



Futures in Biotech, 32: Controlling HIV Evolution

Leo Laporte

Bandwidth for Futures in Biotech is provided by Cachefly at cachefly.com.

Marc Pelletier

This is Futures in Biotech, episode 32 for Wednesday June 4, 2008: Controlling HIV Evolution. Futures in Biotech is brought to you by audible.com, the Internet's leading provider of spoken word entertainment. Get a free audio book download of your choice when you sign up today. Log on to audible.com/biotech today for details.

[Music]

Marc Pelletier

Welcome back to Futures in Biotech. I'm Mark Pelletier. It's kind of the reverse of how I normally do it. Anyway, I just want to start off the show by mentioning how the summer of 2008 is going to be an incredible summer for science. It's no news to anyone, the Phoenix mission just landed a probe into the Martian Arctic and it's there to determine whether or not the conditions exist to sustain life. They're going to be looking for organic molecules which could be relics of past or even present life; there's also the Personal Genome Project that's braving us into a new era of human genomics that's run by Dr. George Church at MIT; and lastly, the Large Hadron Collider is approaching readiness to explore the origins of the Big Bang. Right? They might bring us to an ultimate theory of everything. These are incredible projects of absolutely grand scale.

Nevertheless, there still remains a large dark cloud over humanity and that cloud is HIV. So our guest today is Dr. Ron Collman, who is Professor of Medicine in Microbiology at the University of Pennsylvania Medical Center, and also the Director of the Virus Cell Molecular Core at the Penn Center for AIDS research in Philadelphia.

Now this show should – this episode should have a warning label because it's probably one of the most hardcore biotech episodes we've produced in a while. Dr. Collman describes the molecular structure and pathology of HIV, but also with some great detail how HIV should be tackled. Now, the last part of the conversation was a private conversation, so it gets even higher level of hardcore biotech I suppose, but he did give me permission to air it and it was definitely fascinating. So, enough said; onto the interview.

Dr. Ronald Collman

All right. Otherwise we're going to have a graduate student pounding to show me results or something.

Mark Pelletier

So how many graduates do you have?

Dr. Ronald Collman

I have two students. I have two thesis students right now.

Mark Pelletier

HIV is outside my area of expertise and I guess I could always start the show this way. Kind of absolves me from asking dumb questions.

Dr. Ronald Collman

Right. What will be my excuse then?

Marc Pelletier

I had Ron Desrosiers on the show and he's the Director of Harvard's primate facility...

Dr. Ronald Collman

Sure.

Marc Pelletier

And works on HIV vaccines. He was saying that it was unlikely that we'll arrive at a vaccine, and if HIV is one of the best understood viruses, the big question is why?

Dr. Ronald Collman

[3:19] I think that there are some unique features of HIV that make it quite different from traditional viral agents where vaccines have been really very successful, and the notion that you can just make a decision to make a vaccine and then there'll be a vaccine, it – science doesn't work like that. I remember, gosh maybe 20 years ago – longer than 20 years ago, the Director of HHS at the time, Margaret Heckler, pronounced that within a certain number of years there'd be a vaccine and that's not the case.

So why is HIV different? Well, I guess there may be two or three or maybe more reasons why it's a greater challenge than a measles or influenza. So the first thing is that the diversity in HIV is unbelievable. For influenza, you need to create a new vaccine each year, because there's some diversity, the virus evolves. If you take the level of diversity within the influenza pandemic, in an entire year, that's less than the amount of diversity within a single individual infected with HIV, and the reason for that is that HIV mutates very rapidly. It has an unbelievable flexibility in the ability to accommodate mutations. Most viruses have a very limited repertoire of the mutations they can accommodate. HIV can accept so many different variations.

And then we're talking about an infection that lasts for years and years and years and is cumulative. So, it's not like influenza where people get an infection that lasts for a couple of weeks. Most of them recover, some don't recover but that's it. It's an acute infection. HIV is a chronic infection and all of those mutations accumulate. So the level of diversity in the population for HIV is huge and the notion of being able to come up with a vaccine to hit all of the different variants of the virus that a person might be exposed to is overwhelming. That's one reason.

