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Treatment and Prevention
Prophylactic HIV Vaccines: Where Are We?
International AIDS Society
and Australasian Society for HIV Medicine
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KEITH PEDEN, PH.D.: Good afternoon. My name's Keith Peden. I work at the FDA and I'm co-chair with Jose Esparza here. We're having a very interesting session today. It's supposed to be 20 minutes each presentation. We're trying to have a 15-minute talk plus five minutes for questions, and we're trying to keep us all to time. The idea is that they're really going to present summaries of work, and they're going to tell us where we are in their respective fields, and perhaps where we're going to go, which is interesting.

So let's start today. Dr. Glenda Gray is going to talk about phase 2B trials.

GLEND A GRAY, M.D.: Thanks, thanks very much. Okay, I'd like to thank the conference organizers for inviting me to do this talk and I think that my talk is truly cross-cutting because I'm going to go from the rationale of phase 2B trials and look at some of the trial designs, and then look at - because we may use viral load as surrogate end points in phase 2B trials, to look at some of the natural history of viral load in different regions of the world and look at the impact of Jane Doe age or roots of infection on viral load set points. And then just to discuss briefly the HVTN503 trial that's being conducted in South Africa.

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So we know that a preventive HIV vaccine should mimic the effects of natural exposure to microbes and should provide long-lasting protection against infection and serve as a freestanding prevention method. Presently in HIV vaccine research, there is a lack of knowledge of the quality and quantity of immune responses that can be required to protect against HIV infection or the development of disease. And so the features of a phase 2B test of concept vaccine trial should provide a rapid preliminary assessment of whether a vaccine concept is sufficiently promising to move forward into a phase 3 trial. So it's intended to inform stop-or-go decisions. Normally it would be a randomized specific control trial in an active risk population, and preferably a very high-risk population so you can have your endpoints met. And it would also evaluate efficacy looking at specific endpoints, in this case probably viral load, that would augment immunogenicity. And what's really important in these test of concept trials is to follow our people that still convert on vaccine trials and look at their virological and immunological outcomes, because this will inform the vaccine design in the future.

This slide just shows the difference between a phase 2B test of concept trial and phase 3 pivotal design, and the most important differences are obviously around statistical power and sample size, as well as population. In a phase 2B, you're

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going to look at a very narrow population, and you'll go to places where there's very high risk, so you can optimize your data for getting your endpoints. In terms of a primary endpoint, both in phase 2B and pivotal trials, obviously HIV infection is an endpoint and a surrogate clinical outcome as well, as measured by viral load or CD4 count.

Just looking currently at the phase 2B or 3 trials that have been completed or are in process, we have the two Vaxgen trials which were completed that took place in Thailand and in the developed world, with around 5,400 and 2,500 participants. You can see that obviously phase 3 trials cost double the price of a phase 2B. Currently there are three phase 2B trials that are ongoing and there's the two HVTN studies that are using the Merck product, one in South Africa and one in the USA, Caribbean, Latin America and Australia. And these two trials are around 3,000 people large and are imprint driven trials. STEP trial is fully involved and they're looking for 100 endpoints. In the HVTN503 trial, we are about, I think, 160 enrolled, around 450 people, and this is amongst heterosexual men and women in South Africa.

Presently, there is a 16,000 phase 2B trial in Thailand with the Canary pox [inaudible] GP120 that is going at the moment. There's also a new concept that's also been discussed, which is called the phase 2 screening test of concept trial, or

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STOC, and this is even a more novel approach of trying to gather preliminary efficacy data in a very short period of time and fewer trial participants. So a typical STOC trial would go to an area and they'd look for 30 incident HIV infections to detect a one log reduction in viral load, and for this you would require an HIV incidence in the population of about 4-percent and with 500 subjects with 18-month follow-up, you could answer the issue of whether this is an important or promising vaccine.

Just to go back to the Vaxgen trials looking at GP120, these trials were designed to look at anti-HIV neutralizing and binding antibodies, and they weren't supposed to detect a [inaudible] response. We know that these vaccines did not prevent HIV infection, but it's very important to look at the effect of this vaccine on the natural history of HIV and to ensure that the study did not alter disease progression in any way. And also it's very important background data because it gives us very important data on the natural history of HIV infection in this group. As you can see from the slide, the vaccine did not impact on viral load setpoints or on CTL CD4 count, but it provides very valuable information for future trials which look at CTL responses to see if there's any differences.

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So what about CTL-based vaccines? So the current portfolio of vaccines hopefully will produce a CTL response which we know is important for control of viremia, both in the early part of the disease and in subsequent HIV infection. And we hope that a vaccine that induces a very strong T cell mediated immune response, in the absence of [inaudible] neutralizing antibodies may prove beneficial even if infection is not prevented, and that we hope that these vaccines will blunt initial viremia, prevent the early and massive destruction of memory CD4 cells and help control infection and prolong disease free survival. And also what's important, such a vaccine may impact on secondary transmission and you may be able to find this out by doing community randomized trial. So these are the futures of a CTL-based vaccine.

And we would evaluate a CTL-based vaccine on the following parameters. We would look at the vaccine efficacy in terms of reducing susceptibility, that is they reduce the risk of acquiring HIV infection. We'd also want to see what this vaccine did in disease progression, and indeed one would have to demonstrate that the initial reduction in viral load setpoint resulted in good clinical outcomes. You'd also want to see the durability of the T cell response, and obviously again you want to demonstrate that this vaccine reduces

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secondary transmission and impacts on HIV infection in the community.

So if you have a look at vaccine efficacy, we'd obviously want to, as I mentioned before, reduce peak viremia and impact on the viral load setpoint, improve CD4 counts and maintain CD4 counts, cause viral load to be reduced for a long time and an increase of time to initiate HART. And thanks to Peggy Dunston with a beautiful review in NEJ, just to look at the hypothetical response, what we hope to see in a CTL based vaccine. And you can see what we hope will happen is that there will be a decrease in the window of vulnerability, the smaller [inaudible], the viremia, the less seeding into and destruction of the gut associated lymphoid tissue and a very small amount of HIV in reservoirs being established, which will hopefully delay the time to progression to AIDS or death.

One of the clinical surrogates that one would use is viral load, and it's very important to try and understand how viral load is a measure of efficacy. Well, it may be affected by things like gender, age, subtype of virus, the region where the infection occurs, HLA, and the root of infection. And we'll discuss mostly the impact of viral load on gender and age, look at some of the natural history data coming out of different parts of the world. I'm not going to do anything on

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host immune response. Hopefully Bruce Walker will do it, and then look at some issues around root of infection.

This is a very important slide to try and understand the impact of virus infection on the immune system, and you can see that very early on after seroconversion, or after time of onset of symptoms, within about seven to 10 days, peak viremia is reached. Nadir has been attained at around 76 to 100 days after seroconversion and usually this nadir predicts viral load and CD4 counts at 6 to 12 months of age. Also you see that just after symptoms of seroconversion, your CD4 count is low and then it increases and reaches a nadir at around 30 to 40 days after infection.

If you look at the natural history of HIV infection by region and sub-type, you'll see that there are some problems with the data because people were using different assays. Some were using BDNA, some were using [inaudible], the time to seroconversion was blurred. But basically, based on taking into account all these factors and limitations, it's important to try to ascertain whether we could predict what time to AIDS would be in different studies. Looking at some of the data out of Europe, around 6-percent of people in the sera converter study in France had undetectable viremia even without HART and these undetectable viremia were maintained for about a year, about 11.1 months. Females were more likely to have this

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undetectable viremia, and people with high baseline CD4 counts and low viral loads at the time of seroconversion.

