Recent research developments along several fronts permit some degree of optimism.

The fourth annual Caltech Biology Forum, on October 8, focused on the latest developments and challenges in AIDS research. This article is adapted from the remarks of three of the forum’s speakers. They were joined by Brenda R. Freiberg, vice president and treasurer of the Foundation for AIDS and Immune Research and chair of the Public Policy and Planning Committee of the AIDS Service Center; and by moderator Sandra L. Thurman, director of the Office of National AIDS Policy and member of the Presidential Advisory Council on HIV/AIDS. Sponsors of the event included Glaxo Wellcome Inc., Agouron Pharmaceuticals, Inc., Huntington Hospital, the Pasadena Star-News, and the AIDS Service Center. Videotapes of the forum may be ordered, at a cost of $29.95, by calling 626/395-4652 or 888/2-CALTECH.
The Quest for a Cure:  
AIDS Research at the Millennium

David Ho

David Ho is director of the Aaron Diamond AIDS Research Center of the Rockefeller University in New York, where he is also a professor and physician. His research using a combination of drugs to treat patients in the early stages of HIV infection brought him acclaim as Time magazine's 1996 Man of the Year. Ho graduated from Caltech in 1974 (before going on to Harvard Medical School); he spoke at Commencement last June and this fall was named a member of Caltech's Board of Trustees.

Left: A small molecule designed to fit exquisitely into a cavity in the protease molecule prevents the protease from carrying out its work of replicating HIV particles. Protease-inhibitor therapy, along with drugs that attack another stage of HIV replication, has dramatically slowed the progression to AIDS.

The AIDS epidemic presents a very pessimistic picture. We now have close to 30 million cases throughout the world, heavily concentrated in sub-Saharan Africa, but with a growing epidemic in southeast Asia. It's predicted that in a few more years, the Asian epidemic could surpass the African one. Each day now, there are 16,000 new infections (including 2,000 children), and 90 percent of these cases occur in developing countries, primarily in Africa and Asia. In some countries this disease is killing much of the affected population. In a particular region in Uganda, for example, AIDS now accounts for about 44 percent of deaths in the whole population and, in the 25-34 age group, for about 90 percent of deaths. HIV has become a major killer in the world, at a level comparable to tuberculosis and malaria. In the United States, too, AIDS has been creeping up as a major killer of young people between the ages of 25 and 44, surpassing even accidents and cancer since the early 1990s. Fortunately, in North America and Europe there is actually some decrease in new infections per year.

Recent research developments along several fronts permit some degree of optimism. One very important development has occurred primarily in the last 18 months. For more than a decade we have known that HIV finds its principal immune-system target cell, the CD4 T cell or CD4 lymphocyte, through a very specific recognition site, or docking site, for a molecule called CD4 that sits on the cell's surface. For about a decade, we have also known that a second docking site is required, but that receptor molecule has remained mysterious until the past year, when it was identified as a member of the family of molecules known as chemokine receptors. HIV needs to interact with the first molecule and then with the second, especially one called CCR5 and other related molecules, none of which are there to serve HIV. They're there, in fact, to bind smaller molecules—chemokines—that are released by
Right: A burst of HIV in the blood follows immediately after infection and then settles down to a plateau or set point, where it can remain for years before AIDS occurs. Just how many years is a function of the plateau level; current therapies are aimed at bringing down that set point, in hopes of stalling the onset of AIDS indefinitely.

Below: An HIV, its surface bristling with glycoproteins, infects its target cell by recognizing and docking (red line) at a surface molecule called CD4. In the past year, a second docking site (wavy yellow line), necessary for the HIV to enter the cell, has been discovered—a protein called CCR5 or CXCR4, which is a chemokine receptor. Chemokines might be employed to block this interaction.

various cells in the immune system. We might possibly be able to employ these chemokines to engage this second docking site and block this entry step for HIV, so this now becomes another therapeutic strategy. We could also specifically target this docking site via the development of other small molecules.