Another reason is that the virus actually targets the very cells that are necessary to achieve good protection. It kills immune cells and so – and it may actually kill the immune cells directed against HIV more efficiently and more rapidly because the virus likes to infect cells that are activated. What cells get activated when you get a virus infection? Well, the cells that are directed against that particular virus.

Then, the third reason is that the coating of the virus, the envelope glycoprotein has some really remarkable features. It's covered with sugar molecules, glycosylation, and glycosylated proteins are very difficult for antibodies to attack. And so, it's got what some people have called a glycan shield. It's shielded sort of like Star Trek; when they put the shield up. And then, that viral envelope protein, which is where antibodies would have to attack, is also really flexible. It's both physically flexible in that it's got pieces that probably move around, and it's also genetically flexible in that it can evolve and mutate away from any antibodies.

Now, we know that people make antibodies that can attack the virus, because what you see is that somebody – most people will have antibodies that don't affect the virus that they presently have but they affect the variant that that person had six months ago. And then, the body will create new antibodies directed against the variants that are present at the current time, but by the time those antibodies mature and are able to attack the virus, the virus has evolved away.

Marc Pelletier

Wow. So, the virus has the incredible ability to evolve faster than our immune system? Just fast enough to beat our immune system?

Dr. Ronald Collman

That's right. All it needs to be is one step ahead.

Marc Pelletier

Viruses always seem to be that way.

Dr. Ronald Collman

[7:52] Well, most viruses are not. Most viruses – people generate good immune system – good immune responses and they clear the virus. You know it's only a small proportion of people who succumb to any viral infection. There are really only a few that clearly have the upper hand, and the two that come to mind are Hepatitis C and HIV. Hepatitis C about maybe 80, 85% of people who get infected maintain a chronic lifelong infection unless they get treated with antiviral therapies, and in about half of those people the virus can be cleared. But most people who get infected with HIV cannot naturally clear it. HIV nobody naturally clears it; at least nobody who's been identified so far.

Marc Pelletier

Could you tell us about the anatomy of the virus?

Dr. Ronald Collman

Yeah. So HIV is – it's a member of the retrovirus family and what makes the retroviruses so interesting is that for many years in biology there was a paradigm that information is contained within DNA, and information flows from DNA to RNA which is the intermediary, and then RNA becomes the code upon which proteins are made, and proteins form the building blocks of all life. And so enzymes are made of proteins, structural components of cells are made of proteins. And back in the 1970s, the iconoclastic concept came in that information could actually go from RNA to DNA, and that was called reverse transcription. And so, HIV is a member of the retroviruses because they go retro, they go backwards. Their genome when they are a particle, their genome is RNA, the information is contained in RNA. And what is amazing to me is that the RNA genome of HIV is 10,000 bases long. So it's 10,000 nucleotides and this entire epidemic, 25 million people dead, 34 million people currently infected worldwide is all because of this 10,000 little bases of RNA. So the RNA is within the centre of the virus. There are two strands so it's what we call a diploid virus. Not too many viruses are diploid. There are two...

Marc Pelletier

And they are not attached, it's not two – it's not a double stranded DNA, it's single strand?

Dr. Ronald Collman

It's two RNA strands. So they...

Marc Pelletier

Like two chromosomes, two very short...

Dr. Ronald Collman

Yeah, exactly.

Marc Pelletier

5,000 bases each?

Dr. Ronald Collman

No, they are each 10,000 and they encode for the same information but it's like having – animals may have two copies of each chromosome. And so each virus has two copies. That's actually one of the other reasons that the virus evolves so rapidly is because it can have two copies, they can be slightly

different. There can be recombination between them and that's another way of generating diversity. So these two copies of the genome which carries all of this destruction within its code is contained within a core that's made up of viral proteins that then is contained within a lipid envelope that's stolen from the cell when the virus buds out. And into the envelope are studded these envelope glycoproteins, proteins that the virus encodes. So this RNA genome-containing virus finds a cell. And if it has the right receptors it binds, enters...

Marc Pelletier

So these are proteins on the surface of, for example, a lymphocyte.

Dr. Ronald Collman

Right.

Marc Pelletier

These are little proteins stuck to the lymphocyte, that give lymphocyte it's – definite it as a lymphocyte.

