

**AIDS: Evolution of an Epidemic (2007)**  
**Lecture Four—Vaccines and HIV Evolution**  
**Bruce D. Walker, M.D.**

**1. Start of Lecture 4 (00:01)**

[music] [ANNOUNCER:] From the Howard Hughes Medical Institute, The 2007 Holiday Lectures on Science. This year's lectures, "AIDS: Evolution of an Epidemic" will be given by Dr. Bruce Walker, Howard Hughes Medical Institute investigator and director of the Center for AIDS Research at Harvard University. And Dr. Bisola Ojikutu, director of the Office of International Programs in the Division of AIDS at Harvard Medical School. The fourth lecture is titled "Vaccines and HIV Evolution." And now to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Thomas Cech.

**2. Welcome by HHMI President Dr. Thomas Cech (01:08)**

[DR. CECH:] Welcome to the final presentation in this year's Holiday Lectures on Science. Many believe that the only way to truly defeat the AIDS virus would be to develop a vaccine. But again, this is something that is very challenging because of the diversity and rapid mutation of the virus itself. In this final talk Bruce Walker will discuss the efforts to develop an effective vaccine against HIV and now here's a brief video about Bruce Walker.

**3. Dr. Bruce Walker in South Africa (01:52)**

[music] [DR. WALKER:] Edendale Hospital is a typical hospital within the Department of Health in South Africa, sees huge numbers of patients, health care workers are overburdened. You know you have to remember that all these hospitals were set up at a time when there was no HIV infection and now about 70% of the patients that are admitted to the hospital are HIV infected in that particular area. That doesn't mean that they're all coming in because of AIDS, but a large number of them are developing diseases because of their HIV infection. So it's been a tremendous burden on the health care system. We went to South Africa initially intending to establish a collaboration so that we could get samples to bring them back to our laboratory here. When we got there we rapidly realized that there was an opportunity to actually do the experiments on site. Moreover there were a lot of people that were really interested in doing research. So Africans that were not involved in research, they were watching patients die day in and day out and they wanted to do something about it and the end result of that is that we were able to build at the Nelson Mandela School of Medicine, the first dedicated biomedical research building to ever be built at an African university. The question is can we harness the immune system in a way to get it to do a better job in those situations where it's not doing a good job. The mere fact that some people are infected with HIV and not being bothered by it tells us that it's possible to control that infection and I think that's the direction that we really are headed in right now is trying to understand that and then trying to replicate that in other individuals.

**4. HIV drugs have revolutionized treatment (03:52)**

Good morning and welcome back to this final lecture in the Holiday Lecture series. You've just heard from Dr. Ojikutu about an incredible advance that science provided to the world. In 1987 the very first drug to treat HIV infection was released and now in 2007 there are nearly 25 drugs available to treat HIV infection and this has resulted in just a spectacular improvement in the lives of people that have had access to these drugs. But during that same time we've seen the epidemic continue to spread and as you heard over 33 million people are currently infected with HIV and the sad news is that 90% of these live in developing countries where it's much more difficult to deliver therapies and where the therapies are often not available.

## **5. Hopes for an HIV vaccine have existed for years (04:58)**

In 1984 when HIV was clearly shown to be the cause of AIDS, a statement was made by the then-Secretary of Health and Human Services, Margaret Heckler, essentially that the discovery of HIV would enable us to develop a vaccine to prevent AIDS in the future. We hope to have such a vaccine ready for testing in approximately two years. So let's go back to the timeline. Now in 2007 we still don't have an HIV vaccine and what I would like to do now is give you a sense as to why that's been so difficult and what the prospects are for how we might approach that in the future and finally I'd like to tell you a little bit about what our laboratory is trying to focus on to help in an effort that's clearly going to require contributions from many, many, many different laboratories.

## **6. How vaccines work (53:06)**

So let's start by talking about how vaccines work. Ideally you'd want to have a vaccine completely prevent infection. This is called sterilizing immunity. In general sterilizing immunity is conferred by antibodies that bind to whatever the pathogen is and prevent any even initial infection from occurring. On the other hand there's another kind of vaccine and this is actually the much more common type of vaccine that we have in use today and these are vaccines that prevent disease but don't prevent an initial round of infection by keeping the microbe or its toxins in check and this is called nonsterilizing immunity and this can be conferred by either antibodies or by particularly by cytotoxic T lymphocytes which we learned about yesterday. The goal of both of these approaches is to try and alleviate symptoms in the person who becomes exposed to the pathogen and also to keep the level of the pathogen low enough that transmission is unlikely to occur to somebody else.

## **7. Antibody-inducing vaccines provide immunity (07:01)**

So let's talk first about sterilizing immunity and about antibody-inducing vaccines that might confer sterilizing immunity. B cells, when exposed to a pathogen, are converted into plasma cells and start to secrete antibodies whose job it is to directly neutralize virus by binding to the outer surface of those virions. What we're trying to do with vaccines is to basically trick the B cells into thinking that they're seeing the pathogen so that they make antibodies that will already be there by the time a person may happen to then subsequently encounter the pathogen.