Now, as this discovery was being made, Bill Paxton, a colleague of mine at the Aaron Diamond AIDS Research Center of the Rockefeller University, was working with a number of patients who had been exposed to HIV through multiple sexual contacts and yet remained uninfected. Even in the test tube, HIV cannot infect the CD4 T cells of some of these individuals. This was distinctly unusual. With the discovery of the chemokine receptor CCR5 as an important docking mechanism for viral entry, it became logical to ask if these people had any abnormality involving the chemokine receptor molecule. And it turns out that some of these exposed uninfected individuals have a deletion of a 32-base-pair sequence in the DNA that encodes this molecule, so that, in fact, these people are missing the chemokine receptor CCR5.

This observation was followed up primarily by Dr. Huang in our group and by Steve O'Brien at the National Institutes of Health, who showed that individuals who have the CCR5 defect are principally, perhaps even exclusively, Caucasian. About 1 percent of the Caucasian population, particularly from northern Europe, has two copies of the defective gene (one from the mother and one from the father), and these people are almost, but not quite, 100 percent protected from HIV infection. People with one normal gene and one abnormal gene have a slower disease progression after HIV infection. This is an important development, because we now not only know that these chemokine receptors represent an important gateway for viral entry but also that CCR5, in particular, is dispensable, making it a rational target to go after in drug design.

Over the last couple of years, we have also learned a great deal about the levels of HIV in infected people through the work of John Mellors and others from the University of Pittsburgh. Shortly after HIV infects a person, there’s a burst in the amount of virus as measured in the blood, after which the virus is brought down to a plateau, presumably by the body’s immune system. But the level where the plateau is reached is quite different for different individuals. Through their work, we now know that if a person settles at a high plateau, with a high viral load, there is a great chance of progressing to AIDS in five years’ time. In contrast, if the virus is brought down to a lower plateau, there is a much slower progression to AIDS. This shows in a definitive manner that the level of virus replication drives disease progression. We also now know that, once this plateau is reached, it is typically maintained for many months, even years in some patients, with the level creeping up only slowly over time. We had previously thought that HIV was quiescent during this period, but the work of several groups in the past few years has shown that HIV replication is extremely active, especially when the plateau remains high and continues mercilessly in the infected person. Infected CD4 T cells make enormous numbers of HIV particles each day. Such particles are removed very quickly by the body, although some particles go on to infect new T cells, and this cyclic process continues relentlessly. Throughout this cycle, many CD4 T cells are destroyed either directly or indirectly by the continuous replication of virus.

Now that we can begin to get a handle on the magnitude of this virus replication, it clearly has implications for how we treat HIV. We now view it as a much more active process from the very beginning, and this process destroys a lot of important immune cells in the body each day. So it doesn’t really make sense, now that we have drugs available, to let this continue unchecked. In addition, once we define the magnitude of virus replication, we can calculate how many new cell infections occur daily. As HIV infects new cells, it has to take its genetic material from RNA to DNA through reverse transcription—a process that David Baltimore defined a couple of decades ago. During reverse transcription, HIV will make a lot of errors, generating many mutations. Some of these mutations will begin to confer drug resistance to HIV. So then, if we try to treat HIV with a single agent, the virus will be inhibited only for a transient period, and it will quickly rebound with a drug-resistant strain. This suggested to us, as well as to many others in the field, that we had to attack new infection by using several different drugs, trying to corner the virus so that it can’t mutate sufficiently to evade several drugs at one time. This is the strategy that has generated the most promising results.

The viral life cycle is illustrated above: the
Patients who received combination therapy involving protease inhibitors along with older drugs that target reverse transcription have seen the levels of virus in their blood drop to undetectable levels. The virus may still be hiding elsewhere in the body, but such therapy, although expensive, offers hope for controlling the disease.

In the life cycle of HIV, the virus enters the target cell, creates a negative strand of its DNA through reverse transcription, which enters the cell's nucleus and begins to synthesize HIV components. The protease enzyme cuts these components, the viral proteins, into the smaller pieces necessary to assemble new HIV.

