OK. I think we'll get started. So we're going to continue our discussion today about viruses, finish talking about influenza, influenza A virus and the flu, and then talk a bit about HIV and AIDS. Do you guys know more about the tutorial session and so on and so forth for next week? Has that been decided yet? OK. So you'll get more information Monday and Wednesday. And I'll also have an office hour, probably a couple of office hours at the end of next week. Most likely, if it doesn't conflict with this tutorial session that's being planned, it will be during this hour from 11:00 probably until 1:00 in this room because it's free since we're usually here. But that may conflict with you guys so we'll have to see. I'll let you know on Monday. So, again, the situation with viruses is an ongoing one. And, actually, one of you brought to my attention an outbreak of polio that's happening now both in Africa and Indonesia. This is actually from today's CNN. om where there's a fear that polio is starting to spread. In this case in Jakarta, in Indonesia. And there's a very extensive reaction to this. There's a great fear that polio will spread. This is an area that is not well protected currently by polio vaccination. And so if you look at this you'll see that they are raising lots of money in order to try to vaccinate with standard polio vaccines, which work extremely well, 5.2 million children, to try to stop the spread of this potential epidemic of polio. It hasn't reached anything near those levels to be that concerned, but in order to protect against that possibility they're going to vaccinate. And, obviously, if you vaccinate, individuals cannot be successfully infected. If they cannot be successfully infected, the virus cannot propagate so they cannot pass it on. So you just close off the infectious cycle in a very small circle of infected individuals. Also, in today's Globe, there was a discussion of a local company that makes vaccines against smallpox. And I've mentioned to you that smallpox is largely eliminated, if not fully eliminated in the world, but there are stocks of smallpox in freezers around the world. And there is a concern that an individual could access smallpox virus or make one because the relevant sequences of the genome of the smallpox virus is known. And so, in theory, you could synthesize your own smallpox virus genome, and thereby create your own smallpox virus and expose now unprotected individuals because we don't get vaccinated currently against smallpox. And so there are companies like this one that are making currently and distributing smallpox vaccines. They distributed 182 million doses in this country alone just in case somebody tried to deliberately release some smallpox. So that's what we can do when we know what we're dealing with. We can create effective vaccines. In this case they're effective, but sometimes we get exposed to stuff that we cannot effectively deal with. And I introduced this to you last time. This was the major flu pandemic from 1918. Some people worried that there might be another pandemic this year because in the off season between 1918 and 1919 when this happened, and 20 to 40 million people died, that's when the Red Sox last won the World Series. [LAUGHTER] So the Armageddon folks thought maybe this was the year, but so far so good. So, as I mentioned last time, this was a major outbreak and a major problem worldwide. But you also should know that influenza is an annual problem. And I, for one, didn't appreciate this. Where there are about 25 thousand deaths per year in the United States. And, of course, to deal with that we make available, especially to the elderly and infirmed immunodeficient flu shots. And flu shots are simply flu vaccines. And I'll tell you a little bit about how they're made towards the end of this section, but they're generally effective in combating against the flu that is going to hit the population that year. And they change every year because, as you'll see, flu changes all the time as well. So it's an annual problem. There are occasional epidemics which you could think of as -- -- population-based infection where many individuals within a particular population are infected. And the virus, in this case, is spreading throughout that population. And there are even more occasional pandemics. And that's a worldwide infection. Where, because of the virulence of the virus and its ability to access different populations and the absence of immunity throughout the worldwide population, lots and lots of people get infected. And that's what happened in 1918. So how do we explain this? How do we explain the occasional epidemics of a virus that we're so familiar with? Flu, as I said, we get every year, lots of people get it every year. And, yet, we seem to be susceptible year after year. So why is that? What causes the resistance to this immunity in small measure and in large measure? And that's what I want to review for you today. So this is the responsible agent. It's a virus, of course. More specifically, it's an enveloped virus, which means it has its own lipid bilayer that it picked up from the host cell. And you'll notice on the outside are things sticking out of the bilayer. These are proteins encoded by the virus which are going to be responsible for binding