Dr. Ronald Collman

[11:53] Yeah. The two main receptors are CD4, you know CD, it stands for cluster designation four, but it's a molecule that's involved in immune regulation that lymphocytes have CD4 lymphocytes and macrophages have. And then – so the virus first attaches to CD4 and then some changes occur in its envelope that change the structure of the protein allowing it to then interact with a second molecule. And the second molecule is either CCR5 or CXCR4 and these are receptors for small protein mediators but the virus has subverted them for its own use. And once it's attached to both CD4 and one of the second receptors, CCR5 or CXCR4, it inserts a piece of its envelope protein into the membrane of the cell, disrupts the membrane and allows the virus to fuse and dump its contents into the cell.

Marc Pelletier

So that's a package of RNA encapsulated by protein.

Dr. Ronald Collman

Yeah. The whole purpose of this capsule – the whole purpose of the package is to be able to transport RNA, this genetic material, from cell to cell, its mobile genetic elements. So then once the RNA and its associated proteins are in the cell, the RNA gets copied into DNA and that's the reverse transcription process. And then comes really the most dastardly aspect of HIV infection. That DNA copy that has all the genetic information of the virus migrates into the nucleus of the cell and it integrates itself into the host cell chromosome. And so in essence the virus becomes part of the host cell chromosome and once that happens, it can't be eradicated from the cell. So that little stretch of DNA that contains all the information to code for new viruses can either remain there quiet, which we call latency, or it can be active and direct the formation of new viruses. And so the only way to get HIV that's integrated out of the cell – well actually there is no way, the only way to get rid of it is to kill all of the infected cells. And so that's why the treatments we have for HIV are suppressing the virus. The notion of cure which is eradicating the virus from the body, that's at this point just not possible with our current concepts. It doesn't mean it's not possible with some revolutionary new concept, but the fact that the virus is part of that cell and will be passed down to any progeny cell, means that there is no way to actually eradicate the virus.

Marc Pelletier

Wow, that's an amazing thing. I suppose 10,000 bases, right? That encodes for how many genes?

Dr. Ronald Collman

So there are about 8 or 9 genes. Every amino acid is encoded by three bases and so what that means is in any stretch of DNA there are three possible reading frames and in fact, into this 10,000 bases, HIV is so efficient, all three reading frames are used and there are some pieces of the genome where all three reading frames are used at once. So every stretch – there are particular stretches of

the genome where the virus reads it starting with the first base, starting with the second base going every three, starting with the third base going every three and it's got these overlapping proteins that are generated off overlapping reading frames. It's unbelievably efficient.

Marc Pelletier

That's sort of like a crossword puzzle for genes, right? Where the same base is used by two genes?

Dr. Ronald Collman

Yeah. I think that's a good analogy.

Marc Pelletier

But unlike a crossword reading in three directions in one way and three directions in the other.

Dr. Ronald Collman

[16:00] That's right. Although HIV everything goes in the same sense. It doesn't use – you could say there might be six reading frames but in fact only the positive sense, only the positive strand is used because there is one single primary RNA transcript. So, once that DNA is in the chromosome it then is transcribed into RNA and the RNA gets cut up by cellular processes into different pieces, each piece will encode a different protein or a different set of proteins. That's called splicing and so that's one of the ways that the expression of the viral genome is regulated. Now you know, we think of retroviruses as the enemy and certainly for HIV it is the enemy. But some people will call them mobile genetic elements if you take the virocentric perspective. It's a mobile genetic element that just wants to pop from one cell to another and there is a long evolutionary history of mobile genetic elements and there are certain footprints that retroviruses leave behind. And there is a whole family of these mobile genetic elements that work fairly similarly. And when you look at the human genome you can see that a pretty substantial portion of the whole genome of humans was generated by mobile genetic elements inserting themselves in, in different places in the chromosome. So, in fact it's really contributed to human evolution in a positive way as well.

Marc Pelletier

Wow! So that's pretty scary. So does this mean we have genes within us that are not typically human?

Dr. Ronald Collman

Well, I would challenge you to say what makes them not typically human if they are a part of the primordial human or mammalian genome.

Marc Pelletier

I stand up to that challenge. Okay, here is a potential place. Our genome is inefficiently used, right. There are segments of DNA in a virus that get used by multiple genes where our genes just have a start codon and then it...