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David Baltimore is president of Caltech, an office he assumed in October after spending most of his scientific career at MIT. He is a former president of Rockefeller University. Baltimore helped pioneer the molecular study of animal viruses and won the Nobel Prize in 1975 for his discovery of the enzyme reverse transcriptase, which permits retroviruses, such as the AIDS virus, to replicate. He is chair of the National Institutes of Health AIDS Vaccine Research Committee, a post he will continue to hold along with his Caltech duties.

As you have just heard, these exciting new drugs are too expensive to represent a global solution. Approaches to preventing HIV infection by education and behavior control involves cumbersome mechanisms that have never been more than partially effective. But we do already know how to prevent virus infections. We prevent virus infections by vaccination. So, in the very earliest moments of the HIV epidemic, everyone said we should be making a vaccine.

The United States government has put an increasing amount of resources behind the production of a vaccine. We are now spending more than 100 million dollars per year on AIDS vaccine research (out of a total of $1.3 billion allocated to AIDS), and as effective drugs are developed, I think that a larger fraction of that budget could now go to vaccine development, if we knew how to spend it well. But money is not enough; we need an organized program of research to find a vaccine.

In 1996, Harold Varmus, head of the National Institutes of Health, asked me to establish a committee that would oversee the AIDS vaccine development effort in the United States and make it into a coordinated program that could feed the latest information into the vaccine-development pipeline. This committee consists of molecular biologists, infectious-disease experts, AIDS-treatment specialists, researchers on the history and evolution of AIDS, and people from all aspects of the epidemic, including one member of the advocacy community. The group is small, which makes it easy to work with, but we can expand it if we need to.

Our job is solely advisory. That's an odd charge for a group that's supposed to organize a program. We are supposed to advise the vaccine research programs at NIH with regard to scientific opportunities, gaps in knowledge, and so on. It has to be advisory, because the only people who can spend money on research are Federal employees, which we are not. Over the past year we've performed our role by meeting as a committee to grapple with the issues of what the vaccine program is; by starting a new grant program; by bringing people in the immunologic and virologic communities together in workshops to talk about the issues; by generating new ideas; and, particularly, by trying to bring new people into the vaccine effort, because one of the things we saw early on was that the great strengths of the American immunology and virology communities were not totally focused on this issue.

The innovation grant program that was invented through the committee's efforts is a way of simplifying the process of getting money from Washington—making simpler grant proposals, getting them funded faster, and targeting those grants to problems that we had identified as crucial to the vaccine effort. We targeted three areas: developing better animal models; studying the protein found on the outside of the virus, which is likely to be one target of any vaccine; and finding out how to get the cellular arm of the immune system revved up to attack virus-infected cells.

We were able to announce the grant program in March, have the grants come in by May, and have them funded in September—52 new grants, spending $12 million on new approaches to AIDS-vaccine development.

Even before I started on this committee, the first
question I asked myself was: is it possible to make a vaccine? We don’t know the answer to that for sure, but I had to convince myself that there was at least a high probability of it. And I could do that because some research developments suggested that you could make a vaccine. First of all, there was work with nonhuman primates, the best model we have for HIV. A number of researchers, mainly Ron Desrosier and his colleagues at the New England Primate Center and Harvard, had found that you can protect macaque monkeys against SIV (simian immunodeficiency virus) infection with an appropriate vaccine preparation consisting of a live, attenuated virus particle. The virus is a perfectly infectious live virus, but its genes had been mutated in such a way so that, although it can grow and stimulate the immune system, it will not cause disease. The exciting thing was that it was done by mutating certain critical genes that are particular to the AIDS virus. People infected with such mutated strains of HIV have infected other human beings, and those infected people identified so far are nonprogressors, that is, the mutated virus causes a chronic infection, but the disease symptoms do not appear.

The AIDS virus is a retrovirus, but there are a lot of very simple retroviruses that don’t cause AIDS, or much disease at all, unless they pick up a particular new gene, or if they integrate in a specific place in the genome (in which case they can cause cancer). To a large extent retroviruses are benign. The differences between them and HIV is a series of little genes (see illustration below), which form the heart of HIV’s power to cause disease. The mutations were put into these genes, and the vaccine created from the live mutated virus. Unfortunately, even with those mutations, the virus occasionally causes disease, especially in very young monkeys, so there would certainly be a serious safety problem for human beings with this vaccine candidate. Right now, we’re at a point with this vaccine concept where there is proof of principle, but we don’t know how to carry that from principle into action.