the virus to the host cell. And also allowing the viral membrane to fuse with the host cell membrane. And you'll see that that's done in the case of flu virus in a slightly different way than for some other viruses. And then inside you have the capsid. And wrapped up in this protein, this helical protein structure are the nucleic acids of the virus. And the nucleic acids of this virus are multiple. That is it's not just one. Oops. It's not just one. It's actually several. So, again, it's called influenza A virus. It's an example of an enveloped virus. And I said you should note the envelope proteins, which are largely encoded by the virus itself. And we call these envelope glycoproteins. Glycoproteins because when they emerge through the sorting pathway of the cell they pick up sugars. And so when they're on the outside of the cell, it's not just protein. There is some sugar there, so glycoproteins. And sometimes the antibodies that we make are directed against the sugar moieties alone or in combination with peptides. So the envelope glycoproteins are important. As I said, it's a segmented RNA genome, which means that there are multiple distinct nucleic acids in the viral particle. And in the case of flu there are eight. The RNA genome is single-stranded. And all of the genome segments are in the minus configuration. So their sequence is the complement to the mRNA. OK? And we talked about the significance of RNA viruses that have a minus strand polarity in the sense that they need to have their own RNA polymerase coming in with the virus in order to help, in order to initiate the replication cycle. So, hopefully, that's familiar from last time. This is a diagram of the virus, and it just makes the same points I just made. So, again, it has a lipid bilayer. It has envelope glycoproteins sticking out from the surface. You'll see that they're important for different things in a moment. Inside that is a nucleocapsid which surrounds the nucleic acids. And you can see that there's a series of nucleic acids that are enclosed within this. And, again, there are eight of them. And they're all minus strand. And, importantly, contained within here is a protein, an RNA

eight of them. And they're all minus strand rna. Importantly, contained within here is a protein, an RNA dependent RNA polymerase, the yellow dot. And it has to get incorporated with the virus so that when the virus infects it goes in there, too. And it can then act on the RNA genome and convert it into a plus strand, which will then serve both as the RNA for translation, as well as the template for the production of more of the minus strand RNA. OK? So this comes from your book, and it is a summary of the infectious cycle of this virus. I've modified it a little bit because some of the details which I think are important were missing. So you might want to pay attention to this figure as you're reading the section, the relevant section in the book. But this is a typical viral lifecycle. The virus attaches. Remember, the terms that I used last time? The virus attaches. Here one of the viral glycoproteins binds to a protein on the surface of the target cell. That then initiates an internalization process, a penetration process in which the virus gets brought in through an endosome. So it gets brought in through a separate vesicle, an endosome. That vesicle, the endosome then fuses with a lysosome, not shown here. And you may recall that the lysosomes have very low pH. And it's in the context of the low pH that one of the viral glycoproteins changes its shape. And when it changes its shape it allows the viral membrane to fuse with the membrane of the lysosome. So this is a pH-dependant protein conformational change which turns an inner protein into a protein that facilitates fusion. And when that happens, the viral capsid can then slip out into the cytoplasm. It gets unpackaged. The RNA, which is the minus strand RNA gets released. And then it gets acted on by that RNA dependent RNA polymerase that came in and gets converted from minus strand to plus strand. And the plus strand can then do two things. It can get translated into viral proteins, both the glycoproteins that go through the sorting pathway and make it to the membrane, as well as the structural proteins that form the capsid. The structural proteins then join up with the viral RNA segments, now the minus strand RNA segments. They meet at the membrane and then bud off to form a new virus. OK? So this is a standard infectious cycle for this class of viruses. And because it's going to be relevant later, I just want to emphasize a couple of points. Through interactions between the cytoplasmic tail of the envelope glycoprotiens, the capsid proteins coalesce underneath the plasma membrane. And there's an interaction between these proteins and the tails of these proteins which causes the virus to start to bud off the surface of the cell. Now, there are separate interactions with the proteins on the inside of the capsid which bring with them the different viral RNA. So remember there are eight. And there's a rather amazing process by which the eight distinct RNA segments get brought together into the capsid, and then the capsid goes to the membrane and gets budded. So there is a sorting process, which we don't fully understand, that