Looking at a larger group of people in the Cascade Collaboration, also around 7-percent of people having a spontaneous control of viral load and CD4 count, women again and people without symptomatic infection and people with high CD4 counts and low viral loads at baseline. Again, the median duration of undetectable virus in these cohorts was around 11 months.

To look at the effect of sub-type or region on median setpoints and viral load, you can see that data coming out of Brazil as compared to U.S. men and women, you can see that men in Brazil are sera converting to have similar viral loads to men in the U.S., but their CD4 counts [inaudible]. If you have a look at the data in the U.S., you can see that women do have lower viral loads than men, even though their time to AIDS is similar, which implies that women progress to AIDS at a lower viral load than men in the similar region.

If you have a look at some of the work coming out of Asia, you can see again that although the initial viral loads look similar to those coming out of the U.S., it seems that men in Asia, they tended to increase to AIDS much quicker and the median age to AIDS was about 7.4 years as compared to data coming out of the developed world where the median age of

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progression to AIDS is around 11 years. Also, in India, the viral load trajectory was much steeper than what was seen in the developed world. Looking at Africa with different sub-types, you can see that viral load setpoints seem to be similar, and that even though there seems to be low CD4 counts in Ethiopia, in fact, survival time or time to AIDS was similar as seen in the developed world. So you can see we need a lot more data to look at the effect of region or sub-type on disease progression.

And here this slide just shows that in men and women with high viral loads at baseline, their progression to age is also higher, and also the older you are, particularly if you're a man, the quicker you will progress to AIDS. Just looking at the data coming out of gender, age and route of infection, you can see from this slide that men who have sex with men will have higher peak viral loads, higher setpoints and will have much higher viral loads than men and women. Also you can see the effect of age on viral load setpoints and progression to AIDS. Also here just looking at a quick slide to show you the impact of age - men who have sex with men who were converted at a much older age tend to high setpoints as measured at 10 months after seroconversion.

So let's get on in my last two minutes. I've only got two minutes left. Let's get on to the Pombili [misspelled?] or

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phase 2B HIV vaccine trial that is being conducted in South Africa. This trial started at the beginning of this year, and as I mentioned before, we have to enroll around 3,000 people. The important part of this study is there will be a lot of women enrolled, so we'll get a good indication of the effect of this vaccine, and also of natural history in South African women. And so basically, obviously, we've tried to make sure that this vaccine is safe and tolerable, and we're looking at two co-primary endpoints, one is an infection endpoint and one is a viral load endpoint. And we're looking to see whether the serum response elicited by this vaccine will prevent persistent HIV infection and control HIV replication if infection does occur. We're also interested in the impact of preexisting immunity to [inaudible] 5 on immunogenicity and also because this cade [misspelled?] C area, and whether a cade B vaccine is and whether a cade B vaccine can confer protection in a cade C area.

And so in conclusion, in my last zero minutes, I'd like to just say that phase 2B test of concept trials are very important to move the field forward. It will help us to decide to go onto pivotal trials or to go back to the drawing board, and that phase 2B test of concept trials are important because they will provide further data that is lacking on viral dynamics in early infection. And furthermore, phase 2B test of

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concept trials will further elucidate interaction between the virus and the immune system that will inform vaccine design and development in the future. And I'd like to thank particularly Peggy Johnson for her lovely review in the NEJM. The HVTN503 investigators and [inaudible] from my unit. Thanks very much.

[APPLAUSE]

KEITH PEDEN, M.D.: Are there any questions? If you could come to the microphone and state your name and institution, that would be good. Okay, well, thanks. So our next speaker is Dr. Denis Burton. He's going to tell us a little bit about neutralizing antibodies.

DENIS BURTON, PH.D.: Okay I'd like to thank the organizers for the chance to talk. So I'm really talking about where are we in terms of antibodies, and I'll do that quite broadly and quite in summary fashion. So the way I look at it, there's basically two major highly related questions we're addressing in the neutralizing antibody field. One is, what specificities and levels of antibodies provide benefit on HIV exposure, and how do we develop immunogens that achieve and sustain these levels? And if you ask where we are now, I think we're pretty good on understanding that the antibodies we want are broadly neutralizing antibodies, and we have a pretty good handle on what specificities on many of those antibodies are.

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There's been a recent upsurge of enthusiasm for non-neutralizing antibodies. I personally don't share that enthusiasm. I think that non-neutralizing antibodies could contribute, could provide some benefit, but in the absence of neutralizing antibodies, I don't think they will do that particularly effectively. The levels is still a major issue that we're grappling with and there are ongoing studies there. So in the absence of really understanding what levels will provide benefit, especially in the context of robust cellular immune responses, which we hope we're going to get in a vaccine, we're really focused on problem number two and reduce that to understanding how can we design immunogens to elicit broadly neutralizing antibodies and not worry too much at this stage about levels.

So all neutralizing antibodies against HIV target the envelope trimer and broadly neutralizing antibodies can target conserved exposed regions on this trimer. And there are basically, I would say there are major strategies of immunogen design that are in operation today. And the first, and I'll go through these quite quickly. So the first would be trimer mimics, and here the great problem, and this is a problem that distinguishes HIV vaccines from others, the great problem is that the envelope trimer is very unstable, and so one needs to find some way to stabilize it and this is what is done in many

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recombinant preparations. However, nobody has yet achieved good stabilization of the trimer and good mimicry of the trimer as it appears on the viral surface, despite intense efforts from labs such as Sidrosky [misspelled?] and Mure [misspelled?] and so on. And I think that one of the things that everyone's waiting for is the structure of the trimer, a crystal structure of the trimer, that would allow us to more exquisitely design these stabilized recombinant trimers. And hopefully that's going to be on the way in the next two or three years or so. Certainly, we're getting better and better views of the trimer with for example cryo-EM. But we do need the high resolution of a crystal structure, I think, to really design these mimics.

The next approach would be entry intermediates where one's essentially most likely trying to target the co-receptor site. And the problem here seems to be one of accessibility. The co-receptor site highly conserved, is exposed during viral entry to some degree, but the exposure occurs in the context of two membranes, and is limited, and it does not appear from most of the data that the amount of space that's provided is sufficient for whole antibodies to gain access. So this approach has kind of died a little bit amongst some in recent years, although some are still pursuing it.

And then the third approach is epitope mimics, which essentially is produce epitope mimics of the broadly

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neutralizing antibodies determined from structural studies on complexes. And the basic idea is fairly straightforward. So what one seeks to do is one has knowledge of the broadly neutralizing antibodies, one seeks to gain molecular information on the interaction of these antibodies with envelope, and then use this information to design immunogens. And that's - there's a lot of effort ongoing in this area at the present time.

And what I'll do is just run through the various broadly neutralizing epitopes and discuss what's ongoing in very few slides. So let me begin with the CD4 site, which is the natural target in a way for antibody vaccine development. It is a region that's clearly pretty highly conserved. The virus has got to remain CD4-tropic. So the site needs to be fairly well conserved and accessible, at least to CD4. But over many years now, the only evidence that we've had that this is a vaccine target has been a single monoclonal antibody b12. Now, there has been a great advance there from Peter Kwong's lab this year in February in that Peter published the crystal structure of a complex of b12 with the core of gp120. And this for the first time provided us with molecular view of this broadly neutralizing epitope. And so the race is now on, and that's the yellow color here. But you can see the yellow color there, and we have that in molecular detail now. And so the

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race is on to try and take that molecular detail and design that [inaudible] into another molecule.