Most important, we’ve got to bring new creative ideas into vaccine development, or 10 years from now we may still be wringing our hands.

There is evidence that some kind of protection, probably of an immune nature, is possible in humans. For example, there are sex workers, particularly in Africa, who have been exposed to HIV over and over again and have not been infected. They have some kind of immunity—different from that conferred by a mutation of the chemokine receptor that David Ho talked about. It may be cellular immunity due to what are called cytotoxic T lymphocytes, the cellular arm of the immune system. Also, once a person is infected by HIV, it’s very hard to infect him a second time, even after multiple exposures, suggesting that infection produces some sort of barrier against other HIV viruses coming in. If we knew how to make that barrier without the infection, we would be ahead of the game. These are the kinds of evidence that drive the vaccine program today—proof of principle, or suggestion of principle in the human cases, but no direction as to how the vaccine should be made.

Now, what does a vaccine do? We tend to think that vaccines protect us from virus infections, but they don’t really. What they do is make sure that, if you are infected, your immune system reacts to that infection before any disease occurs. It’s actually an abortion of the ongoing infection rather than what might be called sterilizing immunity or complete protective immunity. If we could develop an AIDS vaccine that gives sterilizing immunity, it would probably be the first virus vaccine to do that.

So what could it do? Well, as David Ho suggested, it could reduce the initial multiplication of HIV to reach a lower set point in the early stages of infection, to increase the time before the body loses control over the virus and AIDS occurs. In the best of cases, it might drive blood virus levels below the detection threshold so that perhaps the disease would never occur. This would involve driving down the plateau level below the point where the body can no longer control the infec-

Left: A graph of estimated annual adult HIV infections from 1980 projected forward to 2000 shows that cases will likely continue to rise dramatically in Asia at the end of the century, taking over the lead from Africa. Expensive drug therapies are unlikely to provide a solution here, underscoring the urgency of finding a vaccine.

Right: The difference between ordinary retroviruses, which don’t cause disease, and the AIDS virus is a series of little genes—vpr, vpu, nef, rev, tat, and vif. Mutations put into these genes have produced a vaccine that works in monkeys but is still too risky for humans.
NIH funding for research on an HIV vaccine has maintained a steady rise from 1985 to 1998.

A fanciful representation of HIV shows the Env proteins sticking up off the surface of the virus. It would make sense to use these proteins in a vaccine, but changes produced by laboratory methods of making these proteins or inactivating the virus have so far compromised their utility.

tion. That's what we imagine a vaccine can do. We're not sure this is possible, but it's certainly suggested by the work with monkeys.

What kinds of vaccines could we use? The historic vaccines that have been effective against virus diseases are of two kinds. One is the live attenuated virus like the one I described for SIV. The Sabin polio vaccine is a good example of a mutated live virus. The other kind of vaccine, such as the Salk polio vaccine, uses killed virus, in which you take a perfectly infectious virus and kill it by some chemical or physical means. It can still induce immunity, but it doesn't produce any infection. Unfortunately, HIV is a very fragile virus to any method of killing that's been found so far; it falls apart and is not really useful as a vaccine. I think it's a soluble problem, but it hasn't been solved yet.