ensures that viruses get at least one copy of each of the genomic segments. And that's necessary because if a virus doesn't have all eight, when it gets into the host cell that it might infect, it's not going to be able to replicate. These RNA segments encode one, or at most two of the viral proteins that are necessary for the infectious cycle that's drawn up there. And you'll see why that's important in a moment. OK. So we get infected, we develop antibodies against the proteins of the virus, and we typically develop them against the glycoproteins that are sitting on the outside. We develop T cells that can recognize virus infected cells and kill them. And yet we keep getting flu. So why is that? How is it that we can, how is it that the virus can overcome this immune resistance? And the answer is that the virus changes. And this is a major problem with all viruses, but it's a particular problem with RNA viruses and a particular problem with flu. So here's a depiction of the virus again. It's got its genomic segments in here. And on the surface it has these glycoproteins. And I'm going to draw a little bit more detail in these glycoproteins now. There are actually two distinct glycoproteins on the surface of the cell. There's one that's called HA and there's another that's called NA. And they carry out different functions. When you get infected, your immune system sees these epitopes sitting on the surface of the virus and you make antibodies against them. And there are three particular places on these proteins where good antibodies can be made inside your body. One of them is here, another one is here, and a third one is here. And different people make different of these antibodies. And these antibodies that are made have a name which is neutralizing or blocking antibodies. They're called that because if you have one of these antibodies, and it will bind to the surface of the viral particle, this virus can now not infect the cell. There's an interference between the, what the hell? Between the glycoproteins on the surface and the receptors to which they bind by the antibody. So they block binding. And if you cannot bind you cannot infect. And this is very efficient. This works. So that's how you can overcome the infection to that particular virus. The problem is that viruses change. During their replication there are mutations. And sometimes these mutations affect the structure of the proteins that are sitting on the surface. So you might imagine the strain here gives rise to variant here which has an HA protein which looks more or less the same, but it has an NA protein which has changed. So now this epitope, which was recognized by antibody three, cannot be recognized by antibody three because it's got a difference sequence. OK? If you were a person who made predominantly antibody three in response to this infection, you would not be susceptible to this virus. So if this one came along next year you'd get the flu. If you were a person who made antibody one or antibody two as your major protective antibody, you'd still be resistant to this. You'd be one of the lucky ones. And because there's heterogeneity within our population, even if these new variants arise, they don't spread all that much because there are enough people in the population who are pretty well protected. So those are low-level spread or low-level epidemics. OK? Now, this process, one day I'm going to figure out how this works. This process of generating variant viruses through this mutational mechanism is called antigenic drift. It's a slow drift of the antigens on the virus. And it happens because the virus is highly mutagenic. And it's true of RNA viruses in general. And there are two reasons why RNA viruses create variants at such high levels. One is that RNA is less stable than DNA. Remember, there's a difference in the structure of RNA versus DNA. There's an extra hydroxyl in the structure of RNA which can cause increased mutation, increased breaks and base substitutions in our RNA molecules compared to DNA molecules. So RNA is inherently less stable than DNA. And also RNA polymerases lack proofreading functions. Which means they have an inherently higher mutation rate. And hopefully, again, this is familiar from our discussions about DNA replication. All polymerases make mistakes but your DNA polymerases have what's called a proofreading function which can recognize the mistakes and correct them. RNA polymerases lack that, and so they make the mistakes and they stay as mistakes. So the mutation rates for RNA polymerases are at least ten fold higher than for DNA polymerases, and

