BARBARA IMPERIALI: What I'm going to do first of all for today, the bulk of today's lecture will be on HIV and Ebola viruses, with more time spent on HIV because it's potentially one of the mechanistically best understood of the retroviruses. And it also has offered numerous opportunities for therapeutic intervention. And there are a lot of themes and terms that I can bring up as I talk to you about the HIV virus, because it's just a great exemplar of the viruses. OK.

So HIV is what is known as a retrovirus. So that sort of designates that there's something working backwards in this retrovirus. Based on its number in the Baltimore Classification, it is the most recently identified mechanism of viruses. HIV stands for Human Immunodeficiency Virus. And when you hear the term HIV, you'll often hear HIV/AIDS where the AIDS part stands for Acquired Immunodeficiency Syndrome.

So what you can start to tell about the name-- why am I hunching down-- is there's something related to the immune system with respect to this virus. And in particular, it is that this virus targets, very specifically, a population of the cells that are critical in immunity. And when the virus attacks those cells, the person infected with the virus becomes immunodeficit.

So I've talked to you about different viruses. Some go to the liver. Some go for other organs. So like the liver, the viruses that really cause a deficiency in liver function, but this one is very specifically deficiencies in immune function. And it has been a tremendous challenge developing vaccines against HIV/AIDS because of the problem with the immune system.

The other thing that I mentioned to you in the last class was that HIV is often found co-infecting with TB patients, people with those microbial infections. And that's because the immunodeficiency makes you more susceptible to tuberculosis. And oftentimes there's a co-infection with the two diseases. And it makes the people with TB really even less able to combat the TB infection. So that is really what gets a lot of the TB patients. So that's an important aspect to know. And it's also an important aspect to be aware of when treating people for any infection is if there's an HIV component, the body is much less able to deal with mounting an immune response because of the fact that the immune cells are targeted.

So HIV is an envelope virus. That means it has a membrane around it. It is this sort of peculiar shape where the outside of the membrane is coated-- the outside of the virus is coated with membrane. And then stuck in that membrane are particular proteins that are very critical for

interaction with the human host cells. So these are often termed GP proteins. And the one very important one is GP140. And that GP stands for glycoprotein.

Now, an important aspect, remember, of virus lifestyle is that they basically exploit all of the human cellular machinery for their benefit to make their proteins for all of the basic needs of a cell. So these glycoproteins look like the glycoproteins that are made by humans, because they're being made by the same machinery that we would make the glycoproteins. In bacteria, when we talked about them, it's a different machinery that glycoproteins and glycoconjugates look different. But in HIV, the glycoproteins look like our glycoproteins. And therefore, you could more or less consider the HIV virus particle to be coated with things that look sort of human. So that's a kind of decoy to the human system anyway with respect to recognizing a foreign entity. So often the glycans there serve as decoys to make the human body think, well, there's nothing wrong here.

Now within the virus, there is RNA. And it's single-stranded but negative strand RNA, so that's what puts it into-- Sorry. Positive strand. Positive. So what puts it into a separate category is the fact that the genetic material in the retroviruses is single-stranded RNA and it's the positive strand.

So far we've talked about two other types of virus mechanisms-- the double-stranded DNA-- remember that was as an example. That was smallpox-- and we've also talked about the single-stranded RNA negative strand. And our best example there was the influenza virus, which is a segmented genome. What you'll learn about HIV is that it has a non-segmented genome. Meaning that this strand of RNA is one continuous strand rather than separate pieces as we saw in the influenza virus, which was the last example at the end of the last class.

Now, let me just take a quick look. So every virion, that would be a single viral particle, therefore will contain within its structure the RNA. And it will also contain two copies of a particular enzyme that are absolutely critical. When the virus infects a cell, there's no time for it to make anything. It needs a particular enzyme to get going with replicating that initial strand of RNA to start to come to make a DNA, double-stranded DNA copy of it, and then make a messenger RNA. So the enzyme that's important and there are two copies of an enzyme known as reverse transcriptase.

So basically, the virus structure is fairly simple. There's a capsid inside with the RNA plus the

reverse transcriptase that I'm just showing as a filled circle here. And then surrounding the virion is a membrane bilayer with GP proteins stuck into that membrane bilayer that are going to be important when one starts to think about the infectivity by the virus. All right. There's a second glycoprotein that's also important, which is known as GP41. And these are very specifically part of this complex, but associated with the GP140.