When these problems were recognized some time ago, scientists began trying to make vaccines that consist just of the surface protein of the virus. (It's called the Env protein because it is in the virus's envelope.) The virus has on its surface little aggregates of three copies of Env protein; they have affinity for CD4 and the chemokine receptors on the surface of cells, and they use these as an entry port to infect the cell. It made a lot of sense to use Env as a potential vaccine. But two problems have emerged: the first is that the methods used to make these produce single units, not trimers, so that they don't look like they do on the virus surface. Second, in the initial work on HIV, it was necessary to make a lot of virus. For this, scientists could not just use the virus taken from people; they had to grow the virus in cells in the laboratory. We did not realize that when you grow virus in the lab, you select for changes in the structure of the virus proteins. These laboratory-adapted viruses are easily killed by the antibodies they induce, giving the impression that vaccination with these strains would be possible. People don't get infected by adapted strains, however; they get infected by field strains. The field strains are not susceptible to killing by the antibodies raised by these vaccine candidates, making their utility doubtful. It has recently been questioned whether antibodies against the Env protein of field strains can be raised at all. I think they can, but it's going to take some pretty subtle tricks to do it.

Because of these problems, the cytotoxic T lymphocyte (CTL) arm of the immune system has come to the fore as a potential way of protecting the body. When an appropriately cytotoxic T lymphocyte sees a virus-infected cell, it releases materials that cause the cell to commit suicide. Such lymphocytes exist in all of us, and their killing capacity can be stimulated by any protein made under the direction of the virus, even proteins that do not become part of the virus particle. Much of the effort today is going into inducing this kind of immunity to supplement whatever antibody immunity can be produced. The kinds of things that will do that are vectors that bring genetic material into cells—things like other viruses or naked DNA. These can be injected into the body, get into cells, and induce the synthesis of proteins that stimulate the CTL arm of the immune system. Vaccine designers today are trying to use many different techniques to induce the two kinds of immunity: peptides representing parts of proteins; vectors derived from other benign viruses to induce synthesis of proteins inside of cells; and the proteins themselves, often carried on particles that look like viruses but aren't viruses.

I've been discussing the search for a vaccine as if all of these techniques were just under development and nobody had ever tried to test a vaccine. Actually, the program is 15 years old. The day the discovery of HIV was announced in Washington, then Secretary of Health, Education, and Welfare Margaret Heckler said: "We now have the virus; in two years we'll have a vaccine." She was optimistic, but that was, in fact, the start of the vaccine program. Many vaccines, in particular some using live vectors such as the smallpox vaccine virus, have actually been tested during these 15 years. Only a little work has been done on immunization by naked DNA, but there will be a lot more. Even whole killed virus has been tried, although not with much success.

So, with all this history, why isn't there a vaccine? I think the defining moment came a few years ago, when we realized that the laboratory strains were different from field strains. Even before that, we had known that adaptation to the laboratory changed the virus, but we didn't know the consequences. But now we became aware that we were working with materials that probably would never give decent immunity. It's not certain that this is true, and these materials are still being tested, but it has forced us to go back and think about redesigning the whole program of vaccine development. This was the genesis of the committee that I represent and of the
attempts to introduce new and more innovative methodologies.

What are the main needs of the vaccine program today? First, we have to integrate into vaccine development the latest knowledge about HIV. Why did it take so long to recognize that the field strains and laboratory strains were different from one another? Partly because vaccine development was running on a track quite separate from the basic research track, and the information transfer was poor. We need to bring the latest information to the vaccine efforts and use it to modify them accordingly.

We have to introduce this information into the human testing process because, ultimately, we can only know that vaccines work when they've worked in human beings. More than 2,000 people have already taken vaccines in a continuing process that has been quite separate from much of the research effort. Research is mostly government-funded and takes place in universities and research institutes, while vaccines are, in the end, developed by industry. Under government direction, we need to integrate into a partnership the many different strong research institutions in the United States and elsewhere along with all the industries that will ultimately make these vaccines. Most important, we've got to bring new creative ideas into vaccine development, or 10 years from now we may still be wringing our hands. One exciting initiative that our committee has helped foster is a laboratory on the NIH campus that can carry out an integrated program of HIV vaccine research. This will help couple the vaccine development effort to advances in basic knowledge about the virus.

What should be the test of the success of our committee? Development of a safe and effective vaccine will not happen quickly. President Clinton has asked for a vaccine within a decade. I have a more modest goal. If we have exciting vaccine candidates that are safe and work well in animals within the decade, I will feel we have been successful. If we don't, I think we will have to consider the possibility that HIV has outwitted us, that a vaccine is not in the cards.