And an example of this would be the work ongoing from Bill Schief, Gary Nabel and Peter Kwong where they are trying to scaffold the binding site. So if you look here at b12, this is b12, you'll see that there are two loops in yellow that are the core of the binding site, and what Bill Schief is doing is to design scaffolded molecules that present those cores, those loops on different scaffolds. And there's now the possibility of *in vitro* evolution of these molecules, and ultimately of testing as immunogens. And there's a lot of interest in whether this scaffolding type of approach can work for vaccinology generally.

At the same time, personally, I think an important development has been the realization that there are other antibodies that are out there than b12 that are broadly neutralizing that target the CD4 binding site. So this is some work from our lab which shows two sera that you'll see the green color indicates neutralization and there are a large number of different viruses shown in this table from different clades and different parts of the world. And these sera are very broadly neutralizing, and they're from donors who have been infected in the early to mid-'80s. And we fractionated the sera and have tried to determine the specificities of this

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neutralization and we've been partially successful in showing that much of the [inaudible] is gp120 reactive. It's not directed to the v3 loop. It's not directed to the gp41 broadly neutralizing epitopes, and we suggested it might be directed to the CD4 binding site.

Now, a beautiful piece of work has been carried out and is in press now from Rich Wyatt and John Mascola showing for two donors, indeed broad neutralization can be assigned to the CD4 binding site. And so in brief, what they did was to fractionate the sera with wild type gp120 and then also fractionate it with a mutant gp120, a d368r mutant that does not bind CD4 binding site antibodies. So they have these two possibilities and the results were very clear.

So if you will just look at patient 1, if you look at the control beads, there's the neutralization curve, pretty good. And then if you deplete with wild type gp120, you can essentially deplete virtually the 90-odd-percent of the neutralizing activity with the wild type beads. If you use the mutant beads, nothing. Very strongly indicating that the specificity that you're looking at is directed to the CD4 binding site. And they did many many different experiments that suggested the same thing. One experiment that is complementary is that they actually luted the fraction that bound to gp120 and showed that that was highly effective at

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neutralization against a resistant virus that was not neutralized by b12. So for the first time now, we have strong indications of broadly neutralizing antibodies directed to the CD4 binding site as well as b12. And I think that's a very significant development.

Let me move on to the glycan shield. So there is this antibody 2g12 which Dan Calarese [misspelled?] in Ian Wilson's lab showed was a domain exchange antibody, and he determined the structure with its sugar ligand, [inaudible] 9 and then we can then model this structure on the spikes, and you can see here, if this will just stretch, that what we're looking at is the recognition, if we combine crystallographic and biochemical data, of an array of three man9's [misspelled?] on the envelope spike as this being the binding site. So the vaccine protocol now is to try to take that array of sugars and reproduce it and use that to try and elicit this sort of antibody.

And there's a couple of approaches that people are adopting. Raymond Dweck's lab in Oxford is looking for natural presentations of this sugar. And they found a few. One is on *candida albicans*. It's not exactly the same as HIV, but it's very similar, and it does bind 2g12 quite well, and they're immunizing with these. An alternative approach that we have followed with Chi-Huey Wong's lab, Will Greenberg, M.G. Finn and Ian Wilson, is synthetic, so try and synthesize these

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sugars and put them in multi-valent presentations. And these are two examples of quite successful presentations where we have reproduced the glycan shield sufficiently well that we can achieve good 2g12 binding. You'll see the first one expresses 279 copies of this mam9 on Q-beta, a phage, and the other one uses an oligodendron arrangement to present a large number of mam9s on BSA. And both of these arrangements, now wholly synthetic arrangements, bind 2g12 with [inaudible] affinities, and they're being investigated as immunogens.

And finally, I'll talk a little bit about the MPER, which is the final broadly neutralizing epitope, with a number of antibodies targeting it. The first two both from Herman Katinger, 2f5 and 4e10, you'll note that both of them recognize epitopes very close to the membrane. 2f5 recognizes a rather extended peptide epitope as determined by Peter Kwong, and 4e10 recognizes a helical epitope as determined by Rosa Cardoso in Ian Wilson's lab.

Now, there are a number of different attempts to reproduce these peptide epitopes. Bill Schief and Rich Wyatt and Peter Kwong are doing this in a number of epitope scaffolds, that you'll see here. We took a look at trying to engraft this epitope, this helical epitope, into the v1, v2 loop of gp120, reasoning that this was quite an immunogenic site. And what [inaudible] did was to move the register of the

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helix, so he kind of turned the helix around in the v1, v2 loop to give different constructs. So we know that the antibody is only going to recognize one side of the helix, so we turn it around and present it in these different fashions. However, although we could get very good binding of 4e10 to these constructs, when we immunized both rabbits and mice, we got no 4e10 like antibodies.

And there are a number of possible explanations for this. One would be that argued originally by Bart Hanes [misspelled?] of tolerance. Another would be the hydrophobicity of the helical epitope, that this is a very hydrophobic, and perhaps when you put it into an animal, it just finds something else hydrophobic and sticks to it, and it's not there exposed to the system anymore. And then there are other possibilities that I won't go into. I'm only two minutes away.

So just on this issue of tolerance, many of you will be aware of this paper from Bart that argues that these antibodies are prohibited by tolerance, that they are anti-[inaudible] antibodies. We don't agree with that, and Erin Shearer [misspelled?] will present an oral abstract on this if you're interested at this meeting.

This is basically my last slide. Just to emphasize that what I am talking about, here is an attempt at rational vaccine

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design that goes through a molecular understanding of antibody recognition of the viral envelope. And the more broadly neutralizing antibodies that we can put in on the left hand side here, and the more rapidly we can screen these on the right, the more rapidly I think we'll arrive at some kinds of candidates that elicit useful levels of neutralizing antibodies. Hopefully I mentioned the many many people in the world that I showed. I'd like to just particularly acknowledge the financial support of NIAID and of IAVI, and I'll finish there. Thank you.

[APPLAUSE]

KEITH PEDEN, PH.D.: Any questions for Denis Burton?

MALE SPEAKER: Denis, I wonder if you could elaborate in your comment about this recent interest in non-neutralizing antibodies, and certainly antibodies have other functions, some of them mediated by DSC.

DENIS BURTON, PH.D.: Yeah.

MALE SPEAKER: Such as ADDC, and they may contribute, not by themselves, but maybe they contribute to build the [inaudible] to infection.

DENIS BURTON, PH.D.: Right. Well, as you know, we have done some work that shows that FC receptor binding seems to be crucial for the anti-protective activity of a neutralizing antibody. But if you have non-neutralizing

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antibodies, then basically as we understand it, generally their activity will be much diminished compared to neutralizing, because they're binding to non-functional falls of envelope. Now they may - there are shed gp120 so there's gp41 stumps, and you could bind to those and you could hope that that might help you clear the virus. I mean those are possibilities, but you know, we have the Vaxgen trial where we had lots of non-neutralizing antibodies and there wasn't much protection. We have other studies that we did in mice where with neutralizing antibodies we got protection, but with non we did not. And also, some of John Mascola's studies with low levels of neutralizing antibodies that were not protective. And generally speaking, viral protection is much much better with neutralizing antibodies, almost without exception I would argue. I don't know if anybody wants to argue that.