instance. So the mutation rates for RNA polymerase are at least ten fold higher than for DNA polymerase, and in some places even higher still. OK. So that's what's called antigenic shift, sorry, antigenic drift. And it happens all the time and is responsible for why we keep getting mild cases of the flu. But that's not what's responsible for the Spanish flu of 1918. Instead of antigenic drift -- -- these are due to a phenomenon called antigenic shift. Rather than generating subtly different variants, like I've shown up here, this is the process of generating essentially entirely new strains. And it happens in the case of flu all the time. And one of the reasons that it happens is that there are lots and lots of types of flu viruses which are kind of similar that infect other species. There are duck flus, swine flus, horse flus, seal flus that are all caused by versions of this influenza A virus. Similar. Not identical but similar. And sometimes those viruses cross over into the human population. And this is an example of a zoonotic infection -- -- which is an infection from another species into the human population. And actually happens all the time. Many of the new pathogens that arise in the human population arise through zoonotic infection. Now, most viruses that are evolved to grow in the cells of one given species, at the temperature of a given species, will not replicate very well inside human cells. So even if they did infect, they wouldn't reproduce themselves very well. And that would be true of these viruses, too, swine flu virus, bird flu viruses. The problem is, in the case of flu, the human virus is so common and the mechanism of replication of this virus is so complex and amenable to generation of recombinant viruses that we can generate new viral forms. And that's what I want to talk to you about now. So here's an example from not too long ago. This is the collection and culling of a lot of birds somewhere in Asia. I'm not really sure where this was. But there was a concern that a new virus was developing in a bird population and was crossing over into humans. And it caused some deaths. And so to avoid that further they just wiped out some millions of these birds in order to prevent further cross infection. But, again, it's not the presence of the virus, the bird virus or swine virus itself that's the problem. It's the fact that the two viruses, the human virus and the bird virus can recombine to form a dangerous new virus which is rather similar to the human virus but carries some segments of the bird virus. And that's illustrated here, and I'll show you on the board in a second. But basically the idea is that a human virus carrying its eight segments, including the segment for HA and NA infects an animal which also gets infected by a bird virus. So that in the same cell there are the segments for the human virus, as well as the segments for the bird virus. And during this process of the generation of a new viral particle, you can get mixing of the segments so that most of the segments come from the human virus and, therefore, will replicate well in the context of the human cell. But two new segments come in from the bird that encode its HA and NA, a version that's never been seen in the human population. Nobody has antibodies against it so the virus can spread like wildfire. And that's what we believe happened then and we fear continues to happen over time. So, again, imagine a cell which has been infected by two different viruses, the avian virus, in this case it was a duck, as well as the human virus in that given year. In that cell there are going to be lots of different segments of virus RNA, the human ones H1, 2, 3, 4, 5, 6, 7, 8 and so on, as well as the duck ones, duck 1, duck 2 and so on and so forth, duck 7 and duck 8. When the viral capsids coalesce underneath the viral glycoproteins to produce a new virus, they cannot necessarily distinguish between the human and the duck subunits. So there might be human 1, human 2, human 3, duck 4, human 5, duck 6, human 7 and human 8. OK? This then makes a virus which is perfectly able to replicate in human cells because it's got mostly the genes that are optimized for human cells, but it's got new HA and NA forms that have never been seen by the human population. So there is nobody who has antibodies against it. So the virus will spread like crazy. OK? So this is an example of antigenic shift, and it's responsible for these worldwide pandemics. OK. Any questions about flu? One thing I failed to mention, sort of trivial but you probably know, is it spread very well because it can spread in water droplets. If you sneeze on your friends they'll also get the flu. It's a very efficient virus so you don't need very many viral particles to go inside of your respiratory system in order to initiate the infection. And this is another good example. When you have a very active infection inside of you, you start producing lots of the cytokines, that I mentioned last time, like interferons. And, again, it's largely those that are causing the symptoms of the flu, and in large enough quantities can cause death. You can also get secondary infections with bacteria when you're that sick, which can be another reason why folks die. OK. So if there are no other questions about flu, we'll now turn our attention to HIV. And I think HIV is probably fairly familiar as a topic. It's, of course, a major, major worldwide health problem. The initiation of this epidemic goes back a very long way. The first cases were reported in 1975, so about 30 years ago. And so that's considered to be the initiation of this epidemic or pandemic. The first case in the United States is considered to have occurred in 1980. And here's another example. We think that this probably occurred by one of the zoonotic infections. But it happened a long time ago. It happened at least before 1950 because there are samples of individuals who died of unknown causes that have now been tested