All right. So now let's take a look at the virus life-- or details of this virus. But first of all, I want you to take a look at T-cells. You've heard a lot about T-cells. They're a major component of the immune system. So I'm going to home you right in on what the virus, the virion, recognizes on T-cells and what makes it such a disaster for the human immune system.

OK. So here are typical T-cell receptors on the surface of T-cells. Just as a recap, I want you to remember T-cells produce unique antigen binding proteins that are put onto the T-cell surface. And they provide cell-mediated immunity by destroying antigens such as viruses.

But what the HIV virus does is it really homes in on the cell-surface receptors of the T-cells, specifically the HIV virus recognizes the CD4 receptor, which is a glycoprotein on the surface of some T-cells that are designated as CD4 positive T-cells. So if you have circulating T-cells, that would be the first place where the HIV virus attaches to the T-cell to cause infectivity, thus debilitating the ability to mount the type of immune responses that T-cells are involved in. So it's very important to now understand why the physiology of this virus is to knock out the thing that's there to protect us from foreign antigens, which makes it so serious.

Now, HIV first started to emerge in the early '80s. It was more or less designated as a death sentence. As you may be aware, HIV circulated around the gay community in the San Francisco area very rapidly. But it then became incredibly widespread. There's a possibility that the jump of the virus from primates to humans, the virus might have originated in Africa. There's a lot of work done on the origin of this virus, but it sort of came out of nowhere. It was something that was unexpected, unanticipated, and hitting people just like a cannonball. And it was literally a death sentence early on.

As the mechanism of the virus became understood, a number of strategies could be put in place with respect to antiviral therapeutics to target this particular virus. And what we will see is the four types of targets that have been named as targets for therapeutic agents, why they work, how they work. And we'll see the mechanisms of those as to how they pertain to the HIV virus. And we'll talk about things like combination therapies.

So early on, there was really nothing. Gradually early, therapeutic approaches started to emerge, but they were devastating treatments because the drugs were so poor against the HIV virus, people were taking massive handfuls of medications a day to try to stay ahead of the virus. As time has progressed, these therapeutic agents have been improved and improved. And now they're relatively simple therapeutic agents where you don't have to take massive doses of fairly non-specific therapeutic agents. But they're quite sort of workable.

The problem is, and you'll see why, people have to take these therapeutic agents for life. Once cells are infected with HIV, there will always be a population of the virus genetic material in the human genome. So you can't just say, I feel way better, not taking these drugs anymore, because there is the chance that in some repository in some of your cells the virus genetic material is still there. And you'll see which enzymes in HIV are responsible for that. OK?

So let's take a look here at the Group VI retrovirus named HIV/AIDS. In 2015, there were 37 million people infected. Nowhere near enough in treatment, about half of those in treatment. And about 1.2 million deaths. This may account also for people coinfected with TB.

I mentioned to you when we started talking about infectious disease, I really encourage you to go to the NIAID website. There is tons of really interesting information, not just from a clinical perspective but from a mechanistic perspective, from a treatment perspective. There's really important things. So I really encourage you if you are interested in infectious disease, the National Institute of Allergies and Infectious Diseases Component of the NIH is the one responsible for all the research on infectious disease. And in fact, for many, many years during the crisis with HIV, there were special earmarked funds for any research towards the development of therapeutic agents.

So what happens? How does this virus, this little particle that I've shown you here-- I should really show you this is the capsid. And I should really show you the membrane a little bit more encircling that with the capsid inside. So the cartoon that you see on the screen is more correct.

So here's the virion particle. It has inside it the capsid that includes the single-stranded RNA. It only needs to code for very few proteins, remember, because the virus just exploits everything else from the human host. And GP120-- sorry for that typo, please correct that. And GP120 is displayed on the surface of the virion. And in fact, it's GP120 that interacts with the CD4 receptor on the surface of a target host cell, a T-cell.

Now there is a second receptor that's quite important. It was not discovered until quite a bit later on. It's designated as the coreceptor. And the interaction of the virion with the host cell is only good when there's both the CD4 receptor plus the coreceptor. And this was found through careful biology that realized there was another critical component for the infectious disease. And that is designated as the CCR5 receptor or CXCR4 receptor. So both receptors on the surface, the virus has evolved or selected out, to recognize cells that have both of those types of receptors on the surface which ends up making them very targeted towards the T-cells.