MALE SPEAKER: So Denis, [inaudible] someone else is coming up. The broadly neutralizing antibodies - you look at a whole series of x4 as well as r5 viruses?

DENIS BURTON, PH.D.: Yes, there's no real difference in neutralizing ability.

MALE SPEAKER: Denis, there's many many harmonized residues on the subject of gp120. What's special about those that induce 2g12?

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DENIS BURTON, PH.D.: Well, I mean just let me say, I don't actually know that they induce 2g12. We never know, but they certainly bind to them. And what's special about them is the arrangement, is that it seems that if you have single - I mean we know quite a bit more about this now, because we've put synthetic manos [misspelled?] onto different scaffolds. And so we did that with [inaudible] mosaic virus, and it turned out there we got lots of groups of two mam9's but we never had three or four. And there we go no 2g12 binding [inaudible]. It was only with the Q-beta when we had these groups of three and four. So I think you need these three and four, and it might be four. It might be that it's binding three, but the other one is nearby and it's stopping the arrangement flopping about. And I think that's only found once on gp120, generally, although Raymond Dweck has also shown that if you treat with [inaudible] and then you can produce gp120 that's all oligomanos [misspelled], then you have more 2g12, or you have two or even three.

FEMALE SPEAKER: Yeah, regarding the synthetic mam9 vaccine candidates, is there not a danger that you might also elicit an immune response to glycoproteins that have a mam9?

DENIS BURTON, PH.D.: Well, yeah. I think the answer to that would sort of be related to the first question, which would be that they will not recognize single manos. And as far

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as we know, the only time that this clusters of manos occurs is on gp120 and certain pathogens like *candida* and there's a schigala [misspelled?] that has it.

FEMALE SPEAKER: But there's no evidence [inaudible]?

DENIS BURTON, PH.D.: I'm sorry?

FEMALE SPEAKER: There's no evidence that there is combinations in [inaudible]?

DENIS BURTON, PH.D.: No, there's no human protein that binds to 2g12 that we've been aware of.

FEMALE SPEAKER: Thank you.

FEMALE SPEAKER: Along the same lines, Denis, I believe it was your lab that showed several years that the skate from 2g12 might be fairly easy to achieve.

DENIS BURTON, PH.D.: Yeah.

FEMALE SPEAKER: Simply by removal or changing of one of the amino acids so that you get fewer of the mano site chains. You still seem to think that this is a reasonable target for trying to elicit antibodies.

DENIS BURTON, PH.D.: Sure. Yeah. I mean, I think those experiments - and now you can add on Alexandra's experiments, [inaudible] experiments and Marty Markovitz, where if you treat with 2g12, the escape will happen just like that, by simply losing one of the sugars. But the difference is, when you're doing protection, the antibodies there before the

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virus and there isn't all that great diversity, and you're probably protecting against a very small diversity of species. That would be my argument, but there's data for that in that John Mascola has shown that 2g12 is very good at protection. So I think protection's very very different from established infection. That would be my argument.

MALE SPEAKER: Denis, I have a question for you. With regard to the data that have been produced in a paper a couple of years ago in [inaudible] Medicine, using this cocktail from Katinger to treat patients in primary infection.

DENIS BURTON, PH.D.: That's Alexandra [inaudible].

MALE SPEAKER: Yeah, Alexandra [inaudible]. Now, it is clear that since there was a very good control, transient but it was a good control, that these epitope are exposed, then in principle, they are exposed but for one reason or the other, they are not highly immunogenic. And also now, there are people using the reverse vaccinology approach, particular also Antonio [inaudible] is working a lot on this. And even using a [inaudible] from a long term non-progressor is having really a hard time to isolate the B cells that are able to produce a neutralizing antibody. Then the issue is, it is only the fact that you cannot expose [inaudible] results are an issue with really poor immunogenicity, because the frequency of research

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of this epitope is very, very, very low compared to other viruses.

DENIS BURTON, PH.D.: Actually, I think Antonio's having a couple of successes recently. But further to that, you are right, I mean the immunogenicity of these epitopes is very low. But that's in the context of the virus that has evolved just for that purpose. It cannot tolerate having high titers of anti-MPER antibodies. But that doesn't mean to say that when we present those epitopes in the environments that we do that we can't change immunogenicity completely. I mean, that happens all the time with peptide epitopes and so on. So what we're trying to do is to rewrite immunogenicity by our design.

JOSE ESPARZA, M.D., PH.D.: So as Denny described, the development of vaccines that use neutralizing antibodies are protective is one of the major challenges that the field is confronting and we certainly look forward to see progress in the future. But most vaccines that are currently in clinical trials are designed to induce cell-mediated immunity. So Giuseppe Pantaleo from Lausanne will tell us everything about vector-based vaccines in 15 minutes.

[LAUGHTER]

GIUSEPPE PANTALEO, M.D.: Okay. That's be 16 minutes. First of all, I would like to thank the organizers, and I have

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been asked to give some overview on vector-based vaccines, particularly limiting to data originated from phase 1 clinical trials.

Now, with regard to the different concepts that have been developed, as you see there are a number, the one that was just mentioned by Jose, in the HIV field, really have been the most developed are those using virus vector-based vaccines and the DNA. And I'm going to limit my talk to these two different strategies.

Now, this is a list of the different clinical trials that you can find on the IAVI web site. This is the source data. And then the point that I want to make, and then there are several, three or four pages, it is the most comprehensive list of phase 1 vaccine trials. And what you will see is that practically, we are talking exclusively about trials performed with adenovirus-based vaccine and with the pox virus based vaccine. And then complement it or not with DNA. Then despite the quite large number of other vectors in pre-clinical, but in clinical, most of the phase 1 clinical trials really focus on the use of adenovirus and the pox virus based vaccine complemented with DNA. There are some interesting also phase 1 trials addressing new adjuvants, particularly there are [inaudible] some trials using some DNA expressing IA15 or IA12. These IA15 or IA12 are important cytokines involved in

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maturation of T cells and also in directing T cell responses towards TH1 cell responses. But as I said, most of these trials they are using all the same types of virus vectors.

Now, let me give you some background about the adenovirus. There are 51 serotypes identified that are classified in these groups, and then generally, in healthy individuals, they cause a self-limited disease and for this reason, there is no drug for the adenovirus infection. In contrast, adenovirus infection can be pathogenic in individuals who are compromised, such as HIV infected individuals, bone marrow transplanted patients and also children. Now, the adeno-5 is the most common used vector in gene therapy vaccination and it has been rendered to be incompetent through the deletion of the E1 gene. It has an excellent safety record. The disease is manipulated. And also we can insert that expression of inserted [inaudible], and then it is highly immunogenic.

Now, what is the immunologic basis for using adenovirus? It comes from original studies performed by the Merck Group, John Schiger [misspelled?] and collaborators and also from Norm Netvin [misspelled?] and Gary Nabel's group. And it was initially shown that, in fact, this vector would be able to induce an effective antiviral immunity. And also a more recent paper was showing that immunizing monkeys with

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these vectors expressing SIV genes, we could preserve the initial depletion of CD4 sensor memory cells.