and can be shown to have HIV sequences by PCR. So whenever this happened, and from whatever species it happened, it happened quite a while ago. Why it then initiated in this epidemic form much later is not so clear. The etiological agent -- -- is a virus called HIV, human immunodeficiency virus. It is a retro virus. And we'll briefly review how it replicated, but I told you more or less how that happens last time. And this was another good example. The syndrome was first discovered in 1975, gained a lot of attention in this country starting in the early 1980s, and the virus was first purified and characterized in 1983. So it didn't take too long to identify the agent and then ultimately sequence its genome. And also to develop tests for the presence of actually not the virus itself. The AIDS test is a test for the presence of antibodies in you that recognize the viral glycoproteins. And this was very important, obviously, in screening populations, screening blood banks and so on for contaminated samples. Now, since this time, in the early 1980s, the situation has, of course, gotten much, much worse. In this country, there are currently -- -- a million people infected with HIV. That's between, you know, one and 300 individuals total. And, amazingly, about 20% of those people don't know it. Worldwide, anybody have any idea how many people are infected with HIV in the world? 40 million. And total about 60 million people have been infected because since the early 1980s, 20 million people have died from this disease. More than 20 million. And the prevalence is remarkable in certain places in the world. In Southern Africa, for example, not the country of South Africa but in the Southern Region of Africa, Saharan Africa, there are 25 million people infected. And in certain countries that's one in four sexually

active individuals. Remarkable infection rates. And the effect of this disease and the death that it causes has also had dramatic effects on the population. So that in Zimbabwe, for example, where the life expectancy in 1990 was 52 years old, not a huge number but 52 years old, in 2003, because of AIDS, it's less then 35 years. So it has dramatically affected the lives of people throughout the world. OK. And, as you know, I suspect you know that transmission for this virus occurs through direct contact of contaminated fluids. Sexual contact, which is both homosexual and heterosexual. As well as blood transfusion, direct blood contact, through transfusion, and also needle sharing. And sometimes occasionally organ donation, but that's very, very rare. OK. So people die from AIDS because they develop infections. And we'll talk a bit more about that in a moment. But here's one patient who has developed an infection with a virus, a particular type of herpes virus now called Kaposi's sarcoma virus. That virus then is able to replicate in certain cells of the blood vasculature and cause tumors. These are actually tumors. And this individual will die from a disease called Kaposi's sarcoma, a type of cancer, another example of a virus-associated cancer of humans. And in this country AIDS, as you probably know, has taken over as the leading killer of young male adults. It was not known in the early 1980s, and by 1992, just ten years later had become the major killer of young male adults in this country. And, of course, likewise around the world. So here's the virus. As I said, it's a retrovirus. Retroviruses also are enveloped. They have glycoproteins on the outside, envelope glycoproteins. They have a capsid like all viruses do. They have a genome. The genome is made of RNA. And, as you know, through the retroviral lifecycle, the RNA is converted into a DNA form. We'll go over that next time. And that's accomplished by an enzyme called reverse transcriptase which gets prepackaged with the virus. Again, it has to be there because your cells don't have an RNA dependent RNA polymerase. So this gets prepackaged. The virus then infects cells. And this, again, is a somewhat oversimplification. I'll show you more details in a second. But the virus attaches via glycoproteins on its surface, membrane proteins on the surface of the host cell. In this case it's a familiar protein to you called CD4. That leads to a fusion event. So now at the plasma membrane, which is different from what we saw with flu virus which happened in an internal membrane, at the plasma membrane there's a fusion event. So now the viral membrane fuses with the host cell membrane and the viral capsid can lead into the cytoplasm. There's a little bit of unpackaging that goes on. And then reverse transcriptase, that enzyme that got prepackaged there acts on the viral genome, which is made of RNA and converts it to a DNA form, a double-stranded DNA form which is called a provirus. And that provirus then gets integrated into the host cell genome in a random process, random integration, the host cell genome. This is also accomplished by a pre-packed enzyme called integrase. Once in the genome it gets treated like any old piece of DNA. It gets transcribed into actually multiple different RNAs. They get translated into viral proteins which come together at the plasma membrane and the glycoproteins, the capsid proteins which incorporate the genome, plus the reverse transcriptase, and