Dr. Ronald Collman

Yeah.

Marc Pelletier

Right?

Dr. Ronald Collman

Well, in general that's true and I'll take it even one step further which is if you take the whole human genome only a tiny fraction actually contains genes. Most of the human genome is made up of repetitive elements where sequences that don't seem to encode proteins, they don't seem to be obvious regulatory elements, and so it looks like it's extremely inefficient. So it used to be that people called the rest of this non-genomic DNA, junk DNA. Now, obviously it's not junk, it's there for a reason and I think maybe there is some accumulating evidence suggesting that it may have some role in

some aspects of regulation, but we just don't know what it does – what all of this non-genomic DNA in the human genome does.

I'll tell you something else that's really remarkable about HIV, is in the last – I don't know – about four or five years it's become clear that there's a dance between retroviruses and mammalian genomes. And there are at least two really well-described examples now of proteins in the mammalian cell, in humans, in mice and monkeys whose only function is to counter incoming retroviruses. And so there are hundreds and hundreds of retroviruses and humans are not susceptible to most of them. Unfortunately HIV is one that it is susceptible to. And these innate antiretroviral defense proteins are what protect the cell from incoming retroviruses. And so on one hand you could say that we are incredibly successful at defending ourselves from all of these incoming retroviruses. It's like a bombardment. On the other hand it's unfortunate that a couple have slipped through the defense mechanisms.

Now some people have looked at the evolution of the genome by comparing humans to monkeys to mice and other species and these proteins that serve as innate antiviral defense proteins are the most rapidly evolving portion of the human genome. So that says they are under incredible pressure. And so this dance between retroviruses constantly bombarding animals, people and the cells' efforts to defend against this incoming bombardment is one of the most powerful forces shaping modern evolution.

Marc Pelletier

I am certainly glad we can keep up with it to an extent.

Dr. Ronald Collman

Well, right, right, right. But not to quite as good an extent as we would like, you know, HIV has managed to slip by both of these innate antiviral defense mechanisms.

Marc Pelletier

[21:26] Well, let me ask you, see if I understand this. We have got an RNA which is the genetic information and it's wrapped around a capsid protein which is sort of a – how many different proteins are there in the capsid?

Dr. Ronald Collman

Well it's wrapped around – it's encased in the – there's nuclear capsid which is intertwined with the RNA, that is encased in a cone-shaped core made up of capsid protein and in fact the shape of that core looks just like an old-fashioned space capsule. It's got this cone shape and by electron microscopy it looks just like a space capsule. That then is contained within a spherical core – a spherical envelope that's made up of a lipid envelope underneath which is another viral protein call the matrix protein.

Marc Pelletier

The outer surface is the lipid with the glycoprotein?

Dr. Ronald Collman

Right.

Marc Pelletier

Where is the dance ultimately played? I am just trying to visualize where the evolutionary step is.

Dr. Ronald Collman

Yeah. Well, so when a virus comes into a cell, if you take a non-human cell. For example, people have been trying – people have tried very hard for a long time to put HIV-1 into monkeys and it doesn't work, it doesn't go. You cannot infect standard monkeys with HIV-1, and the reason is that they have this antiviral defense mechanisms. So the virus can bind, it can fuse, it can put its interior contents into the cytoplasm of the monkey cell and then they are degraded and they are degraded

because these antiviral proteins, one is called TRIM5-alpha, another is called Apobec will either actually degrade the capsid or it will screw up the process of reverse transcription.

Marc Pelletier

You were saying that the virus itself there's more variance in one human than the entire influenza population?

Dr. Ronald Collman

In an entire year's epidemic.

Marc Pelletier

Wow! I am trying to get an idea of where the virus is changing. But maybe I misunderstood. Is it the capsid? Obviously it's the RNA that mutates, but is it the RNA that encodes for the capsid or the glycoproteins?

Dr. Ronald Collman

Well, so there are two answers to the question. Where does the genetic mutation take place and then where does it have its consequences.