Now, with regard to the adenovirus vector that is in, as we have heard from Glenda Gray, in the phase IIB trial, these slides have been kindly provided by the Dana [inaudible] and Mike Robertson. You see it's a three valent vaccine expressing [inaudible]. It has been engineered to be replication defective through the deletion of E1AB and also to be genetically stable and manufacturer product is producing a cell line, which is [inaudible] 6 that eliminates reversion to [inaudible] type and the formulation is 111 with [inaudible] with those of three times 10 to the 10 total virus particle. And this vaccine, as you have heard, is in phase IIB trial.

Now, with regard to immunogenicity data, this slide gives me the opportunity to raise an issue with regard to adenovirus based vaccine. As you can see here, there are three different concentrations, more or less induce the same level of immunogenicity, which is in the range of 300 spot-forming units per 10 to the 6 blood nuclear cells as measured by interferon gamma [inaudible] assay. But what you can see, the black bullets, they are the individuals, volunteers, with the antibody type as for adenovirus below 200, and then the empty bullets are the volunteers with the antibody type that's greater than 200. And then you can see, however, that in these

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studies, there is a tendency of the concentration of the responders, the individuals with the antibody type that's below. Therefore, there is an issue about the preexisting immunity with adenovirus. In part, and then you can see also when they look at the longevity of the response, you can see that in the volunteers that have lower type antibodies in blue, generally we have a better percentage of responders in this group as compared to those who have higher antibody type as here in pink.

Now, one of the strategies that has been used by the Vaccine Research Center is to prime these individuals with DNA and then to boost with adeno, in order also to minimize this preexisting immunity. But also, this strategy has been different from the adenovirus program from the Vaccine Research Center. In fact, there have been multiple sub-types. And then as I said, they have used a combination approach using DNA plus adeno.

And then these are slides kindly provided by [inaudible] from the VRC. And then as you can see it's quite at the same time a complex vaccine regimen which involves the production of ten different products, three DNA expressing the different [inaudible], three DNA expressing individually [inaudible] from [inaudible] B, and then with regard to the combinant adeno-5, the three adeno expressing of AD and C and

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then one expressing [inaudible]. And then the vaccine regimen involves three immunizations with DNA, at months 0, 1 and 2 and then a boost with adeno at month 6. Now these are just the cumulative data, just concentrate your attention on the DNA prime adeno 5 boost. And what you can see here, the response against envelope, is in there is a median number of the spot forming units per 10 to the 6th cells in the range of 300 per 10 to the 6th cells. Whereas the response in [inaudible] are present but are a little bit lower, in median 100 for [inaudible] and then about 50 spot forming units [inaudible].

Now, as I said, there is potentially a problem with resistant immunity, and for this reason, there is a line of research that is looking at different strategies to overcome the issue of resistant immunity. And then this is centering on the development of a de novo adenovirus vector, and then the goal is to develop a novel vector with a similar immunogenicity of adeno-5 with the serologic profile of real adenovirus serotypes which also [inaudible] manufacturer ability. And then the two key strategies that have been identified are novel serotype recombinant vectors and novel chimaeric. Now this data that I'm going to show you has been provided by [inaudible]. Here, you can see the comparative adenovirus seroprevalance in adults from sub-Saharan Africa. You can see that the higher titer of antibodies are against adenovirus 5.

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However, there are a number of other serotypes, particularly 35, 26, 48. As you can see, most of the titers are very very low, below 16. These data, generated by [inaudible] in my mind are very exciting. This is showing what is the immunogenicity of chimaeric adeno-5, adeno-48, or adeno-26 in monkeys with preexisting anti-adeno-5 immunity. As you can see, there is a substantial increase in immunogenicity that seems to be dependent from the presence of preexisting anti-adeno-5 immunity.

And also another extremely interesting piece of data is this one showing that actually you can use some of these rare serotypes such as adeno-26 in order to prime adeno-5 responses. As you can see here, you have an increase of about fivefold of the response of the immunogenicity of adeno-26 plus adeno-5, using adeno-26 as its prime.

Now, let's move to the pox viruses, and most of the development is being on viruses originating from vaccinia virus. And the two that are most commonly used are the modified vaccinia ankara [misspelled?], and also some [inaudible] from the vaccinia virus Copenhagen. Now why there is so much attention on poxviruses? There is a broad [inaudible] are easy to regenerate recombinance. There is a robust production process available. We are able to induce both human sera [inaudible] response and excellent safety

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records. Now something - these slides are put up because sometimes we say that these three viruses are exactly the same, the most commonly used. But as you can see, the origin of these viruses is different. The vaccinia, the AVA has been only generated through [inaudible] 571 in chicken embryo fibroblasts. In contrast, the Nivoc [misspelled?] has been generated through a massive deletion of 18 genes that are involved in viral [inaudible]genicity, and then the canary pox, the one that is used, from which [inaudible] has originated using the phase III trial in Thailand, has been originated attenuated to 200 passages in chicken embryo fibro cells. And these viruses are quite different from one to the other in terms of the attenuation process.

Nivoc is the one that I will show you some data that we are using. I will not go through. And then these are the data that we have generated in the EuroVacc II trial. This is a phase I trial now this vaccine combination as entered in phase II and then a trial recruiting under 50 persons has already started a couple of weeks ago in different centers in Europe. And then the data, in this trial, we compared immunogenicity of [inaudible] of DNA plus [inaudible] versus [inaudible]. They are expressing [inaudible]. These are [inaudible] inserter. It's a monovalent vaccine for Nivoc, the same construct expressed in [inaudible]. And then it is a divalent vaccine

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for DNA, we have two plasma DNA, one expressing DNA envelope, and the other [inaudible].

Now, these are the immunogenicity data. 90% of responders, the primary endpoint, which is weeks 26, 28, two weeks and four weeks after the last vaccination. The response remains as you see very high over time. These are the individual data of the responders, but if we look at the cumulative data, you can see that the median of spot forming units per 10 to 6th blood nuclear cells is in the range of 300, which is exactly comparable to what it has been shown with the DNA plus adeno or with adeno alone. There is also a response against [inaudible] which is in a similar range of about 100 spot forming units, and this response is also present in 50% of individuals in addition to envelope. What about the function of Pro-5? I think that based on our knowledge of immune [inaudible] as we will hear later from Bruce, is the best that we can expect, we can hope, is for fully functional responses for CD4, the CD4 T cells induced by the immunization, they secrete [inaudible]. They proliferate in response to the peptide. The same is for CD8 in addition. They also, we can show the presence of the generational activity, which is a measure of cytotoxic activity. And then the breadth of the response, there is a mean number of 4 epitopes recognized per volunteer, and then most of these epitopes are CD4 epitopes,

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but we have also identified a new HLA 1 restricted CD8 epitopes. The durability of the response, these responses are highly durable, as you can see. One year after the infection and still the response is there.

And the conclusion then, I think the vectors that are currently under development, they have been shown to be highly immunogenic, to have induced a vigorous response. These responses are fully functional, they are durable. Now what is the future? Now in my mind, I strongly support the initiative from the IAVI regarding the symposium that was organized, because I think the future of these virus vector based vaccines now that they have been shown to be safe is to move [inaudible] replication of competent virus vectors. A number of these vectors are already in pre-clinical studies, and I think that one of the possibilities of developing replication of competent vectors is to simply [inaudible] regimen, which they are very complex, to induce a more balanced CD4 T cell responses as it has been shown for live replication of competent attenuated vaccine already available. Improve the durability of the T cell responses and then the most also very important is to reduce the cost and also the problems associated with large-scale manufacturing.