you make a new particle. OK? So this is more or less the lifecycle. There is some detail that I want to share with you in a moment because it's interesting. This is what it looks like in real life. These are HIV particles budding off the surface of an infected cell. They have a very characteristic electron dense structure. You can actually tell it is HIV from just the way it looks in the electron microscope. And this is an HIV infected T cell. Now, CD4 should have rung a bell that it's one of the proteins on the surface of helper T cells, CD4 positive T cells. That is a major cell of infection for HIV. And here is one such cell infected. And all these little blebs budding off the surface of this cell are viruses getting out. This process actually doesn't itself, the budding process doesn't itself kill the T cell but, nevertheless, T cells, this class of T cells dies. And, actually, that's a critical event in the development of HIV-AIDS, the disease. We'll come to that in a second, but let me first tell you one of the interesting details. The situation is a little more complicated than the figure that I gave you on that slide. There are two glycoproteins on the surface of HIV. One of them is called GP120 for glycoprotein 120. It's linked by a disulfide linkage to another protein called GP41. And we now know that both of these proteins participate in binding and fusion. And on the surface of the infected cell is CD4, as was indicated on that slide. And that is the major viral receptor that is present on helper T cells, CD4 positive T cells. It's also present on macrophages. As well as certain cells in the brain called glial cells. So there are CD4 positive macrophages and glial cells. AIDS-associated dementia, which you may know about, is caused by the destruction of the glial cells in the brain. Now, there's another protein which participates, not so much in the binding but in the fusion event that allows the virus to get in. And this is actually a class of proteins called chemokine receptors. There are actually multiple chemokine receptors on your cells. And, in fact, different HIV forms bind to different of these. But just to simplify, let's say that there is one of these which binds to the GP41 portion. And it's that binding that allows the virus to fuse. If you don't have this you cannot fuse. And, therefore, the cells are not infected. What's interesting about that is two-fold. One, it could represent a therapeutic target. That interaction could be a therapeutic target. But it came to light in an interesting way which was there are people who have been followed a long time who lived in high-risk populations, drug-users or homosexual men with multiple contacts with known HIV infected people who have not themselves gotten HIV. They seem to be naturally resistant to the development of HIV, despite documented exposure to the virus. And they were studied. And when that observation was made it was found that these individuals lack the chemokine receptor. And if you lack the chemokine receptor, you may have CD4 on the surface of your cells but you don't have that critical co-receptor. And so there can be binding but no fusion. So your cells are resistant. So these individuals are naturally resistant to HIV. OK. So HIV infects. It infects a lot of T cells, helper T cells and other cells in the body. Why do you get sick? Well, as I mentioned, people get sick because of the loss, the absence -- During infection, the CD4 positive T cells get eliminated. How exactly they get eliminated, why exactly they get eliminated we don't yet know, but we know that the levels of these cells goes down inside the infected individual. And, as you probably remember, CD4 positive T cells -- -- are required for the development of these cells. So if you don't have CD4 positive T cells you cannot make antibody producing cells. You cannot make functional antibodies. And, to some extent, they're also required for the development of cytotoxic T cells. So that part of the immune system is also compromised. So the virus wipes out these cells and, therefore, the immune system crashes. And that's why it's called AIDS for acquired immunodeficiency. Acquired because it comes

system crashes. And that's why it's called AIDS for acquired immunodeficiency. Acquired because it comes through an infectious agent. Immunodeficiency because your immune system crashes. And when your immune system crashes you cannot fight infections. And AIDS patients largely die because they get one or another type of infection. So this is a graph which shows the time course of HIV infection. And it kind of makes the point that T cells go away. Shortly after you are infected, the level of HIV in your blood goes way, way up, as you can see here. It spikes in the first several months after your infection. Some people actually manifest that aspect of the disease. Many don't so they don't know that they have such a high concentration of virus. And, importantly, your body deals with it so you make antibodies, in black. You make anti-HIV antibodies. And, remember, it's these that are detected by the AIDS test. If you've been exposed then you make antibodies. And they stick around so that you know that you've been infected. Likewise, T cells, cytotoxic T cells, CD8 positive T cells go up. And that