So it takes place typically at the reverse transcription step. So when the virus gets into a cell and it reverse transcribes there is an error on average one out of every 10^4 bases. So what that means is that in a virus whose genome is 10^4 bases long, each replication cycle, each virus will have an average of one substitution. Now in the average infected person, there is something like 10^{10} new viruses made per day. So what that means is that 10^{10} new virus every site is substituted at one out of 10^4 times, you can imagine that's something like 10^6 substitutions at every site in every person every day. Now, most of those may be deleterious ultimately but some of them are going to end up providing the changes that will allow that virus to escape from immune surveillance. Escape antibody control. Escape from antiviral drugs.

So the mutation can be anywhere, any one of the viral proteins. If it is in the reverse transcriptase protein, the enzyme that is the target of one of our most important class of antiretroviral drugs, some of those mutations will lead to resistance to the drug. Same thing if it's in the protease protein. If it's in the viral envelope it's going to lead to escape from antibodies, and so that's why there is so much diversity generated – if it's in the envelope it's escape from the antibodies; if it's in the reverse transcriptase, it's escape from RT inhibitors.

Marc Pelletier

So, it's everywhere.

Dr. Ronald Collman

[25:42] It's everywhere. That's the reason you almost always need to use three drugs in an infected person. If you just use one drug, let's say for the sake of argument that it only takes one mutation to become resistant to a drug. Fortunately for most anti-HIV meds it takes more than one mutation, but let's say for the sake of argument it only takes one mutation. So, what that means is if you only need one mutation and a person produces 10^{10} new viruses per day and each base is going to be substituted one in every 10^4 or one in every 10,000 viruses that means it's 10^{10} divided by 10^4 . That means that in a given day the average person is going to have 10^6 viruses produced that happen to have that resistance mutation and that's why developing resistance to antiviral drugs if you only give one is inevitable. If you give two, what that means is that somebody is likely to have 10^{10} divided by 10^4 times 10^4 .

Marc Pelletier

We are almost there.

Dr. Ronald Collman

Which means they have only a 10^2 – they are only going to have 10^2 viruses with two resistance mutations. A hundred viruses, well you know it may take a while for those 100 viruses to grow up and replace all the other drug-sensitive viruses but it will happen. Only when you get to three drugs do you have, 10^{10} new viruses, but you require 10^4 times 10^4 times 10^4 ; you would need to have 10^{12} viruses to have a virus that has the right mutation in each of the three required sites.

Marc Pelletier

By having the combination therapy you are actually suppressing evolution of the virus?

Dr. Ronald Collman

You are both suppressing evolution and you are making it extremely remote that there will be a preexisting variant with that combination of mutations.

Marc Pelletier

So you have to get this started early too?

Dr. Ronald Collman

Well, that's certainly been a complicated area. Does it really matter if you started early? It looks like, you know, the advantage of starting early is you would think that there might be less likelihood of resistance and you might think that there would be less immune damage. On the other hand these drugs all have toxicities and giving the drug always does lead to the risk of viral resistance. People miss doses, they may not be absorbed properly. There may be compartments in the body that don't get as high a concentration of drug. So the studies seem to show so far that it probably is just as good to wait until somebody has had some evidence of immune damage and that starting super early doesn't have on balance as much benefit as one might guess it would when you count the negative aspects, early drug resistance, toxicities of drugs. Every once in a while somebody is identified who has their primary infection – they initially get infected and there are studies going on now to see whether – if you actually start drug therapy when somebody first gets infected is there a benefit to that. But I don't think the answer is in on that yet.

Marc Pelletier

I would like to take a minute to thank Audible.com for sponsoring Futures in Biotech. It's a great source of audio content and I think they have over 45,000 maybe even 50,000 titles now, not entirely sure. Anyway my pick of the week is Freakonomics: A Rogue Economist Explores the Hidden Side of Everything by the economist Steven Levitt and co-authored by Stephen Dubner. Here's a short clip.