Once that association occurs, then becomes the process of fusion. So remember, I told you that large things can get into cells by merging a membrane with the membrane on the surface of the cell. So what is happening as those two receptors are engaged is the virus membrane is coming close to the host cell membrane and starting to fuse through the action of GP120 along with the CD4 receptor and coreceptor. And I'm going to show you a movie that shows that part of the fusion, because it's really cool to see it in action.

Once that fusion occurs, the virus membrane actually just merges with the remainder of the host cell membrane. And you drop that capsid into the cell, which then becomes on packaged to release the single-stranded RNA that's the contents of that. And what's important to know-- whoops going backwards-- is that the minute the single-stranded RNA gets into the cell, that needs to be processed. But it needs to be processed with an enzyme that is completely unique to the virus. And that's the reverse transcriptase. Without the reverse transcriptase being there, it's just RNA that's just going to get chewed up in the cell.

So what reverse transcriptase does it reverse transcribes RNA into DNA. So that gets you on the track to the infectious process. In the absence of RT, you don't get there. You're not able to make a DNA copy to then make a copy of the RNA and so on.

So reverse transcriptase, that is why the reverse transcriptase has to be delivered along with the virus. It can't be just a virus with the genetic material. It's got to have this particular enzyme with it. So reverse transcriptase is responsible for converting RNA into DNA. And as you all know, that is not the usual direction with respect to the central dogma. We're always talking about DNA to RNA.

But you've also heard throughout the course, how handy this reverse transcriptase is material is. When we talk about arrays and loads of biotechnology, using reverse transcriptase is super

valuable. Let's say, for example, we don't want to sequence a genome, we want to look at the transcriptome, the part of the genome that's really important for being translated into proteins. What we do is we collect the transcriptome but reverse transcribe it into DNA, which is a much more stable, tractable material for sequencing and so on and so forth.

So RT has become kind of a gift to biotechnology and biological research. And it was inspired from the RT in the HIV virus. So that's very important.

So the reverse transcriptase, then, makes a DNA copy of the RNA. And then a second complementary copy of that DNA is made to make double-stranded DNA which represents the viral genome, but in the format of a double-stranded DNA. That DNA can be, then, taken-- here's the fun part. That DNA is actually, then, taken into the nucleus and it's zippered in to the host genome by an enzyme known as integrase.

So it's not that you have this virus junk and then you send it back out again with the mature particles, you actually put this in the permanent copy of your DNA in the nucleus. And that is why people don't get cured from HIV/AIDS. They have to take treatment for the rest of their life, because that DNA is permanently in the copy of your genome that gets replicated every time your cells divide and so on. So you can understand the seriousness of that event.

So that gets taken into the genome. But also when it's time to make new virions, the DNA is transcribed into messenger RNA. The messenger RNA leaves the nucleus, gets translated into proteins. And those proteins form the basis of the new virion particles.

Now, I mentioned somewhere here that it is a non-segmented genome. So initially, the messenger RNA is a single segment that then gets translated into a single segment of protein, which is known as a polyprotein. So there are no stops between the various genes encoding frames within the messenger RNA. So that gets transcribed by the host machinery straight into a polyprotein that's all glued together in a single long piece. But then there is another important HIV enzyme, the protease, that chops those portions of the polyprotein up into useful and usable portions. So you also need the protease readily available rapidly to start dicing up the polyprotein into usable protein segments for the new virus assembly.

So let's take a look here what happens. The messenger leaves the nucleus. You make a polyprotein that gets digested into protein segments. And then everything accumulates at the inside surface of the membrane and starts budding off the virion. When it's first budded off, it's not really ready to go. It's not intact. It has to develop a little bit further to become a mature

virion moving forward to go infect another cell.

So the initial stage, that budded stage, of the virion is not completely competent to go infect. And in fact, there has been some thoughts about can you ensure that the immature virion doesn't develop into the mature virion, because that would be one way of targeting the system.

