	INI	ViiV Healthcare/Shionogi	Phase II
Bevirimat	Maturation inhibitor	Myriad Pharmaceuticals	Phase II
Ibalizumab	Entry inhibitor (monoclonal antibody)	TaiMed Biologics	Phase II
Unknown	Vpu inhibitors	Biotron	Preclinical stage
Unknown	Vif inhibitors	Gilead Sciences/ Gladstone Institute	Preclinical stage
Truvada	Combination pill (two reverse transcriptase inhibitors)	Gilead Sciences	Approved in 2004
Atripla	Combination pill (Truvada + reverse transcriptase inhibitor)	Gilead Sciences/Bristol-Myers Squibb	Approved in 2006
'Quad' pill	Combination pill (Truvada + elvitegravir + boosting agent)	Gilead Sciences	Phase III

INI, integrase inhibitor; NNRTI, non-nucleoside reverse transcriptase analogue; NRTI, nucleoside reverse transcriptase analogue.

viral levels in check. However, people who have been on HAART for long periods tend to carry HIV strains that use the CXCR4 receptor instead of CCR5. Drugs that block CXCR4 are also being developed, but animal studies suggest the drugs are toxic.

Daily dose

No matter how effective the available drugs, the harsh reality is that they must be taken for life — although when to start them is an equivocal point (see sidebar, Perfect timing). If treatment is interrupted, HIV rapidly springs back into action, rising to detectable levels within weeks.

Partly to make taking the medications more palatable, and partly to keep the HIV drug arsenal stocked with new compounds, many companies have invested in making pills that combine multiple drugs.

"Taking one pill a day is much easier than taking two or three," says Michael Mullen, acting chief of infectious diseases at Mount Sinai Medical Center in New York. "Pharma is realizing that the most important challenge in antiretroviral therapy is improving adherence by simplifying regimens."

So far, only the commercially available pill Atripla — one of the most widely prescribed HIV drugs in the United States — combines drugs from different classes. More are on the way, however.

Last year, GlaxoSmithKline and Pfizer together launched ViiV Healthcare, a company that will focus on creating new antiretroviral medicines, particularly combination pills. In April, Gilead Sciences began phase III testing of its 'Quad' pill, which combines two reverse transcriptase inhibitors and one integrase inhibitor.

These combinations might make taking the pills less cumbersome, but they will not be able to alleviate the harsh side effects. Depending on when therapy is begun, the lifespan for those on HAART is 10 to 30 years shorter than average. They also suffer from a host of conditions including heart, kidney, liver and bone disease, cancers and serious cognitive problems.

Some studies suggest that these complications are more the result of a sustained inflammatory response to the virus than of drug toxicity. In either case, the chronic problems are a natural consequence of long-term infection.

The ideal HIV drugs would not just suppress the virus, but would eliminate it from the body — something that is just beginning to look feasible.

ROBERTO BOREA/AP PHOTO

Although antiretroviral drugs are effective, they must be taken for life, and can cause harsh side effects.

Elusive target

To eradicate the virus from the body, scientists must first pinpoint its hiding places. HIV is particularly good at staying invisible. In 1997, three teams independently discovered one HIV reservoir: resting memory T cells. These cells stay quiet for decades, and are activated only when the immune system encounters an invader that it has seen before.

An HIV-infected individual carries about one million infected resting memory cells. However, while in this state, these cells are invisible to the immune system. The only way to destroy the reservoir, scientists reason, is to stimulate the cells to begin making virus, thereby rendering them vulnerable to antiretroviral drugs.

In the late 1990s, researchers used agents such as interleukin-2 growth factor, which activates all T cells, to prod the cells out of their resting state. Provoking a global immune response is dangerous, however, and can trigger massive leakage of fluids into tissues.

One alternative is to tickle the latent cells so that they express HIV proteins without replicating themselves. For example, inhibitors of histone deacetylases — enzymes that suppress HIV transcription — could stimulate latent cells to produce HIV.

Screening for such drugs is technically challenging because resting T cells tend to die in culture. In the past year, however, several groups have genetically or chemically engineered the cells so that they can survive longer in culture, allowing scientists to screen for drugs that target the viral reservoirs.

"It's actually fairly easy to find compounds that turn on latent HIV without causing global T-cell activation," says Siliciano. "We've already found several." Although the compounds identified so far are probably too toxic for use in people, he notes, it is encouraging that the technique works on the small scale.

There might also be many other reservoirs in which HIV replicates at low levels. These could be blood stem cells, other immune cells such as macrophages, or inaccessible caves such as the brain or the gastrointestinal tract.

"If we're going to come up with an eradication strategy, it's not going to be just as simple as purging the virus from T cells," says Mario Stevenson, professor of molecular medicine at the University of Massachusetts.

Last year, several leading researchers called for a large collaboration involving academia, industry and the government to identify HIV's hiding places and investigate ways to drag the virus out of them[5] (see page S21).

The ultimate test for any treatment that attempts to clear out the reservoirs is to take patients off therapy and see whether their viral loads stay in check — which some deem unethical.

Even the most enthusiastic researchers admit that the field is far from understanding latency, and is at least a decade away from producing compounds that could clear out the viral reservoirs. If history is any guide, however, they will not stop trying.

"Scientists are stubborn," Stevenson says. "That persistence on the part of the scientific community hopefully exceeds the persistent qualities of the virus." ■

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Perfect timing

How early should someone infected with HIV begin treatment? Doctors in the developed world recommend drugs when the number of an infected individual's CD4 immune cells falls to less than 350 per cubic millimetre of blood. Many doctors in the United States say treatment should begin as soon as HIV infection is diagnosed, even if CD4 counts are normal.

"This very aggressive approach is new, and it's based on non-definitive data," says Steven Deeks, professor of medicine at the University of California, San Francisco.

Still, Deeks supports early intervention because research has shown that lower CD4 levels pre-therapy lead to more age-related problems. "The longer you wait, the more inflammation, the more immunologic dysfunction and perhaps more of these diseases will occur," he says.

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In April 2009, a report in the *New England Journal of Medicine* found that beginning therapy when CD4 levels are less than 350 cells per cubic millimetre of blood carries a 69% higher risk of death compared with levels of 351 to 500 cells per cubic millimetre.

This was an observational study, however, so researchers could not determine whether certain characteristics of people who get early treatment — such as better drug-adherence rates or less recreational drug use — explain why they fare better.

To resolve the debate, an international research team last year launched a randomized, prospective clinical trial, called the Strategic Timing of Antiretroviral Treatment, which is projected to run until 2015.

Even if early treatment is beneficial, cautions Gregg Gonsalves, an AIDS activist who has been on antiretroviral therapy for 15 years, "actual clinical practice may play out entirely differently, in terms of people's ability to stay adherent to medications and deal with side effects." –*V.H.*