Now, the last slide is to acknowledge the people within the EuroVacc consortium, that I have shown you the data, and

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also Rick Culp from the Vaccine Research Center that kindly provided the slides and also other people who contributed to this presentation, [inaudible] and Mack Robertson from Merck, [inaudible] and Katarina [inaudible] from [inaudible] and [inaudible] from Harvard Medical School. Thank you.

[APPLAUSE]

JOSE ESPARZA, M.D., PH.D.: This presentation is open for discussion.

MALE SPEAKER: I'm just wondering if you have done any or know of any information about [inaudible] in terms of the number of T cell repertoire, and also in terms of the mucosal immunity and information about this vaccine and whether or not cells could be recruited to this site.

GUISEPPE PANTALEO, M.D.: Yeah, well, there was the piece of data in one slide using [inaudible] that we can at least for the CD8 T cell responses we have developed some [inaudible] so that we can monitor very well the response. With regard to the type of T cells that are induced by these type of immunizations, there is a very categorization of the type of T cell response, and then with regard to CD8 T cells, these T cells they have a phenotype which is rate positive and CCR7 negative, which is typical of [inaudible] T cells. With regard to the mucosal immunity, we have not tested, we have not

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performed biopsies in these volunteers in order to determine whether or not T cells were also present at the mucosal level.

MALE SPEAKER: Guiseppe, my question is, do you have any animal protection data with the Nivoc? And maybe many years ago people actually used Nivoc or other vaccinia for animal protection, but I don't know if your construct has been tested in an animal model.

GUISEPPE PANTALEO, M.D.: Well, we have a parallel analysis and use of this combination in both immunogenicity and protection data in the monkey model and then in fact it is highly protective. For what, these monkey models are important, but it induces complete protection, this immunization scheme on the monkeys. There is a paper that [inaudible] about these data. Now with regard to the Nivoc, I think that what is important to keep in mind is that it is not only the vector but also the antigens. And then I think that it has been used in the past for cancer vaccine, Nivoc, and the only explanation that I have that it is possible that the antigens that were inserted were not highly immunogenic. But based on the data that we have, it seems to be an extremely promising poxvirus candidate.

MALE SPEAKER: Because we have some time, let me ask another question. [Inaudible] are not just mechanical carriers

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of [inaudible]. Poxviruses are very complex viruses, and they have many genes that can actually modulate immune response.

GUISEPPE PANTALEO, M.D.: Yeah.

MALE SPEAKER: And I wonder if you can tell us what is [inaudible].

GUISEPPE PANTALEO, M.D.: Yeah, I mean the future - I think that it's a good point. There is now an entire program that is funded, supported by the Bill and Melinda Gates Foundation about development of more immunogenic poxviruses and there are two strategies, in fact. One is a true demodification deletion of some genes expressed by poxvirus that are known to be immuno-suppressive. And then the second strategy is to develop some replication competent poxvirus. And then I think that in my mind if we are able to combine the two, I think that we are going to be in a situation of probably using only one vaccine and not having also the need to prime with DNA which definitely adds a lot of complexity to these vaccine regimens.

JOSE ESPARZA, M.D., PH.D.: Any other questions? If not, we'll move on.

The Holy Grail of vaccinology is to identify the immune response or immune responses that confer protection in vaccinated individuals. And we have some clues from animal protection experiments, and also from HIV-infected people who

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somehow control infection. And in the last presentation of today, Bruce Walker from Harvard will brief us on correlates of protective immunity. Bruce.

BRUCE D. WALKER, M.D.: Thanks very much. I want to thank also the organizers for inviting me. So I'm going to talk about CD8 T cell responses and try and be a little bit provocative about where we need to go and what the focus should be. So basically, the problem that we're all trying to deal with in terms of vaccines is how do you prevent cells from making more viruses? The more they make, the worse people are going to do. And so this is really the issue. And the question is, how can you limit virus replication?

The important point to remember is that we're dealing right now with the realization that the first generation vaccines are going to be vaccines that allow infection to occur, so we're going to be in a situation where we're going to have to limit the amount of virus that's produced by an infected cell.

So there are ways to do this, neutralizing antibodies can do it, and what we've pursued is induction of antibodies that neutralize infectious virus. And so basically what's guided development thus far in terms of neutralizing antibodies is trying to look at those antibodies that actually have an effect on the ability of virus to replicate, because at the end

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of the day, that's really the issue. And we really have not particularly pursued antibodies that don't appear to have any function that we can measure.

In terms of vaccine development for CTL, I guess the question I'll ask is, are we essentially measuring the equivalent of binding antibodies with the current assays that we're doing? And I think this isn't a question that just I am asking, but lots of people are asking. You know, we do these assays where we're not looking at infectious virus, what we're looking at are cells with a bunch of synthetic peptide plastered on their cell surface that is not physiologic. And then we look at interferon gamma production, which basically tells you that some interaction is happening at the surface of that cell when you have a cell that has a bunch of peptide plastered on the surface.

You can make this more complicated by looking at other things that might get tickled to be produced by a cell and you can start looking at polyfunctional responses, but again, that doesn't really tell you a function that's actually having something to do with limiting virus replication, it just tells you that cell's getting tickled and some cytokines being produced. So the question is, can we think about this in a more functionally relevant manner and try and look at the issue of virus production by infected cells? And in fact, there is a

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way to do this. If you, instead of looking at synthetic peptides you actually look at infected cells. And why is that important? Well, there are a whole bunch of steps that are completely skipped when you do an LE spot type assay. You know, the process of infection, of antigen processing and presentation, binding class I presentation to cell surface in a physiologically relevant amount, and then not only recognition by CTL but also killing by CTL where the measurement then is the inhibition of virus production as a measure of efficacy.

So let me pose some hypotheses here. The ability to inhibit virus replication is important. Current LE spot assays really give no indication of this ability. The hypothesis is that in fact we can identify CTL responses that neutralize infectious virus, and that vaccine induction of these responses is going to be key for current vaccine strategies.

So let's take a situation where we have somebody who's spontaneously controlling virus, a so-called elite controller, and let's look at the ability of their cells to inhibit virus replication, rather than just doing a simple LE spot assay. And so what you do here, and this particular experiment is based on some studies that Otto Yang had done 10 years ago, is you expand their CD8 cells just so you have enough of them to put in this assay, you infect their CD4 cells with HIV. It's actually easy to do in these patients, because if you just

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stimulate their CD4 cells, they don't produce autologous virus, so you exogenously infect with a virus, and then you look to see if you can inhibit replication. So basically, this is using anti-CD3 just to get kind of a representative expansion of the cell specificities that are there, and then you put them in this virus neutralization assay, and what you see is somewhere between 1,000 and 10,000-fold reduction in virus replication by those cells over a seven to ten day course of infection. So that's a huge amount of neutralization in an assay where you're actually measuring what I think is something functionally relevant.

Well, then, if you've got a way to measure that, then you can start to dissect this in the same way that Dennis was talking about with antibodies. We can sort of elute different kinds of antibodies by their specificity and see what is it that's important in terms of mediating this. What I just showed you were bulk CD8 cells showing really profound inhibition of virus replication. Well, what if you now just try to elute out specificities and measure those to see what happens? Well, let's do that first for a gag-specific clone, just deriving CTL that are specific for this KK10 epitope, which we know is relevant, because if you look at people that target this particular epitope, they have a low viral load, and

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B27 positive individuals that don't target it have a high viral load.