Stephen Dubner

So there is this economist at the University of Chicago named Steven D. Levitt and I, Stephen J. Dubner, was asked by The New York Times magazine to go write an article about him. And I like most people assume that he, as an economist was the kind of guy whose questions about life were whether interest rates should be raised by an eighth of a point or a quarter of a point. Or to try to figure out whether the economy was going to generate 250,000 jobs or 260,000 jobs, which are the kinds of questions aren't really all that interesting to most people. But Steven Levitt even though he is an economist uses economics as a kind of tool kit to ask an entirely different kind of question than most economists do. And as he explained it to me, if you think about it, this thing that we call 'the economy' is really this very complicated thicket of information and data and cause and effect and the idea is to try to measure what causes something else. Well, those same tools that can be used to measure the economy can also be used to measure things that to you and me might seem a whole lot more interesting. And those are the kind of things that Steve Levitt works with. So here are some of the kinds of questions that Steve Levitt comes up with instead of worrying about interest rates and so on. If drug dealers make so much money why when you go to a housing project where a lot of drugs are sold are so many of the drug dealers still living at home with their moms? Or when you hire a real estate agent to sell your house for you, how do you know that she is really looking out for your best interest and trying to actually get the best price for you? Steve Levitt is more of a kind of intellectual detective than anything, using the tools of economics to ask the kinds of questions that

you or I would love to know about the world but which we figure out somehow too complicated to actually figure out.

Marc Pelletier

[31:51] So, if you want a copy of Freakonomics head over to audible.com/biotech. It's a 14-day free trial and even if you decide to unsubscribe you get to keep the free book. So either way it is a win-win situation. Back to the interview.

This is pretty amazing. I don't know if I want to ask this question right away, maybe it is a good time.

Dr. Ronald Collman

Go ahead.

Marc Pelletier

This is a podcast, it's not – we are not bound by the rules of interviewing in the 15-minute sound bite. Have we learnt something by watching this virus over the years from the perspective of a bioengineer, a Drew Endy or a Craig Venter, who is trying to synthesize an entirely synthetic organism for the, you know, under control. And I am not saying engineering a virus here, but I am just saying let's make a protein that does a specific job, for example, convert biofuels, starches into ethanol at incredible rate. Have we learned something about the possibilities of engineering, I mean, evolution is just selective pressure where you just keep pressuring, but is there some way, some thing – some intelligent design elements that we figured out on the limitations based on the HIV that carry it over to bioengineering?

Dr. Ronald Collman

Well, I think there are a couple of aspects of that question that are interesting. One is that, we are still trying to look for the magic combination of resistance mutations that are incompatible with the protein's function. You would think that if you would require enough changes to become resistant, say to a drug that you would come up with a combination that just screws up the protein's function so completely it couldn't possibly be an effective functioning viral protein.

Marc Pelletier

It's amazing that it can keep mutating and mutating and still remain a functional HIV virus.

Dr. Ronald Collman

Yeah. Some of these mutations decrease the efficiency of the virus, that's why sometimes people who acquire drug resistance actually still do better if you keep the drug on board to maintain the resistance mutation than if you stop the drug. But we haven't found any magic combination that's completely incompatible with viral replication.

But I will tell you something relating to bioengineering that has been remarkably successful coming out of what we have learned with HIV. So there is a lot of effort for many years trying to use gene therapy approaches to either correct genetic diseases or introduce new genes into host cells and there are a whole bunch of different vectors that have been used. The one that is really pretty darn good are gene therapy vectors that are based on HIV. So the terrible thing about HIV is that it has the machinery to very efficiently integrate its genetic material into the host cell chromosome and then that genetic material is a permanent part of the host cell chromosome. That's what makes the virus so dastardly. At the same time that's what makes it potentially really useful as a vector.

And so a really widely used approach to gene therapy is to take the sort of the skeleton proteins of HIV and replace its guts instead of with the genetic material to create a new virus, replace its guts with the gene of interest. So if you want to put a particular gene into a cell and have it become part of that – insulin, for example, I don't know whether insulin has been transferred with gene therapy, but you know adenosine deaminase, the genetic absence of that or cystic fibrosis.

Marc Pelletier

Exactly, that would be phenomenal.

Dr. Ronald Collman

So put it in an HIV genome and use the virus's machinery for integration as a way of delivering it. The other thing that HIV does, which is also quite nasty is it does not require a cell to be dividing in order to transduce. Transduce is the word we use for viral insertion into the cell. HIV doesn't need a cell to be dividing. So HIV based vectors can be used for non-dividing target cells like neurons in the brain, gene therapy approaches to Parkinson's disease, to Alzheimer's disease, to Huntington's disease, other neurodegenerative diseases, you can use HIV to get genetic information into non-dividing cells. So, that's one place where although none of those therapies have come to fruition yet as useful clinically applicable therapies, that's one area where it has been very useful with lots of potential. It's nice to have some silver lining to this cloud.