OK. So there are a few different pieces-- the reverse transcriptase, the integrase, the protease, and then, finally-- these are all critical processes that have been the targets of therapeutic agents. They are molecular targets, such as enzymes. And we know how to address enzymes with inhibitors. And one last feature of the virus life cycle that has been focused on respect to therapeutics is the viral fusion. So there have also been efforts at a molecular level to try to inhibit this process where your virus particle docks down on the cell and somehow the membranes fuse and then the virus dumps its contents into the cell.

OK. This is just sort of a quick sort of more beautiful view. But in a minute, I'm going to show you a video. But I thought it was kind of nice because it really allows you to look at the different steps in the virus life cycle-- recognition, fusion, delivery, making the double-stranded DNA, integrated the DNA into the genome, and making new viral proteins. So those steps, they're pretty logical. It's just like step one, got to find my target. Step two, I've got to stick to my target. Step three, I've got to deliver my genomic content into my target and so on. So I don't want you to think this is a bunch of steps to memorize. They all sort of make sense in a logical progression of events to make the virus be able to go all the way through to budding off new, immature, and then mature virions.

And so as I mentioned over there, these are the four targets that I'm going to describe to you with respect to therapeutic development. And this is where they all work-- the fusion early on, the reverse transcriptase taking that single-stranded RNA to a DNA copy, the integrase which zips the genomic material into the genome, and the protease that cleaves up the long polyprotein to make mature pieces of protein that are part of the virus.

OK. So let's talk first of all about viral fusion. And as I mentioned, when I get to showing you-- it's a really beautiful video from the Howard Hughes Institute that really shows the fusion very well. But I want to describe the fusion to you at a molecular level. And then later, you'll see it a little bit more real life.

So what I said to you is that GP120 and GP41, which are these proteins that are in the surface of the envelope then recognize the coreceptor and CD41 and form a complex. So it's a complex that involves what's on the virus. It's actually in a trimeric structure with the two receptors that are on the host cell. And they form a pretty close union. And then what happens is there are a series of events that tug the two membranes together, mechanically linked to conformational changes of the protein, and basically splosh the two membranes together for a fusion event.

So it's through the confirmational dynamics of that large complex happening in sequence after the first interactions that get you to a fusion event. So these initially look like this, but then they start changing their shape, and making a fusion event. So you could look at it as, initially, you have the complex formed, but then, later on, there's been a twisting of the structures, the large macromolecular structures to fuse the membranes.

So for quite a few years-- and I don't think it was the most successful of the approaches with respect to therapeutics-- people thought that maybe you could inhibit the interaction between GP120 and those receptors with small molecules that might inhibit the progress through the fusion process. So that was deemed a viable target. It's not the same as an enzyme target, but it's definitely a critical point in viral infections.

And what was made were short peptides that might stabilize this complex and avoid the structure moving forward to the fusion-related complex. So that was one sort of series of events. So those peptides were designated a C-peptide. They would bind to this portion up here that's pointed to with the N. They'd stick and they'd basically jam the cogs. They'd stopped that event occurring by binding quite tightly to part of the fusion complex. OK?

The drugs, the therapeutic agents, in that case were peptidic, kind of large. They don't diffuse into cells, but that's not a problem because this target is outside the cell. So there's no need for that to get into cells for its effect. All of the other therapeutic agents I'll describe to you are molecules that need to get into cells in order to do their job. But this particular strategy didn't involve that. Which was why it was fairly attractive early on, because it was a very different target from the others.

So the next step I want to describe to you is inhibition of reserve transcriptase. So this is a RNA-dependent DNA polymerase. OK? So different from the other DNA polymerases. We have DNA-dependent DNA polymerases, but we don't have the RNA-dependent ones.

And so the types of strategies that were used initially was to use types of analogs of the nucleosides that will be used in polymerization. So the reverse transcriptase, the first types of targets-- what's going on down there-- the first types of targets were nucleoside analogs because they can inhibit the polymerization. And you're going to realize you know quite a bit about these when we first look at the structure.

So I'm going to describe to you what are known as nucleoside analogs. What does this term mean? Nucleoside means it's a nuclear base plus a sugar. There's no phosphates. Because, remember, there's an s there. And it's an analog meaning it's not one of the native ones, but it-- that looks good.

AUDIENCE: I'm looking for [INAUDIBLE]

BARBARA IMPERIALI: OK. I'll grab her. And you get the bag.