So with this particular assay, now you can go back and you can add that clone at a 1:1 ratio, or you can have one of those per 10 CD4 cells or even one of those per 50 or 100 CD4 cells, and you can see a statistically significant inhibition of replication, and in fact, in this case it's almost five logs of inhibition that you're seeing with that particular clone. That's a huge amount of inhibition. That's a huge amount of virus neutralization.

Well, how specific is this inhibition? We want to test the efficacy of the immune responses in a relevant fashion that will reflect what we think is going on *in vivo*. So let's look at variant and wild type viruses and see if this assay can actually show us those differences and let's also look at the issue of antigen-processing mutations that would never be picked up in an LE spot assay. And when we do this, we're looking at wild-type epitope versus an escape epitope. In KK10, what you see is that you see this huge amount of inhibition of replication on the left-hand panel when you add the clone, but if the naturally arising variant that occurs under immune selection pressure is the virus that you use now for this assay, you get no inhibition of replication showing in fact that this is replicating exactly what we might expect.

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Well, what about if you have processing mutations? Here are three panels. The first panel is just wild-type virus, the second panel is a virus that has a mutation outside of the epitope and what that does is it abrogates processing and you get no inhibition of replication, and on the other hand, if you have a mutation there within the epitope that does not affect processing, on the third panel you get complete inhibition. So we've got an assay now that we can feel is fairly robust.

So now we can start to ask some more relevant questions in terms of vaccine development. Does virus neutralization by CTL depend on the epitope that's being targeted? Pretty fundamental question. We've got a lot of effort put into making vaccines. There's only a few things that we can do. One of the things that we can do in terms of designing those vaccines is to decide what to put into it. Should we put the kitchen sink in, or should we be selective about what's being put in, and are some HIV antigens better than others? So here's the simple experiment that you can do now. You take somebody's PBMCs and you enrich either for gag-specific response or an envelope-specific response by stimulating *in vitro* with an envelope peptide. And then you put them into the same assay and you say do these things now that we can measure

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as enriched populations for envelope, or enriched for gag, do they inhibit virus replication?

Now, we published a paper in January that suggested that gag responses by LE spot, the broader the gag specific response the lower the viral load, and the broader the envelope specific response, quite surprisingly the higher the viral load, which was a little unsettling to us to see. Because here are immune responses in the same individuals that are heading in completely different directions. Well, now we have a chance to look at this same question in what we think is a more physiologically relevant situation and here's the result we get. Same HLA allele that's restricting here. On the one hand, in this individual we've enriched for an envelope specific response, and we don't get inhibition of replication, but when we enrich for a gag specific response, we get a huge amount of inhibition of replication. So that at least in this one single incident looks kind of like what we were seeing in this paper that we had published earlier.

Well, can we take this a little bit further? So let's do that, and [inaudible] Chen in our group did that by getting 14 different lines from individuals that are HIV infected for gag and 12 for envelope and then looked at inhibition of primary isolates, both R5 and S4 viruses. And so these are all the specificities. The only thing I'll point out is that we're

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matched for - we have the same restricting allele for envelope and gag specific responses in most of these individuals. And so here's one of the individuals. So all we're doing is we're enriching for the different specificities that are already present there and look at what you see. There are huge differences in the antiviral efficacy of these different responses that are floating around. As you enrich for some, the ones in green are gag, and you get relatively better inhibition of replication than you do with the envelope specific lines that you generate from the same individuals. So these are taking all the specificities that happen to be there and just asking which of them are really mediating antiviral activity?

If you look at this now in two different patients, the patient on the top against an X4 and an R5 virus, you'll see essentially the same thing. Gag does better than envelope. If you take a second subject down on the bottom, again in the green are the gag specific responses and the red are the envelope specific responses. We didn't go into this with a bias, we just generated all those responses. But this is what the data basically showed. We can do a statistical comparison now in terms of these outcomes by basically using something called log inhibition units where the greater the inhibition, the higher the log inhibition units. And basically for all

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four of these individuals, gag specificity wins, envelope specificity loses. In every single one of these individuals that we looked at, when we enriched for envelope specific responses, we got less virus inhibition. When we enriched for gag, we got more virus neutralization.

Is a simple explanation just that we've got mutations that are arising? So let's sequence virus from every one of these patients that we looked at, and let's also sequence virus at the end of the inhibition of replication assay when you've got ten days out, your growing virus. Let's make sure that that's not mutated virus. And in fact, that's what we found, that the mutations would actually not explain this, and when we look on the top two panels here, just at all of the responses in general, we see a highly statistically significant difference. The gag responses are doing much better than the envelope specific responses. On the bottom, we've taken out those isolated cases where there's a mutation within the envelope epitope or adjacent to the envelope or gag epitopes that only got rid of a couple of the envelope and gag specific responses that we were evaluating, but now with mutations completely taken out of the picture, we see the exact same thing.

Well, can we confirm this in another way? Let's do this in kind of a sequential way where we progressively enrich

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for gag and progressively enrich for envelope and see if we get the same effects. And in fact, here's what you see - on the left hand panel, we're measuring the percentage of the response in the bulk cells that's gag specific. And you can see that as you stimulate a second time, that goes up. So you're stimulating with gag peptides. As you enrich, in the middle panel, what you see is that the log inhibition units go up as you enrich, and in fact this is highly statistically significant.

Look at what happens with envelope. On the left-hand panel, you enrich for envelope, but as you enrich for envelope, you actually get less virus inhibition, and that's highly statistically significant and goes exactly in the opposite direction of what we're seeing with gag.

So based on these data, I think we have to think about how to put these into perspective. A couple of conclusions and then some questions. The relative ability of CTL to neutralize HIV can clearly be quantitated in *in vitro* assay. Whether that's relevant *in vivo* I don't know, but it's certainly suggested to be relevant based on the assays that have been done and these issues about viral escape and antigen processing where it all fits in with what we're seeing *in vivo*.

HIV-specific CTL differ by over a thousand fold in their ability to neutralize HIV. A thousand fold difference in

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terms of their specificity, in terms of what their neutralizing activity actually is. That's a lot of difference for a response that we've generally sort of thought about as CTL or CTL and whether it's envelope or gag or pol or nef, who cares? Let's get the most we can. The third conclusion is that gag-specific CTL responses consistently neutralize better than envelope-specific responses. And perhaps more importantly, enriching for gag-specific responses enhances neutralization, but when you enrich for envelope-specific responses, you actually diminish neutralization of virus. And this is all independent of the restricting HLA allele.

I think these are very striking data. The big question is why, and is there any potential explanation for this? Is this all something that's completely irrelevant because it's done *in vitro*, or is this something that really we need to think about in terms of where we're heading with vaccines? Well, in terms of why, there was a really terrific paper published by David Watkins and a post-doc of his, Joan Asasha, basically showing that when virus gets into a cell, there's pre-formed gag protein in that virion, and that preformed gag protein can immediately be taken up into an antigen processing pathway and presented at the cell surface. It doesn't have to undergo *de novo* protein synthesis. But for other things like envelope, before a cell is sensitized for recognition, you have

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to first generate those proteins through *de novo* protein synthesis. So that's at least one potential explanation for this.

I think another potential explanation is that envelope is just very, very plastic, and tolerates a lot of mutability. And so that during the inductive phase of the immune response, if mutations are arising, you may never have a constant enough antigen to be looking at to really get the same kind of affinity maturation that you get with, for example, gag-specific responses.