## **8. Ways of creating a vaccine (07:45)**

Now there are a number of different approaches that people have used for developing vaccines. One is to use a killed virus which then still contains a lot of the proteins that can be engulfed by antigen-presenting cells and induce an immune response, but you don't get any infection. Another is to change the genetic makeup of a pathogenic virus, meaning a disease-causing virus and turn it into a virus that causes less disease but still induces an effective immune response. Another approach in these days of molecular biology has been to develop recombinant proteins that one can administer again to give the body a sense that it's seeing the pathogen even though it's not the infectious construct of the pathogen that they're seeing and finally another example, and this is by no means a comprehensive list here, another example is recombinant viral vaccines, such as a common cold vaccine—adenovirus—that can be genetically engineered to express some of the genes of something like HIV.

## **9. Vaccination primes the immune system for future exposure (08:52)**

So what are we trying to do with a vaccine? With vaccination when the vaccine is first administered, the antibody response ideally is generated and that goes up to some peak level. You start out with naïve B cells

that have never seen this particular vaccine before and what happens as they see the vaccine is that they convert into activated cells, they produce a lot of antibodies and some of those cells then go off to become memory cells and stay in the body persistently, able to make antibody and able to respond quite quickly if they see the virus or other pathogen again. Now if you become exposed, even with the low level of antibody, that may be enough such that, let's talk about viruses in this instance, the virus may never have a chance to actually infect a cell because there's enough antibody there to neutralize it before it even has its first round of replication. On the other hand if infection does occur hopefully there's enough B cells that can quickly convert to plasma cells to make lots of antibody really quickly and stem the symptoms and actually keep the viral load low enough such that people don't get sick and even though people still become infected, you rapidly clear the pathogen by that mechanism.

#### **10. Past successes with vaccination (10:17)**

And, you know, vaccines have had just an unbelievable impact on the world's population. When I was in grade school I remember going to have a polio vaccination, a sugar cube that I ate that was just a godsend for parents at that time who had been terrified about polio and you can see from this slide that in fact polio has become essentially not a problem, at least in the US. It still is a problem in pockets around the world. Another example of a spectacular success from a vaccine is the smallpox vaccine. In fact the success rate here was so great that by 1979 smallpox was officially designated as eradicated in the world and it only exists now in a few laboratories in a frozen form under lock and key. Now, a large percentage of you in this room would not be in this room today were it not for the smallpox vaccine because your forebears would have been killed by smallpox. So these are stellar examples of science having a huge impact on human populations. So it raises the question as to why I'm standing up here now, so many years into the HIV epidemic and not talking about some spectacular success we've had in getting rid of HIV in a similar way with a vaccine and to address that I think we really need to understand a little bit more about the virus, where it came from, and how it's evolved since it's gotten into the human population.

#### **11. Video: HIV's origin in Africa (12:02)**

So to address that I'd like to show you a video from my colleague Beatrice Hahn who has spent her career trying to uncover the origins of the AIDS virus through her work at the University of Alabama and her work in West Central Africa.

**[DR. HAHN:]** We have actually traced the closest relatives of the epidemic form of HIV to very distinct chimpanzee communities in south-eastern Cameroon. So it is very likely that the initial cross-species transmission occurred in these geographic areas. We believe the virus was carried to Kinshasa. The reason we believe that is that in the area of the Democratic Republic of Congo, that is where the greatest diversity has been identified. It's like a pebble that you throw into the water and the rings go from there. That's where this epicenter of the diversity is and then from there over the Congo River which is a major route of transportation and commerce to East Africa where AIDS as a clinical syndrome was first discovered, "slim" disease in Uganda. And then from there it was spread around the world.

#### **12. How HIV was first transmitted to humans (13:15)**

**[DR. WALKER:]** So HIV infection really is what we call a zoonosis, it's an infection that came from another species into the human population. It jumped from chimpanzees and it probably has been around for some time but in fact there were a couple of factors related to growth and development that facilitated its spread. One of those was that new roads were built into the deepest parts of jungles for logging and that allowed contact between humans and chimpanzees. The other was an economic factor where chimpanzees were being harvested for meat and loggers going in to pick up logs would also, as you see here, would take back butchered chimpanzees to sell at the markets on the road that you just saw that runs from Congo all the

way to East Africa and along that road, there was also sex trade, sex workers. So a person that may have butchered a chimpanzee, that was infected, might have gotten infected themselves, then passed it on through a sex worker who then passed it on to many, many more people and so the spread of this infection began.