[LAUGHTER]

OK. So it's a nucleoside analog meaning it isn't the standard nucleosides that you put into your DNA. And the most critical first line actually became azidothymidine. So look, that's the thymide nuclear base, hits a sugar, but it's different from the regular ribose that's either in RNA or DNA. And it's two deoxys so it looks more like a DNA building block.

But check it out. On the three prime site, there is azide. What do you think this thing does? Or have I already told you? Yeah. What do you think this thing does to stop the virus marching forward and turning its RNA into a DNA copy? Can it polymerase? No. So what do you think? So what how does it work?

AUDIENCE: It blocks [INAUDIBLE]

BARBARA IMPERIALI: Yeah. Yeah. It just grinds to a halt. What technology does this remind you of? Yeah?

AUDIENCE: The sequencing with the [INAUDIBLE].

BARBARA IMPERIALI: So it's really cool. I mean, we're using something like this for sequencing blocking the chain growth to get pieces so we can tell the sequence, but this is a very viable drug for reverse transcriptase for kind of similar reasons, because it stops translation. It stops polymerization.

Now, there's one important thing that I need to tell you about this AZT, as it's termed, azidothymidine. AZT is what is known in the industry as a prodrug. It's not the actual drug. Why is it a prodrug? What has to happen to it to be useful? Why is it handy to have a prodrug? I know there's a lot of questions there, but they all kind of tie together.

So it's a prodrug. It can get into cells, right? What has to happen to it for it to be useful in inhibiting reverse transcriptase? Because it won't do it all on its own. Look at that structure. There's something missing in that structure. What are the building blocks in the polymerases? Are they nucleosides? No. Up there.

AUDIENCE: [INAUDIBLE]

BARBARA IMPERIALI: Yes, exactly. So you have to convert this neutral molecule that can sneak into a cell by kinases that put phosphate groups onto that molecule. So that that molecule can get engaged in polymerization, you can put one nucleotide onto a growing strand, but then it can't go any further because of that azido group at 3, which would be the normal place you grow.

So prodrug strategies are very useful. It's a way of having drugs that aren't quite drugs yet but they become drugs once they are at their location. It's not handy to use the triphosphate of AZT as a drug, because it can't get in the cell. So we deliver a cellulary available molecule. We let the promiscuous enzymes in the cell convert it to a triphosphate. And then it becomes a real drug, a mature drug.

So I think that's a very important thing about these types of therapeutic agents. And what I want you to know is that many, many viruses are treated with nucleoside analogs performing similar sorts of things. But in some cases, there's a lot of different tailoring goes on with the nucleoside analog. It doesn't always work to have the azide at C3 prime. Some of these features don't always work. So these have to be tailored in different ways to target different viruses.

Because in this case, AZT hits reverse transcriptase. But there may be a different flavor of polymerase enzyme that you need to inhibit in other organisms. So it's a lot, lot, lot of options. Yeah.

AUDIENCE: Does it only affect RT? Or could there be side effects of the cell, like an old using it by accident?

BARBARA IMPERIALI: There can be, but I think the most important thing is that RT is very promiscuous. So it's much more likely to pop this building block in. And that doesn't have the mechanisms to reverse that reaction, all of the clean up that gets done with normal DNA polymerase. So our DNA polymerase is going to be a lot more tuned up to avoid these mistakes. But the viral reverse transcriptase just churn through in making it. So we're protected by our own proofreading and other types of mechanisms to avoid it really messing up replication. But that's a very good point.

OK. So remember the central dogma. And this is why this process confounds that. And that's azidothymidine. And we've already discussed that.

So the next enzyme that became quite an important target, because it was a new target, was integrase. So I will tell you, though, that reverse transcriptase inhibitors like AZT were used early on, but people with the virus rapidly developed resistance to that therapeutic agent. It was one enzyme that was just mutating like crazy. And the organism could slip through that net. So the development of therapeutic agents didn't stop with AZT. It was, like, we need to hit other targets for us to have a therapeutically viable combination of therapies that can really stop this virus effectively.

So a next enzyme that was targeted is integrase. So this is the one that's been targeted most recently. And there are currently several integrase inhibitors. Integrase is a fascinating enzyme because it really is the enzyme that actually stitches that piece of viral DNA right into the human genome just so it's able to be replicated, transcribed, and translated at any time. So integrase has been targeted. It was a tricky target to hit. But as you can see with several of these drugs, they are successful drugs.