It is tremendously gratifying for us to see one of our early efforts go from molecular to design through extensive testing to actually extending the life of a patient.

Mel Simon is chair of Caltech's Division of Biology and is the Anne P. and Benjamin F. Biaggini Professor of Biological Sciences. He came to Caltech in 1982 from UC San Diego, where he had spent most of his previous academic career. His research centers on how organisms detect and respond to chemical changes, and includes studies of the mechanisms involved in sensory cell function and investigations into the nature of the biological circuits that process information from a variety of cell surface receptors.

About 15 years ago, it became clear to me and to some of my colleagues that we were in the midst of a technical revolution in biology and biochemistry that could provide novel strategies for dealing with infectious disease. The dream was that, using molecular biology, we could identify the molecules intimately involved in the mechanisms of infection, and then characterize them in atomic detail, and design inhibitors that would bind only to those target molecules and inactivate them. The notion of specifically designing drugs atom by atom was different from previous approaches to drug discovery. Many of the drugs that were used to fight infection by microorganisms were natural products that were derived or extracted from plants or other organisms. In fact, there were very few drugs that could cope with viral infection.

By the early '80s, molecular biology had developed enormously, enabling much of this dream to become possible. First, we can, in fact, identify target proteins required to initiate and propagate disease—in the case of HIV, the reverse transcriptase, the protease, and the integrase. These proteins are part of the process of building the virus, and they are absolutely necessary for propagation of infection. Second, in order to wage this war at the atomic level, we have to know the atomic structure of the target molecules. This requires knowing the position of every atom in the target molecule. This picture of the target molecule also tells us a bit about how the molecule works. You can't see the virus with a light microscope; you can just make out gross viral structure with an electron microscope. To actually determine the atomic structure of components of the virus, we need x-ray crystallography, a technique...
that has been around for almost 100 years but whose development has really accelerated in the last 20 years. The great advances in computational techniques, computers, and software for computational chemistry have greatly facilitated protein crystallography. Sophisticated computer displays are available to help us visualize these molecules in three dimensions, to stimulate their interactions with other molecules, and to try to understand how molecules recognize each other. Advances in organic chemical synthesis permit us to optimize molecular designs, and to build molecules that can interact with each other in a very specific way.

Bringing all of these elements together involved uniting a variety of different sciences. A group of us at the University of California at San Diego decided to form a company to do just that. The corporate structure is in many ways ideal for blending cultures and approaches and for focusing the efforts of diverse people on a specific goal or product. It was at this time that we also became aware of the proportions of the AIDS epidemic, the grief that it was causing, and the discouragement that had been experienced in attempts to develop methods for dealing with it. We realized that we could very quickly describe the proteins that make up HIV and that are essential for its replication—the reverse transcriptase, the integrase, and about 12 other proteins, including the HIV protease, whose function is to tailor the viral proteins into smaller pieces. These targets were relatively easily available and provided an excellent model to test the notion of drug design.

Our company, Agouron Pharmaceuticals, used the techniques of molecular biology to isolate large amounts of these proteins, to determine their crystal structures, and to try to design drugs that would block their function. Thus, for example, the HIV protease has to digest a larger protein at specific places, in order for the virus to make an effective "coat." If you block protease from acting, then you don't get a mature virus particle, and the particle that is generated cannot infect cells. First, we and a number of other companies worked out the atomic structure of the protease using X-ray crystallography. The HIV protease is made up of two subunits, which cleave a protein substrate that specifically fits between them. In the close-up (below) of the heart of the molecule, you can see the surface of the protease and the substrate of the virus that it is going to have to chop. (The scale here is in angstroms and fractions of angstroms.) This is a static picture, but these parts are actually all wiggling around, and you can see that the fit is exquisitely perfect. This atomic fit is the source of the protease's ability to recognize a specific substrate.