### **13. HIV mutation leads to staggering diversity in HIV genome (14:37)**

The other critical issue, once it got into the human population, is this issue of the reverse transcriptase being somewhat sloppy and having a poor proofreading mechanism. And because of that it didn't end up staying the exact virus that it was when it jumped over into humans but it started evolving. And we can measure this in a graphic way by showing these sorts of diversity plots that I have on this slide here. The fewer branches and the shorter branch lengths mean less diversity. The longer branches and the more branch points mean greater diversity. So you all are familiar with influenza, we make a new vaccine for influenza every year because there's variation that occurs in influenza. Well let's compare influenza variation for which we need a new vaccine every year to what we see with HIV infection. So on the left you see the diversity in Canada during an epidemic from 1997 to 1998, it's really hardly any diversification there and over the course of a global epidemic in 1996 you see that there's some diversity that's developing. But let's compare this now to HIV on the same scale. So in Amsterdam in 1990 to 1991 they sampled a number of individuals who were infected in that city and the amount of diversity greatly dwarfed what was seen with influenza. But look at the Democratic Republic of the Congo. There, the amount of diversity is just astronomical compared to what we see with influenza and in fact what we're dealing with here is an unprecedented scientific challenge to try and come up with a vaccine for something that has become as diverse as HIV has become.

### **14. Subtype variation complicates finding a universal vaccine (16:29)**

In fact if you look at this you see that there are different branches and HIV has been grouped into different subtypes. An example shown here in pink where all of these come off the same branch, so those are closely related but they're still quite distinct. So if you look on a global level, in the United States for example, the colors here indicate subtypes. In the United States, if two people came into my clinic and I sequenced their viruses they would likely both be subtype B because that's the prevalent strain in the United States. But if I sequence those viruses and compared them, they could be 20% different one from another particularly in the envelope protein. If you go to the Democratic Republic of the Congo and do the same thing, take two patients, sequence their viruses and compare them, it's a staggering amount of diversity, they can be almost 40% different one to another and, you know, comparing this back to influenza for which we need a new vaccine each year, you can see that this is just an enormous problem, an enormous challenge to try and come up with something that will simultaneously protect against all of these different viruses.

### **15. HIV mutation means antibodies are always a step behind (17:41)**

What does this variability mean for making, let's say, an antibody-based vaccine to provide sterilizing immunity? What it means is that HIV starts as one thing and becomes another even within a single infected person and that variation occurs largely in the outer envelope protein. Antibodies are generated as the virus evolves, but the antibodies to one virus are not going to be cross-reactive with another because there's so much specificity to the antibodies that are being generated that they don't recognize the variants that are coming up. So if you look just in a single individual, here we see antibodies arising in response to the virus that's initially infecting. What happens is that by the time those antibodies are there, the virus has become something else and they're actually not effective. Well now the body makes new antibodies to these viruses, but by the time they're on the scene the virus has yet again become something else and the immune system never quite catches up with the virus in terms of this variability.

### **16. Problems with targeting HIV's envelope proteins (18:48)**

Well, why not just try and target with a vaccine a part of the envelope that doesn't vary? It turns out that the outer envelope protein is the most accessible, but it's also the most variable and antibodies readily bind to this but they don't cross-react against other viruses as mutations arise. On the other hand the least variable part of the envelope is the stalk, but the stalk actually is also the least accessible. If you look at this, this is drawn to dimension here, antibodies just can't get in there to do their job.

### **17. Is using cytotoxic T lymphocytes (CTLs) an option? (19:22)**

So then the question is, are there other opportunities besides antibodies? Well, to prevent infection you'd need sterilizing immunity with antibodies, but you might be able to prevent disease by keeping the microbe in check and there we have to turn to cytotoxic T cells and ask if they can do the job. We talked about this before from the virus' standpoint, it's like commandos going in, taking over a machine tool factory and turning it into a bomb factory. You have a factory worker there in blue which is an HLA class I molecule, waving a piece of the bomb out the window which is a viral peptide. That elicits a CTL response and cytotoxic T cells can come in and kill the virus infected cell by directly recognizing it. So the question is, you know, if CTL appear as we talked about before, if they're present in people that are HIV infected, why are they not doing a better job? In fact they persist as viral load increases. Well, the hypothesis is that maybe the same issue is happening here, that sequence variation leads to immune escape from CTLs as well. So let's follow up on an experiment I talked about yesterday where we infect CD4 cells with HIV and then we add cytotoxic T cells. And the infecting virus we're going to use is one that's isolated early from an infected patient when they're doing relatively well and one that's isolated later in the course of their disease when they're doing badly. So here's the early stage virus and you can see that by adding CTL in orange you're completely shutting down virus replication in this experiment in tissue culture following the cells over a seven-day period of time and measuring the amount of virus produced by p24 antigen, one of the viral proteins. Now let's do the exact same experiment with the same cells, but now let's use a different virus, the virus that's isolated later in infection from that person and what you see is now there's no inhibition of replication in this individual, even though you've added cells that, on the left hand panel, were completely able to inhibit.