One thing I want to remind you about, a lot of the names of the agents, if you see A-V-I-R within the name of a therapeutic agent, it means it's an antiviral. So that's a clue that all of these compounds are given names-- raltegravir, blah. I don't know who comes up with these names, but nevertheless, they're all names that end in A-V-I-R. So if you see a medicine and it ends in A-V-I-R, it's an antiviral agent.

Now, the last of the really fascinating enzymes that was targeted is the HIV protease, because the HIV protease is very, very different from many proteases in mammalian biology. It's a simple dimeric structure. So it has quaternary structure. It has two monomers that are 99 amino acids each. And each monomer contributes an aspartic acid to an active site. And those

are involved in the mechanism.

And what generally happens when substrates or inhibitors bind is these two flaps-- one of them I've shown you in magenta-- close down and close up the active site. And ritonavir was one of the first generation protease inhibitors that sat in the place where the substrate would normally sit in the HIV protease.

And remember that the HIV protease is critical for maturity of the virus because it chops up the polyprotein into the necessary enzymes. They can't be functional enzymes in the polyprotein. They have to cut up into the appropriate pieces. So the HIV protease had unusual specificity. And as soon as it was discovered and structurally characterized, every major pharmaceutical company jumped on-- this was in the late '80s-- to make successful protease inhibitors that just got better and better. Ritonavir is an example of first generation, but I think now we're on third generation-type inhibitors which are just a better and better and much more selective and specific for the HIV protease.

And because they're much more potent, instead of needing grams and grams of an inhibitor, you can get away with milligrams of inhibitor, which made the therapeutic dosing for HIV much more palatable is sort of a good word. Because when you have a very promiscuous protease inhibitor, like the early ones that were discovered, they mess up your entire GI because your GI runs on proteases that digest your food. So the original, the early protease inhibitors had a lot of side effects. The most modern third generation are very viable.

And this just shows you what other topic I want to hit on next is here's the green and cyan two monomer units of HIV protease. In yellow is an inhibitor that is bound. And just on one monomer of the two monomers, I've highlighted, in red, different resistance sites. So when any of those sites mutates, the protease stops being inhibited by the HIV protease inhibitors. Because you want to think about it, one mutation in one subunit, actually, will have a corresponding mutation in the partner subunit that is in the dimer. So each mutation ends up being two mutations in the full enzyme that has quaternary restructure.

So these are major. They go all the way out to the outer parts of the enzyme. It's not just around the active site. It's just all over the enzyme you can get mutations that stop the typical drugs that are targeted at the protease's binding

So here I just show you these are data that show for the protease and for the reverse transcriptase. Within the length of the protease, for example, there's mutations all the way

along. I don't know why this is 101. The numbering system is a little different in some variants. But you can see which mutations will add to drug resistance. And there's a similar picture here for reverse transcriptase. OK? So this is a very serious issue.

So now I'm going to give you a little break here.

[VIDEO PLAYBACK]

- So this is HIV. It's a typical retrovirus, meaning that it has an outer envelope. And in the center, it has two copies of RNA, as well as an enzyme here in blue that's reverse transcriptase, which will ultimately turn that RNA into DNA. The virus itself, with this outer envelope PROTEIN actually directly infects T-helper cells.

The way that it does this is that as it comes up to the cell surface, it uses receptors that are on T-helper cells and exclusive to T-helper cells, which are CD4 molecule which really defines T-helper cells. It's a surface receptor that binds to the envelope protein. That causes a conformational change and allows a second receptor to grab hold of the envelope. This is the chemokine coreceptor. It's also called CCR5. And we'll talk about that more.

What happens now is that the stock of the envelope protein pierces through from the virus into the host cell and starts to draw the cell membrane and the viral membrane together. And what ultimately happens is fusion of those two membranes and the viral genetic material is injected, essentially, into the cell. And the envelope protein is left at the cell surface.

- So that's the fusion process that's so hard to explain.

- The virus has a matrix and a capsid protein, shown here in green and red, that essentially are digested when it enters into the cell. That releases the viral enzymes and the viral RNA. And here we have reverse transcriptase, which takes the viral RNA, and using host nucleotides converts that viral RNA into a single strand of DNA. While it does that, it makes some random errors, which is characteristic of reverse transcriptase. It has very poor proofreading activity. That single-stranded DNA, now, is again reverse transcribed into a double-stranded DNA.