But there are a lot of unanswered questions right now. How relevant is this *in vivo*? I think we don't know. What are the implications for vaccine immunogens? Should we use this to think that in fact maybe we should focus on gag? Should we take these data and say oh, my God, is inducing envelope-specific responses actually exactly what we don't want to do? Or in fact is this just kind of another one of these *in vitro* assays that has no real relevance and we have just kind of keep thinking about new assays to develop? And I certainly would be a proponent of trying to develop new ways to look at antiviral function of immune responses, and I'm not sure this is the best.

And then finally, what are the implications for vaccine testing? Do we need to look at responses that are being

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generated, if we've got a way to measure neutralization in terms of cellular immune responses, should we be measuring that with vaccine candidates to try and understand what's going on? These are laborious assays to do, but we've been able to get them very highly reproducible. We're working with Jill Gillmore to develop this with IAVI for a vaccine, a high-throughput assay for vaccine testing.

And I guess I'll stop there and I want to just acknowledge [inaudible] Chen, who is the post doc in my lab who's been working on this. We've had help from a couple of other labs at the Partners AIDS Research Center, Marcus Altfeld's lab and Todd Allen's lab, and a lot of helpful discussions and collaborations from people like Eric Lander, Denis Burton, David Watkins, Otto Yang, Jill Gillmore, Phillip Goulder and Jerry Kavatia [misspelled?], and funding, in particular recently from IAVI to do these particular studies. Thanks very much.

[APPLAUSE]

JOSE ESPARZA, M.D., PH.D.: Thank you, Bruce. I'm sure we have to have some questions. I know that there is.

MALE SPEAKER: I have a question related to methodology. So you're expanding the cells with specific gag and env. epitopes and then looking at responses in killing the virus. Is there a possibility that you're missing some

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autologous epitopes in envelope so you don't expand the populations that might be most responsible for the killing at the time?

BRUCE D. WALKER, M.D.: It's possible, and there may be some stronger responses in envelope that we're not able to detect, but we've done this with - we know what the autologous epitopes are, we know that by LE spot they have strong responses to these particular epitopes. So, in fact, if you look at the expanded cells in terms of the LE spot responses, there's no statistically significant difference between the envelope and the gag-specific responses.

MALE SPEAKER: And if you look at the population of the virus that you're pulling these cells out of, is there any evidence that you have escape or [inaudible] envelope epitopes?

BRUCE D. WALKER, M.D.: And that's a good question as well and we don't have evidence of that, and so I don't think that's the explanation. You know, it's interesting - there seems to me to be something fundamentally different between these envelope- and gag-specific responses and I really have not understood it and I still don't understand it, but envelope is processed differently - we know that from Bob Silicano [misspelled?] - than gag, and that may be part of the issue here. It may also be that mutability within envelope also makes a difference. But what I've shown you is taking some

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people who have circulating responses and trying as best we can to show what inhibits replication. We can consistently show that the gag specific responses do really well, and even though they have strong envelope specific responses, we can't translate that into something that shows us inhibition *in vitro*.

MALE SPEAKER: I have a number of points that I would like to discuss.

JOSE ESPARZA, M.D., PH.D.: We have four minutes to vacate this room.

MALE SPEAKER: Yeah, but just a few points that I think, however, are important. Still, I am of the opinion that it is very difficult to translate data generated in chronically infected individuals and then to transfer in a preventative setting. Now, I think it would be important to mention that there is a paper also from David Watkins published in *General Virology* showing that if you vaccinate monkeys with gag or gag plus env., the gag plus env. performs better than the gag alone in terms of protection. That is the first point. The second point, I think that I would be a little bit cautious with underscoring the importance of gag, because as you know, 100-percent of people with chronic HIV infection have gag responses. And as the people progress in any case, then I think to say that the gag is protective and env. it's non-

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protective, I think it's really a risky call because as I said, actually it's very hard - or minority of individuals have env. specific responses during chronic infection, but 100-percent, they have gag responses.

Now the last point is about the feasibility of [inaudible], because for people like us and others in the field that have spent the last, five, six years to validate an assay, which is the earliest part, I think that in order to validate this assay, it's going to take a long time. I don't think that this type of assay can be implemented very easily when you have 200 volunteers with 15 determination for each volunteer. It's going to be very hard.

BRUCE D. WALKER, M.D.: You know, and I think to take the last question first, I'm not advocating that everybody needs to have this assay done. I think, though, to take selected individuals that are getting vaccine-induced responses and try and look at something that reflects virus replication is something that just makes sense to me and that we ought to try and do. So I think it's not a field assay, I think absolutely you still want to see what the specificity is by interferon gamma LE spot, but you want to know as you're looking at these vaccines, I think it's really critical to know and to evaluate outcomes based on the specificity of these

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different responses that are being generated. And so I think that that's one of the key issues here.

Now, you had some other criticisms.

MALE SPEAKER: Not criticisms, just comments. I mean, everybody has gag responses, but not everybody progresses.

JOSE ESPARZA, M.D., PH.D.: You need to either go very quickly or have a conversation after the conference.

BRUCE WALKER, M.D.: Okay, well, we'll talk after, that's fine.

JOSE ESPARZA, M.D., PH.D.: Okay.

MALE SPEAKER: First, David Watkins' work also has raised with the [inaudible] they've depleted the CD8 T cells from chronically infected animals and looked at the responses and come back when the control is reestablished. I think it's brought up the question, you can measure a lot of things *in vivo* but maybe immunodominance is keeping us even with looking at activity of CTLs of picking up what the right CTL responses are. So in both David's work and in Otto's work, they also have sort have emphasized not just gag and not just looking at envelope, but also thinking about Nef and the fact that immunodominance and perhaps proteins expressed earlier in the life cycle might in fact be the place to go to target and to get CTL activity that might be more focused and better able to control HIV replication. You've commented really on the

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differences between gag and envelope and I think that mirrors your studies, but have you, or do you know if there's anyone else that's tried the use of these assays to look at gag versus nef?

BRUCE D. WALKER, M.D.: Yeah, we're doing that now, and I think other people are adopting these assays also to look at that. In terms of early proteins, in fact what David's study shows is that from a CTL standpoint, gag probably is the earliest protein because it's preformed when it gets into the cell, and within 4 hours of a cell being infected, it can be targeted by gag-specific CTL, whereas envelope-specific CTL come up about 24 hours later. I think there are other issues that are involved here as well in terms of trying to think about the fact of chronic infection and there are a lot of immunoregulatory mechanisms that at play here as well, PD1, CTLA4, et cetera. So it's a complicated pattern.

I'm just trying to be provocative here, I think, in terms of saying that we've developed a whole antibody field around the ability to neutralize virus, I'm showing you some provocative data that say we can do the same thing for CTL, we can elute out those specificities and we can see something that's really striking in terms of the difference. What does that mean? I don't know. But I guess we as a field have to address that.

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MALE SPEAKER: And I think that just because so much effort has been invested in looking at LE spot assays, I think we still have to acknowledge that we may be measuring the wrong thing, or that that assay, even though it's very doable, may not take us as far forward as looking on a smaller scale.

BRUCE D. WALKER, M.D.: Well, I think again LE spot assay tells you something very important, because it tells you the specificity, and I think what these data suggest to me is that specificity matters.

JOSE ESPARZA, M.D., PH.D.: So thank you to the four speakers for their presentations, for all of you for great questions and for staying until 6:00.