### **18. HIV variability disrupts antigen presentation to CTLs (21:31)**

If you look then at what's happening and go in and sequence what's actually being targeted, what you find is that sequential mutations arise within these targeted epitopes, these tiny little fragments of virus that are being recognized by CTL and ultimately you get mutations, for example, in the second position here that prevent the peptide from even binding to the MHC class I molecule. So now instead of having a factory worker showing a piece of bomb, you've got a factory worker and there's no indication that there's anything wrong. The MHC class I molecule can no longer bind those peptides, so the body doesn't know that there's something foreign inside the cell and if you look at individuals that have this mutation, versus don't have this mutation, what you find as shown here is that when the mutation arises, viral load goes up, the amount of virus increases. So this is clear evidence that the immune system actually does target through this epitope but as sequence evolution occurs, what happens is that the response that's been generated no longer becomes effective, again because of this issue of viral diversity.

### **19. Additional reasons for failure of CTLs to respond (22:44)**

Well are there other reasons besides escape that explain why CTL responses fail to control HIV infection? You all know from our earlier lectures that the central orchestrator of an effective immune response is the CD4 lymphocyte. Those CD4 cells have T cell receptors that engage through CD 4 and the T cell receptor and get triggered to orchestrate this response. Additionally those same cells express the co-receptors for HIV entry so that they can be infected and ultimately die. Those cells are supposed to lead to activation and proliferation of cytotoxic T lymphocytes, so part of the problem may be that we're losing critical cells that

help to orchestrate effective CTL responses over the course of infection and as you lose those, the CTL become less effective. Well let's experimentally address this. So let's set up an assay we can do in the laboratory to assess CTL function and that is basically taking blood from an infected individual, stimulating it with HIV peptides and seeing if those cells proliferate. If they get angry and start to divide and do what they're supposed to do, which parenthetically should require the presence of a good HIV-specific helper cell response.

## **20. CTLs can be induced to work by functioning helper T cells (24:10)**

So now let me just explain to you how this experiment is done. We have to have a way to measure the fact that these cells are proliferating, okay? So what you do is you take the blood sample and you expose it to a dye that becomes intercellular called CFSE and then through this particular type of assay we can measure the dye intensity and as cells divide they lose the amount of dye that they have and actually migrate from the right hand panel into the left hand panel. This is done by flow cytometry. And so if we look at somebody with acute HIV infection, primary infection and we stimulate their cells with HIV peptides, what we see is that there are a lot of cells that are expanding, that are trying to do a job to defend against HIV but the panel on the right is from the exact same patient a year later. And now you do the same thing and even though they have CD8 cells, the CD8 cells now don't proliferate anymore. So the question is are those cells somehow rendered dysfunctional? Has a subpopulation of those cells been eliminated, or in fact are they still able to do their function, they're just lacking this critical help from HIV-specific helper cells? So here's the key experiment. You go back to your freezer where you'd frozen down some CD4 cells from the early days of infection and you thaw those cells out and you add them back to CD8 cells that you got at a time when no proliferation is occurring and you see if you can restore function and in fact what you see there with migration of those cells into the left hand, on the far right with migration to the left, you see 14% of those cells are actually now proliferating. The only difference is that you added cells that were obtained early on, so that tells you that in fact these cells are there, they're able to function, but there's a critical piece that's missing in order to get them to be able to function and this, to me, suggests an approach, potentially, to immunization and that is trying to figure out a way to restore HIV-specific helper cells with the hope that if you can do that it will be just like this experiment and you'll be able to get now the CD8 cells to start doing their job again. In other words, restore the conductor and get the orchestra to play in harmony again by doing that.

## **21. Summary (26:43)**

So let me make a couple of initial conclusions and then we'll go to some questions. HIV reverse transcriptase makes frequent errors during replication. We've hammered that point home and that's a really central part of the problem here. Sequence variation leads to escape from antibodies and from CTL so virus is evolving, that makes it very difficult and the loss of CD4 cells leaves the immune system unable to continue to respond and make new responses and orchestrate effective responses to the newly-emerging variants and to even the variants that were originally there because they can no longer function as they should. So let's stop there and...

## **22. Q&A: If HIV is changing, how can it still attach to receptors? (27:22)**

Question here?

**[STUDENT:]** Yes, you said that because the activation sites that are attached to the CD4 and the CCR5, if those are always changing, why isn't it that they change and they can no longer attach to this CD4 and CCR5?

[DR. WALKER:] Well so that's selective evolution here, that if you have a mutant that arises in the person that can no longer feed off the fuel there, it's going to die, but the ones that can are going to be the ones that survive. So there's a selection for viruses that will continue to infect cells. In the middle in the red... pink tie?

### **23. Q&A: Why are there locations that have more HIV variation? (28:12)**

[STUDENT:] Why is it that you see such extreme variation in the virus in different geographic locations?

[DR. WALKER:] That's a very good question and Beatrice Hahn my colleague from the University of Alabama alluded a little bit to that. We believe that's really where the viral infection started, was in that area. We also believe that there were a couple of different cross-species transmissions that occurred resulting in slightly different viruses getting into the human population. There's also a lot of human diversity in those regions and the immune pressure that was applied in those different genetic backgrounds was probably slightly different and helped those viruses to evolve in different directions. So let's go back and we'll take some more questions afterwards.