controls the level of the virus. And it stays low for a while, but after a while, because of the affects on the CD4 positive T cells, the other cells in the immune system are no longer sustained so the B cells start to drop off and the T cells start to drop off. And now you're starting to lose the battle against the HIV, so the levels of HIV go up. And as they go up, you lose more CD4 positive T cells and the situation gets worse and worse. So after a while, usually in the several years out time point, your immune system is now no longer functional and you're very susceptible to opportunistic infections. So AIDS patients will develop infections, and the infections will progress in a way that won't happen in a healthy person. Certain bacterial infections that you would clearly very easily take hold and can cause major problems. Kaposi's sarcoma is caused by infection of this particular virus. AIDS patients are very susceptible to tuberculosis, this particular fungal infection, certain other viral infections. And in aggregate, the exposure to these various infectious agents and the damage that they're causing leads to the death of the patient. OK. So that's bad news. And obviously it's have major and devastating consequences around the world. So what can we do about it? Well, there is some good news here. There are antiviral therapies. Remember that viruses largely use your enzymes. And you cannot make good drugs against your own enzymes for treatment of a viral disease, but I've told you about one viral protein to which you might be able to make drugs. And indeed we have. HIV has an RNA genome that gets converted to a DNA form by an enzyme reverse transcriptase. There have been drugs produced that block that enzyme. And you've probably heard of the drug AZT. There are other drugs like dideoxyinosine and dideoxycytosine that likewise bind to and block the activity of this enzyme. They actually cause chain termination in the same way that DNA sequencing works. And they work because this enzyme is sensitive to these drugs at concentrations where your polymerases are not, so that gives you some selected effect in the treatment of the virus. And these agents, when used singly, work for a while. And then the virus becomes resistant and comes roaring back. How does it become resistant? It becomes resistant through mutation, exactly the way cancer cells become resistant to targeted therapies. Variant forms of HIV arise which have slightly altered reverse transcriptases that now won't bind to AZT or DDI or DDC. And the problem is very, very serious because viruses, RNA viruses in particular are highly mutagenic. They create variant forms at high frequency. An HIV infected person makes ten to the tenth viral particles a day. At a mutation rate of ten to the minus fifth, that would mean ten to the fifth variants of every nucleotide. OK? So variants come up frequently that are resistant to this therapy. Oops. Fortunately, there are alternatives. The viral RNA gets translated into a large polyprotein -- -- which is processed by a viral protease encoded by the virus into different mature proteins. Reverse transcriptase is one of them. The integrase that I mentioned is another. And there are others. This is a viral protein, a viral enzyme. And so, in theory, it's possible to make inhibitors against the protease. And that's been successful. Several companies have made inhibitors that will specifically inhibit the viral protease that work well. They work for a while and then resistant forms arise for exactly the same reason I said. You can create versions of protease which still work but won't bind the drug. OK? So what do you do? Does anybody know what you do to overcome this problem? You put the drugs together. So currently HIV patients, in this country anyway, and hopefully soon around the world will get triple therapy when they're diagnosed. And triple therapy includes two reverse transcriptase inhibitors and one protease inhibitor. Since the development of resistance against each of these agents is independent of the other, to get resistance to all three is the product of the individual frequency. So I said that the frequency of developing resistance to any one is ten to the minus fifth. To be resistant to all three would occur at ten to the minus fifteenth, which is highly, highly unlikely. And so people tend not to develop resistance to all three and, therefore, triple therapy tends to keep the virus in-check for sustained periods of time. As you probably know, this works. The fear is that it is starting not to work. And there are starting to be some resistant forms developing here and they are starting to make their way into the population, so we're going to have to develop even better and more different inhibitors to now combat these variant forms. Before I let you go, the last thing I wanted to mention, and I'll just throw it out there and you can think about it, is that what we'd really like to return to for HIV is what we can do for polio. We'd like to be able to treat people routinely in this country or out in the field in more distant regions with an effective vaccine, which isn't possible today. We don't have one for HIV. You might think about what you would do to make one and why it has been so hard. And I'll touch on that next time.