The protease sees a very specific part of the virus and cuts it. What we wanted to do was to design a small molecule that would sit in the cavity between the two subunits and fit so well that it will not allow the usual substrate protein to work. This small molecule would go into all the viruses, get into the middle of all the proteases, and block them from working. You need perfect molecular recognition at the atomic level for this strategy to work. At right is one of the first molecules that our company made. You can see that it didn't fit snugly, and it fell out of the "active site"; the protease was therefore still able to "do its thing." In other words, you would see very weak inhibition of protease activity. So the designers had to go back to the drawing boards. Each time around, they take an "X-ray snapshot" of the molecule, that is, they generate a co-crystal of the "target" and determine exactly how the putative inhibitor sits in the active site. They see what parts still have to fit; and then they redesign the small molecule inhibitor.

In the case of the protease inhibitor, this design process involved more than 40 iterations. Different small molecules were built and inserted into the active site; the complex was crystallized and its
structure determined. In this way the small molecules were tested to find the inhibitor that fit the site best. You can see how much better this final small molecule on page 24 fits the site. Electronic calculations indicate that it recognizes the active site of the protease with great specificity. When it gets into that crevice, it binds to the protease extremely tightly, and prevents it from acting. The putative inhibitor had to then be tested in a variety of ways to see if, in fact, it blocked virus replication. Then we had to determine if it was harmful to people or if it had side effects that were deleterious to living organisms. Finally it needed to be tested for efficiency in clinical studies. It became clear that the protease inhibitors represented one part of a strategy that David Ho was instrumental in inventing and pursuing—that is, the notion of using multiple inhibitors of viral replication—which lowers the amount of virus in the blood and keeps it down for an extended period of time. The idea that is essential to this treatment was presented by Dr. Ho. Since the virus replicates very rapidly and mutates rapidly, the application of multiple inhibitors that block different steps in replication lowers the number of replicating viral particles, and at the same time requires multiple simultaneous mutations in order to bypass the inhibitors. This lowers the probability that effective resistant viral particles will arise.

Unfortunately, it doesn't work for everybody, but for a large fraction of the patients (more than 80 percent) the cocktail of protease inhibitors and multiple reverse transcriptase inhibitors does have a dramatic effect. Many people who have been taking this combination for over a year have improved in various ways. It is tremendously gratifying for us to see one of our early efforts go from molecular to design through extensive testing to actually extending the life of a patient. The effect that biologists hope to see in their work has been realized in this case—to use our understanding of nature and the tools of molecular biology to improve, or even save, lives.

We know that HIV replicates at an enormous rate. Because it can replicate so prodigiously, and because it can mutate at a high rate, the virus is able to evolve rapidly. Thus, the probability of a mutation that can bypass the drug or cause resistance to the drug is high. The use of multiple drugs raises the barrier to complete resistance, but nonetheless, resistances arises. How does it happen? One of the things that Agouron has found in patients and in tests in the laboratory is that the virus can sustain a particular mutation that will change the protease at one particular position. This change breaks one of the bonds that holds the molecule in the active site, but still allows the protease to function. The same inhibitor no longer fits as perfectly as it did before, and the protease can bypass the inhibitor and work again.

By using multiple drugs and prescribing them early in the course of the disease (along with high compliance by patients), we can lower the probability that these kinds of mutations and this kind of resistance will occur. A variety of drugs is now available, and combinations of these drugs are being used and shown to be effective in averting resistance. Many companies are working on other drugs. Some of these might fit the active site differently and thus augment current treatment. A tremendous amount of research is currently going on to try to perfect this method of recognizing the targets and designing specific drugs fitted to them. Agouron and other companies are working on the HIV-Integrase, the HIV-RNAase H, and other proteins that are necessary for viral replication. It may eventually be possible to design inhibitors that are so clever that they can actually minimize the effects of mutations to resistance. This is clearly an enormous problem, but one that is being pursued at different levels and that will lead to a new generation of antiviral therapies.  

One of Agouron’s first designs for a protease inhibitor clearly doesn’t fit the site (whose surface contours are represented by the small dot pattern) very well—hanging out on both ends and leaving spaces unfilled. Many redesign attempts finally arrived at the successful molecule illustrated on page 24.