### **24. Vaccines to reduce viral load could reduce the epidemic (29:04)**

So let's go back to talk about sort of where we are with an HIV vaccine today. A CTL-based vaccine, as I mentioned to you, would be a vaccine that hopefully keeps the virus in check despite the fact that it wouldn't be able to prevent that initial round of infection. The idea would be that you'd have to get viral load low enough so that there'd be no disease progression and no transmission to others. So do we know anything about the risk of transmission in relation to viral load? Well it turns out that we do and in fact through this study that was done looking at the amount of virus in the semen of individuals, and linking it epidemiologically to the risk of infection of partners, it's clear that in the weeks following infection when viral load is really, really high in the blood, it's also very high in the semen and the chance of transmission there can be as high as 1 in 25. Interestingly, it's not every time that somebody gets infected, even at that level of exposure. On the other hand, as viral load comes down, the chance of transmission drops quite dramatically. So the question is, could you get viral load low enough and maintain it low enough that you'd have such a low likelihood of transmission that even if people had unsafe sex, the likelihood of transmission would be lower and you'd actually start to see the epidemic contract because there wouldn't be as much chance of transmission to other people.

### **25. Disappointing results in the first HIC vaccine tests (30:32)**

Well, that sort of strategy has been employed with a vaccine that was developed by Merck, this was a recombinant cold virus vaccine, it's a virus that doesn't grow well but still will allow for an infection. Certain genes of HIV were spliced into it and it was then given to 3000 people, 1500 got placebo, or saline, salt solution, and 1500 got this vaccine. And they followed these patients in a data safety and monitoring board which basically is set up for this sort of clinical trial, were the only ones who knew who was getting vaccine and who was getting placebo and they would monitor the data as they were coming along and they had previously determined that when they got up to around 30 or 40 infections in this group they would likely be able to tell statistically whether the vaccine was really working because you'd see hopefully more infections in the placebo group than in the vaccine group. And the data were just recently released and are probably the biggest disappointment that we've experienced in the last 10 years, maybe in the last 20 years in the HIV field. This was a vaccine that induced CTL responses, there was a lot of hope for it, but in fact the vaccine didn't show any efficacy at all. In fact if you look carefully at these numbers it even looks like there are more infections in the vaccine recipients than in the people that got placebo. So here's hundreds of millions of dollars that have been put into trying to rationally develop a vaccine and we've got a huge disappointment here, so lots of questions as to what's going on. Is this real? Well, it's not really statistically significant that more people got infected with the vaccine than with the placebo. Vaccine itself could not possibly cause

infection, but could it have done something that might enhance the ability of one of these high-risk people in the trial to become infected and so the trial basically was terminated and we've been left as a scientific community to try and figure out what went wrong and I'm chairing a national task force that's just getting together now to go back and really look at the data and figure out what to do with the remaining specimens that we have from those patients to figure out what experiments can we do to figure out why this happened.

#### **26. Possible reason for failure for the HIV vaccine (33:15)**

And I want to talk about just one possibility that hopefully from what we've talked about, about how immune responses are induced, you guys will understand. And that is could it be that the vector itself actually caused an increase in helper cell responses to the vector, meaning the adenovirus that rendered people more susceptible to infection because they had more activated cells? And what this slide shows is that if you had no titers to adenovirus, so you were unlikely to rapidly develop an antibody response to it, or a cellular response to it, your chances of becoming infected with the vaccine or the placebo were equal. But as the titers increased in terms of adenovirus titers, that meant that those people were more likely to make an immune response to adenovirus, perhaps also made a helper cell response to HIV and might have created more fuel for the fire. Now we don't know the answer to this yet, this is one of these real-world problems. We also can't say that this is really statistically significant in terms of truly being a greater risk of getting infection from the vaccine. The numbers are very small, but there's a trend here that's worrisome and definitely requires us to go in and look at, you know, as carefully as we can to try and understand this. But the reality is that 25 years into the epidemic right now, we are still really desperately searching for a solution in terms of a vaccine.

#### **27. Studying vaccine targets in Durban, South Africa (34:51)**

So where do we go from here? What I'd like to do now is just tell you a little bit about what our laboratory is trying to do in terms of addressing some of the issues about this global problem. One of the issues is, remember we talked about the adenovirus having different genes of HIV spliced into it. Well maybe it makes a difference in terms of whether you put in Envelope or Gag or some other viral gene in terms of the immune responses that you elicit. Is it fair to think that targeting any part of the virus would be equally effective? Not necessarily. So we realize that we could address this issue if we could find a lot of patients who are HIV infected who had not been treated with therapy so that we could look at the relationship between viral load level and amount of immune activity and how broadly directed their immune responses were and what they were actually targeting. So in order to do that we went to Durban, South Africa. You've heard about Durban already. Sadly when we got there about 50% of women that were presenting pregnant for prenatal care were already HIV infected, so a staggering amount of infection there and it was very easy then to get blood samples from patients, and begin to study the epidemic.