Marc Pelletier

Well, the way I sort of got involved in the biotech industry and really those are just engineering problems to tackle more so than things that we will never be able to accomplish it.

Dr. Ronald Collman

[37:35] Yeah, I think it's true and you know, on one hand the last 25 years of HIV research – on one hand it's been disappointing in that we don't have eradication and we don't have a vaccine. On the other hand it's unbelievable what's been achieved in the last 25 years, we have more than two dozen medications that can suppress the virus. I remember when I was in medical school the notion that drugs could block viruses was iconoclastic. People said, oh, you can't block viruses, you know, because they just use host cell machinery. So, now we have more than two dozen drugs that can block HIV and just a couple of whole new classes have been introduced in the last couple of years. So the fact that you can take somebody and treat them in a way that can keep them healthy and doing well for, if everything goes well, an indefinite period of time is really a tremendous achievement. We have also learnt an unbelievable amount about the immune system, about immune system function, immune system regeneration through trying to understand HIV pathogenesis.

Marc Pelletier

Absolutely, this is an amazing story, is that an approach that's being used?

Dr. Ronald Collman

Yeah, it's a great idea and a lot of people are trying to use gene therapy techniques to insert something into the cell that could prevent expression of the virus. So, for example the virus has these control elements, promoter elements that turn on the virus when the cell gets turned on. I mentioned earlier that the virus can either be latent, not expressed, or if the cell gets activated, turned on, the virus gets activated and turned on.

So, people have developed HIV based vectors that can insert into the cell either an antisense piece of DNA that can bind and turn off or that can act as a decoy, so when the same signals come along that would turn on HIV it will turn on this anti-HIV protein or some sort of a gene. So, there's a lot of efforts to try and do that. None have really been very efficient so far. What we really need is a way to disintegrate the virus from the host cell chromosome, that's the only way that you really can eradicate the virus, other than by killing any cell that has the virus integrated. But we don't have the tools to do that. We don't have the understanding to do that. Is it impossible? I'd say it's impossible with our current understanding. But that doesn't mean that it's not going to be possible with the next revolutionary step forward in understanding.

There have been a lot of efforts to find ways of taking every cell that may harbor latent genome, turn on the genome by stimulating the cells in various different ways and then use that as a target for some type of anti-viral approach that could then kill the cells.

You know, one of the newest – or probably the newest anti-viral agent to be released, to be approved for clinical use, blocks the viral use of CCR5 on the cell surface. And that is a fascinating story. So we

know now that HIV requires CD4 plus, either CCR5 or CXCR4. And of those, CCR5 is by far the most important one. CCR5 is the secondary receptor required by virtually all viruses that transmit person to person. And it turns out that there's a naturally existing mutation of CCR5 of unbelievable prevalence.

So in the Caucasian population, ten percent of all of the alleles for CCR5 have a 32 base pair deletion, the same deletion that leads to failure of protein expression. And that means that one percent of Caucasians of European descent don't make CCR5. They're homozygous for the Delta 32 mutation. And those people are for all intents and purposes immunologically normal. So that suggested that blocking CCR5, which should block HIV entry, should be very well tolerated. And it turns out that that's true. And so just released within the last few months is a drug from Pfizer that's a CCR5 antagonist and it blocks viral entry.

Marc Pelletier

[42:35] Wow! So is this like a peptide mimic molecule? Peptidomimetic.

Dr. Ronald Collman

Well it's – fortunately it's not, it's a small molecule. It turns out that – I don't know, the number is I think about 60% of all drugs on the market are small molecules that block seven trans-membrane G protein coupled receptors.

And CCR5 is a 7TMGPCR and they happen to be very amenable to blocking by small molecule agents. There has to be a really perfectly aligned configured topography of the receptor in the membrane, it goes through it seven times. If you screw up the topography, it screws up the function of the receptor. And so small molecule agents are generally pretty effective at blocking 7TMGPCRs. And so this particular small molecule blocks the ability of HIV to use the receptor. And there's no –

Marc Pelletier

It's kind of like a small molecule vaccine.