At that point, another enzyme that has come in with the virus in the beginning, called integrase, essentially grabs hold of that double-stranded DNA and carries it through a nuclear pore into the nucleus of the cell. Within the nucleus of the cell, it finds the host chromosome.

And basically, the integrase enzyme, makes a nick in the host's DNA and allows for HIV to insert itself into the host chromosome. And that right there is what establishes lifelong infection.

Now, RNA polymerase comes along and makes its messenger RNA.

- That's the host polymerase, it's just back to normal now.

- Those messenger RNAs encode for different viral proteins. They end up associating with ribosomes at the surface of the rough endoplasmic reticulum. And here is a piece of mRNA that's making envelope protein which is directly produced into the endoplasmic reticulum. And its shuttled, then, through the endoplasmic reticulum and taken to the cell surface, where, at the cell surface, it becomes embedded in the cellular membrane. And at this point, coalescing with other envelope proteins that have been produced you have this cluster of envelope proteins now on the surface of this infected cell.

At the same time, there are other messenger RNAs that are being produced that allow for translation of other viral proteins. So here are additional viral proteins being made which are going to be used to make up the key components that the virus, ultimately, is going to need. These are transported again to the cell surface, to the area where these envelope proteins are. And a strand of RNA, as well as some of the enzymes are part of that complex.

This then buds off at the cell surface at this point, but it's still not a mature virion because the polyprotein chain needs to still be digested into its component parts. That's done by an enzyme called protease. Protease breaks up those polyprotein chains and, ultimately, allows for them to coalesce and form the mature structures that make up the final virion. And now you have a mature infectious virion that can go on now to infect other cells. Once that happens, now, the cell can produce tons of viruses. And this is really what, then, keeps the whole process going.

[END PLAYBACK]

BARBARA IMPERIALI: OK. All right. So I have gone monstrously long on HIV. So sadly, I cannot get you to Ebola. But I'll post those slides anyway.

This question just speaks to combination therapies. So combination therapies are very, very important, particularly in cases where resistance to individual therapeutic agents against an individual target can happen. So HIV mutates its proteins very, very rapidly. But the likelihood

that it will simultaneously mutate two targets in one cell is much lower. So combination therapies basically are there to avoid drug resistance that occurs, usually, because of the high mutation rate, not because one drug has a short half life, not so that multiple strains can be targeted, or to decrease the number of side effects. Obviously, combination therapies give you more side effects, but they are really important in mitigating the disease.

So I was going to talk to you about vaccine development, but I will discuss this with Professor Martin to see if he wants to take that over. But I want to leave you with one last thing. OK. Three small points. On next Wednesday, this is Fred Flintstone. I tend to channel Fred Flintstone because he wears this nice blue scarf.

Next Wednesday is the last of the classes. We've got some good stuff lined up for you. We're going to have the topoisomerase demo from some people who submitted a topo demo for us. And then we're going to do 7.016 *Jeopardy.* Now, don't think this is going to be cheesy. It's going to be tough. And it's going to be fun.

And we're all going to be involved. Professor Martin and I will be the hosts. He's promised to dress up. Ha ha ha. Jackie will help me run the program, because it's in a real *Jeopardy* program. And Hannah and David will keep scores. And we'd like to encourage you who come to be in teams of two. Otherwise, there will be kind of a lot of you.

So if when you come in, if you sit on that side of the room, put your two names on these boards. And if you're on the other side, put them on these boards because Hannah and David will be posted there to keep score. And we will muster up some small prize for the winner here.

So, yes, question.

AUDIENCE: Will this count as the final?

BARBARA IMPERIALI: No. That would be a heck of an incentive, wouldn't it? What this will do for you is actually kind of help you refresh a lot of things, maybe identify blind spots. It was worth asking. It was a good question, yeah. So I'll post the questions afterwards as well. So you'll be able to kind of say, guy, I don't know what this question was about at all. And you can go and review a little of the material. Nice try, though.

Oh, and there was one other thing. I just want to remind you that a vote is a vote. This is a democratic society. So you should please take time to fill in the evaluations on the course. OK.