#### **28. Building research facilities in South Africa (36:18)**

Our initial plans were to go to Africa, get some specimens, bring them back to Boston and research them in my laboratory in Boston, but as soon as we got there we realized that there was an opportunity actually to try and build some scientific capacity locally but also to be able to potentially more effectively address some of those problems by building research capacity there and there certainly were people that were interested in getting involved, although there wasn't really a culture of basic science research in that area. In fact, the model that has been so successful in the United States and other places, of putting research and clinical care right next to each other, really didn't exist, at least for the very basic science efforts. So we started trying to sell this idea of building capacity and ultimately were able to get a grant with our colleagues in South Africa to build a biomedical research institute which we were just ecstatic about. Now we have the space in which to do this, we had the connection to patients and things were really rolling along.

## **29. A responsibility to treat research subjects (37:29)**

The problem that we had was a problem of conscience and a problem of ethics. We as physicians felt that there was just no way that we should be doing what we were doing if we weren't providing something back to patients. I would get on a plane in Boston, we no longer had AIDS patients in the hospital—it was that dramatic from the drugs here. I would fly and land in Durban, get out of the plane and be in a place where HIV was a death sentence. There was no access to drugs in the public sector at that time and we just felt that we could not go on with those studies. We did see an opportunity though. We had no funding to treat patients, but we had funding from the National Institutes of Health to do immunologic studies and part of those immunologic studies allowed us to do CD4 counts and viral loads. The people that we were studying were all living in poverty, had no ability to get viral loads or CD4 counts, so it wasn't even clear who needed to be treated. Remember when the CD4 count gets to 200 or less that's when it's really critical to start treatment. So knowing that we had these funds from our research dollars and we actually had researchers, you know, nurses and outreach workers helping us with this research project, we did something sort of backwards to what we usually do here where we build research onto clinical care. There what we did was we built clinical care onto research and we used our research dollars to do the research we were supposed to do, but we kept that information, went back to the patients and through philanthropy we were able to raise enough money to buy drugs to start the people that needed them on therapy and at this point then we really felt that we could go forward with what we were doing, we felt that with good conscience we could do this. Now we were doing what medical research is supposed to do which is really give back to patients.

## **30. Antibody response and relationship to viral load (39:23)**

So what I'd like to do now is tell you about some of the things that we learned from studying those patients. We had an extraordinary group of African collaborators there, we were able to generate a cohort of over 500 patients who were HIV infected. Those that needed treatment we were able to start on treatment and we were able then, to, at the same time, when a patient first came in, get a blood sample and figure out what the relationship was between the number of immune responses to each of the viral proteins and viral load. So the idea here was to determine the relative targeting of Gag and Envelope and other proteins and then determine whether having more CTL to one of those proteins made a difference. And in fact what we found was in this large population where we're just looking at this one factor, on the X axis here you have the number of Gag-specific responses, so if people had no Gag-specific responses viral loads, we've seen in the median there in a yellow bar, was higher than if you had more responses to Gag. So this was striking in that the more Gag you had, the lower the viral load. Each of those points is a single individual patient that was studied, and all of those studies were done in South Africa. What really surprised us though was when we looked at the same population of patients and looked at their Envelope-specific responses and the viral load tracked in exactly the opposite direction. So here was a group of patients where it was clear that the more your immune system went after the Envelope protein as a target, the worse you did basically, and the more you went after Gag, the better that you did. And so what these studies have done is to indicate to us in relation really to a question that was asked earlier, could you possibly immunize people and get them to target the Gag protein more selectively and actually get people to do better and those are experiments that we have ongoing right now that are partially funded through the Howard Hughes Medical Institute and that may or may not pan out in terms of having an impact, but this is something that clearly you can state as a clear hypothesis and you can test to see if you can make a difference.

## **31. Can HIV infection be controlled? (41:45)**

I want to talk about one other project that we're working on now that I'm really excited about. So, you know, we've talked already about the fact that some people do better than others and in fact some people do really, really well. Some people after they become infected will drop down to a viral load level of 1000 to 2000 copies and some people will go below 50 copies, the lowest level that can measure with our test. The

question is, how do people do that and the important point to note is that at that level the likelihood of transmission and the likelihood of disease progression is markedly diminished. So when you get down to a very low level like this, epidemiological studies that have been done, a lot of those in Africa show that your chance of transmitting to another person, although not zero is much, much, less and your chance yourself of progressing is much, much less. So doesn't it make sense to try and understand how people naturally do that? So in order to address, really, the issue of, do people ever control HIV over the long term

### **32. Video: Bob Massie: Infected but AIDS free (42:55)**

I want to introduce you to another patient of mine and I want to make the point again that a lot of the important things that I feel that I've worked on over the course of my career have come from talking to patients and from finding out about outliers, people that are different than the norm, that lead you to new scientific questions. So let's listen to Bob Massie.