Dr. Ronald Collman

Right. Well but what people are working on now is trying to use gene therapy approaches to down-regulate CCR5. Can you knock out CCR5 from a cell, or maybe even a stem cell? Suppose you take a stem cell, knock out CCR5 and then repopulate an individual's immune system with cells that lack CCR5? Will that shut down the virus?

Marc Pelletier

Well wouldn't the easiest way to go be to use a small molecule to block CCR5? Because at this time it's a whole lot easier to deliver a drug than to knock out a gene.

Dr. Ronald Collman

Yes, absolutely. Although the small molecule will require lifelong administration and it has to be done in the context of multidrug therapy because if you don't the virus develops resistance, it just finds another way of using CCR5. It adapts to this screwed-up CCR5 molecule. To me, one of the most exciting things would be to actually knock out CCR5 in a stem cell and repopulate the genome.

At a meeting that I was at a couple of months ago, I saw a poster from a clinical group that did an amazing experiment. They had a patient with HIV infection who developed leukemia. A common disease in a common disease. And the best option would be a bone marrow transplant. There was no related donor. And so they searched the bone marrow donor database. And they found not only a six out of six matched, unrelated donor, but they found one who was within that one percent of Caucasians who lacked CCR5, homozygous for the Delta 32 mutation. They took this guy, gave him a bone marrow transplant with matched stem cells that don't express CCR5, the transplant was successful, and they stopped the drug therapy. And they found no virus replication.

Marc Pelletier

That's an amazing proof of concept.

Dr. Ronald Collman

Unbelievable, unbelievable; there is a company called Sangamo, I guess out in California that has the patent rights on just a really cool technique that can do targeted knockout. They're called zinc finger nucleases and it's an approach that can actually knock out specific genes. And so one of the things that I think many people are waiting to hear about is whether they can target this in such a way as to knock out CCR5 from stem cells. And to me, one of the great pie in the sky but maybe not so pie in the sky therapeutic approaches would be to take an infected individual, knock out CCR5 from their bone marrow with one of these really Star Wars type of techniques, repopulate them with their own bone marrow that doesn't have CCR5. And see whether you have a new immune system that's resistant.

Marc Pelletier

[47:03] One thing I have learned in science is that you have to do lottery style experiments, right? You have to have your hook in the water to catch a fish and keep a really positive outlook. And when you see that there is evidence that this kind of thing can work you have to get to it, right? And how do you sleep?

Dr. Ronald Collman

Yeah, yeah, we call them high-risk, high-reward. Most of them are going to fail but boy! If it works it's really cool and important.

Marc Pelletier

Well, thank you very much for coming on the show.

Dr. Ronald Collman

It's been my pleasure.

Marc Pelletier

Before we get to the acknowledgements, I have an apology going out to Alexander Griekspoor and Tom Groothuis. They are the creators of a fantastic application called Papers. I did interview with them a couple of months ago and I promised to get it out this netcast but there is still a little bit of work to do on the audio. So it will have to go out next episode. Now Papers does for PDFs what iTunes did for MP3s. It is a fantastic tool for scientific writing. So for anybody who has piles and piles of papers on their desk or in their office, you can get rid of those. This is the ultimate writing tool.

By the way, this is not an advertisement, it's a software pick, but it's a software pick where I managed to convince them to give FiB listeners a listener discount. And we will talk a little bit about that next episode. You can go to their website at mekentosj.com and download a free trial and we will be talking about it next episode. So if you have any questions or thoughts about it, you can send me an e-mail and I will forward it to them. Again sorry, Alexander and Tom, I promise next week we will get the interview out.

So, I would like to thank Dr. Ron Collman for his time. He is from the Penn Center for AIDS Research in Philadelphia. I would also like to thank Will Hall for the opening and closing themes. I would also like to thank the listeners of the TWIT Network. Thank you for your donations. You help make Futures in Biotech possible. The transcripts for this show are being kindly provided by Pods in Print and you can get them at futuresinbiotech.com. For Futures in Biotech, I am Marc Pelletier.

[Music]