[MR. MASSIE:] People would say "HIV is a fatal illness, although you "can go as long as *blank* years before you fall ill." And the blank was always whatever number it had been for me. So when people would say "you could go as long as five "years," and I was five years out, and then "you can go as "long as seven years" and I was seven years out. "Some people can go as long as ten years," and I was ten years out. So there's always this sense that I was right sort of at the limit.

[NARRATOR:] But against all odds, Massie an Episcopalian minister, has stayed completely healthy.

[MR. MASSIE:] You know there's sort of two pieces of this, one is, they want to take what they've learned from studying my immune system and apply it to others, and that's a wonderful piece. I also hope that they eventually figure out why this happened in me, because they still don't really know that. That's the great mystery.

### **33. Massie's immune system controls the infection properly (44:22)**

[DR. WALKER:] So Bob Massie is a hemophiliac, he was getting blood products while he was in college and in the seminary at Yale and became quite ill with a viral syndrome. Because he was a hemophiliac they were saving his blood for other studies on a regular basis and when the HIV test became available in 1985 they realized that he was HIV infected and they went back and could see that that acute syndrome was actually new HIV infection that had affected him. But when I met him in the early 1990s he came in basically saying, you know, I don't know what's going on and I don't know if you can help me, but everybody else is dying of this disease and I have a normal CD4 count. Viral load had just become available to us as a research tool, so we tested his viral load and we got the viral load test back and it said he was undetectable and I had him come back in because, I said, we have to test you again, I'm not sure that you're infected. But in fact he had antibodies, and subsequently we were able to identify viral sequence in his blood. So there's no question he was HIV infected, but to me, this really changed the way that I was thinking about HIV infection. Here was somebody that seemed to be doing to HIV what all of you are doing to viruses like mono and chicken pox. Seemed to be living with it without it causing any problem.

### **34. Significance of elite controllers (45:48)**

It turns about that about 1 in 300 people are like Bob Massie and the way that I found that out was by speaking to an audience like this of HIV practitioners and I just casually asked... I was talking about this unusual patient and I asked "Have any of you had ever seen one of these?" and about half to three-fourths of the hands in the audience went up and it turned out that these patients existed. They weren't coming into the academic medical centers because they weren't sick, but they were out there in the population and that led us

to believe that we could recruit their patients and do something based on advances of the Human Genome Project to try and determine the underlying genetic basis for being able to control this.

### **35. Scanning genomes to find a genetic basis for elite controllers (46:36)**

So the question that we were asking was can one define a genetic basis for the ability to have a better outcome? What we're attempting to do is to do something called a genome-wide association scan, which uses genetic signatures called single nucleotide polymorphisms, that I'll explain in a second, to identify short gene segments that are unique to a particular population of individuals, and this ideally allows for rapid detection of gene segments that make some people more susceptible or more resistant to disease. So it turns out that the human genome is about three billion nucleotides long. Now, to sequence all three billion nucleotides would be a massive effort, but the HapMap Project has basically identified regions of variation that occur at sort of spaced intervals across the genome and allow you, by making about 650,000 determinations, to get a sense of the variability within the entire genome.

### **36. Genome scanning and big dogs vs. little dogs (47:41)**

So let me tell you an example of a study that was done using this kind of approach. Big dogs versus small dogs: what's the difference? Well, let's apply a genome-wide association scan to try and figure that out. You get DNA from a whole bunch of big dogs, you get DNA from a whole bunch of little dogs and you do one of these analyses where for each dog you do the SNP analysis and you ask, "are there any SNPs that segregate with big or small dogs?" And it turned out that there was a single nucleotide at a single SNP that if it was one variant you were a big dog, if it was another variant you were a small dog. And in fact in going back then to where that SNP was, and identifying the gene, the gene responsible for it was identified.

### **37. Using genome scans on elite controllers (48:27)**

So the issue that we're trying to address is, we're trying to recruit enough people and this will be 1000 to 2000 patients who have undetectable viral loads, having never been treated, normal CD4 counts, and we're trying to look at their genetic makeup compared to people who had progressive infection and have gone on to develop AIDS and die. I have to say that when you sit down with one of these patients as I do, we try and bring the patients who come in and enter into this study over to see the laboratory and speak with them, you really get the sense that the answer is right there, it's just for us to fish it out. You know, if you could replicate what's going on in these people you could have a tremendous impact on this infection. And it may not be vaccines. It may be that we discover other things like mutations that conferred resistance to infection to Steve Crohn, that kept him from getting infected. We're very excited about doing this and really believe that this is a group of people that has to be vigorously investigated and hopefully will reveal to us what we need to know.

### **38. Summary (49:44)**

So I'd like to make a few conclusions and then a couple of final comments. Conclusions are that viral mutation is a huge challenge for vaccine development, it's a huge challenge for drug development also. The virus keeps changing, we have to keep coming up with new drugs as people develop resistance and we likewise have so much diversity that we're trying to make a vaccine to protect against tons of different viruses. An antibody-based vaccine might prevent infection but thus far our ability to generate one that recognizes all those variants out there has really not been successful and my own feeling is, without a major scientific breakthrough, this is going to be a huge challenge. We need some fundamental breakthrough to get us past where we are right now because the traditional ways that we've tried to do this thus far have not worked. A CTL-based vaccine might work. Unfortunately the first test of this concept was a failure. That doesn't mean that the whole concept is a failure, it means that this particular approach was. And maybe we

don't use adenovirus, maybe we use a different kind of vector. Maybe we put Gag in instead of a bunch of other proteins, but those are questions that still need to be answered and what's important is that this vaccine trial has not only given us the disappointment of a failure, but it's given us new hypotheses that we can pursue in a very targeted way to hopefully make more progress. And finally there are some persons who control HIV naturally, providing hope that inducing long-term containment of HIV might be possible. And maybe we can turn this infection into something that will be a chronic disease that will rarely get transmitted from person to person because it will be kept in check so much.

### **39. Tuberculosis and AIDS (51:36)**

The other thing I want to mention is something that is not HIV at all but is a huge problem that has arisen because of HIV infection and that's the problem of tuberculosis and I think you'll all understand this now. With a weakened immune system because of HIV, your chances, if you become HIV infected, of protecting yourself against tuberculosis is diminished. You may have noticed when in the video clip earlier on, Bisola and Zinhle and myself with some of the workers at the hospital at Edendale are walking on the ward with masks and the reason is not because of HIV infection, HIV is not transmitted casually, but tuberculosis is. So now we've got a... basically, a culture medium for tuberculosis, meaning a huge number of people who are HIV infected and we've got an infection that can spread easily from person to person. And this is a major, major challenge. If you look at where the infection is really prevalent, back in 1990 certainly Africa was most affected, but you all know now that Africa is also the place where HIV has really spread over the last 15 years and if you look at what's happened to the TB incidence rate it has just skyrocketed in those regions and it's because of HIV infection really fueling this other epidemic that we're faced with both of these challenges. So I think we all have to ask ourselves where do we go from here and the challenge to all of you is to ask yourselves that question. There's a huge need for scientific advancement to help people that are suffering in the world and each and every one of you can help to make those advances and it's your generation and the people who are sitting in this room who are the ones who are going to be called upon to make those differences. So I hope that we've been able to give you a sense of the enormity of the problem, I hope we've also been able to give you a sense of the enormity of the opportunities to get involved and do something really meaningful. Thanks very much.

### **40. Q&A: Could CCR5 drugs be given to uninfected people? (53:45)**

[applause] T-shirt time. In the purple sweater... jacket... blazer.

[STUDENT:] As a preventative would it be possible to use CCR inhibit drugs in non-infected people and if they were exposed to HIV and possible transmission would that...

[DR. WALKER:] That's a great question. So the question is could you give drugs ahead of time to prevent virus from ever entering a cell, like the CCR5 inhibitors, and in fact you might be able to do that. One of the ways that these drugs are being developed is as microbicides and I think that's an incredibly important area of investigation. The idea would be to give women the ability to protect themselves by having a vaginal microbicide, maybe perhaps made out of something like the CCR5 inhibitor that would prevent any infection from occurring. Unfortunately those have not been successful thus far, but you also could potentially give drugs to people. There it becomes a policy and ethical question as well though. It's an enormous expense to do that and there are toxicities to the drugs that occur and there are some studies that are looking at people at very high risk and treating them prophylactically with drugs to see if you can prevent them from becoming infected, but this is a very controversial area right now because of the broader implications of trying to take that approach. We'll go here. Okay. My arm's getting sore.

### **41. Q&A: Is HIV infection similar to being a TB carrier? (55:42)**

**[STUDENT:]** I work for a doctor and he is a carrier of TB but he does not actively have TB. Is that kind of the same thing that happens when someone has undetectable viral loads of HIV or is it completely different?

**[DR. WALKER:]** Well it's probably different because somebody with an undetectable viral load who is HIV infected still has the ability to transmit. The doctor you're talking about may have been exposed in the past to TB but has probably taken a course of drug therapy that has eliminated TB from his body, even though he may still have an immune response to it. So there is a difference. Are we done?

**[DR. CECH:]** We are.

**[DR. WALKER:]** Okay.

**[DR. CECH:]** Bruce, thank you. **[applause]**

#### **42. Dr. Thomas Cech announces speakers for next Holiday Lectures (56:36)**

Thank you for two fantastic lectures and Bisola also for two fantastic lectures. I want to thank everyone who helped make this lecture series possible. Thanks to our production team, thanks to you who are in our live audience and especially thank you to the speakers. Our topic for next year's Holiday Lecture series is going to be the brain. We are going to have two HHMI investigators, Eric Kandel and Tom Jessell from Columbia University in New York City who will talk about how the central nervous system conducts everything from movement to memory, so look forward to that. And until then I wish you all goodbye and have a great holiday season. **[applause] [music]**