Understanding HIV Transmission Mechanisms: Microbicides and PrEP
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ELLY KATABIRA: Let's start. For those who are still standing, please take up your seats. We are about to start in half a minute.

Welcome to our session this evening. It is Understanding HIV Transmission Mechanisms: Microbicides and Prep. I'm Elly Katabira from Uganda, Professor of Medicine in the Department of Medicine Makerere University Medical School and also I'm the President-Elect for the International AIDS Society and God willing, I will tackle that tomorrow.

My co-chair is veteran Tim Mastro who has been around in the field of research and now working with the FHI who has been involved in a lot of work not only in the research but also in the education across the globe.

This is an important session and since we are already late, we would like to start right away. Our first presenter is going to be Professor Eric Hunter who is a Professor of Pathology and Laboratory Medicine at the Emory Vaccine Center. He completed his undergraduate studies in bacteriology at Birmingham University in England back in the late 60s. His graduate work has been on immunology and he has carved out most of his cancer research at the Imperial Cancer Research Fund in Bruno University. However, the work he is going to talk about he has done it mostly in Rwanda and Zambia. Eric.

ERIC HUNTER: Thank you. I would like to thank the organizers for an opportunity to discuss our work today.

We started out with a hypothesis that an effective prophylactic vaccine or microbicide against HIV-1 has to protect against those viruses that initiate infection at the mucosal surface. Realizing that these may really be distinct from the bulk of variants that have evolved within the host to survive during their growth in this immunologically unfriendly environment.

What are the nature of these viruses and where do they come from? We first reported on this in a paper where Cynthia Derdeyn was the first author about six years ago and showed that in eight out of eight transmission pairs, heterosexual transmission pairs, and a single genetic variant from the quasispecies and the donor initiated infection in the newly-infected partner.

A few years later Brandon Keele and the CHAVI group headed up by George Shaw looked in a Tour de Force at almost 100 newly-infected individuals and using very novel phylogenetic approaches showed that about 80-percent of these infections had been initiated by a single genetic variant.

That bottleneck actually can be broken down to a certain extent by inflammatory genital infections as we showed in 2009. Ron Swanstrom's group showed similarly that this multiplicity infection was a non-quasi distribution suggesting

that other factors might be involved in determining how many genetic variants might initiate infection in a new individual.

In some cartoon form then what we are seeing is this very heterogeneous quasispecies in the donor being funneled through a genetic bottleneck where one or very few genetic variants initiate infection and diversity reemerges under the influence of both the cellular and humeral immune responses.

The studies that I am going to talk about have been done in two HIV discordant couple cohorts. One in Kigali Rwanda and the other in both Lusaka and Ndola in Zambia. These cohorts are directed by Dr. Susan Alan at Emory University. The advantage of studying discordant couples where the rate of transmission decreases significantly upon counseling and testing and disclosure of HIV status to both partners is that we can actually analyze both the donor and the recipient viruses.

When a low frequency transmission occurs, we can then obtain samples from both the chronically infected partner and the newly infected partner very close to the time of transmission. In our cohorts, around 80 to 85 percent of transmissions are what we call epidemiologically linked that is once spouse transmits to the other.

This is a classic example of a phylogenetic tree from such a transmission pair. What you can see in green here is a very diverse virus population. The horizontal lines that are

connecting each of the in this case plasma and PBMC viral sequences in the region of envelope indicates the genetic distances that are between the different variance. We have to add up the horizontal distances going from one variant to the other.

What is striking shown in blue is a very homogeneous virus. Most importantly is that all of these viruses, all of these variants present in the newly-infected partner emanate from a single branch on the donor tree indicating that they all come from a single genetic lineage most likely from a single virus infected cell that has initiated infection in the newly infected partner.

I mentioned that in our discordant couple cohort about a large percentage of these transmissions are epidemiologically linked also and that in those linked transmissions, about 90-percent are initiated in the newly-infected partner by a single genetic variant.

Recent work from gay men has shown that is somewhat lower, about 80-percent with maybe 20-percent having two, three, or four variants being transmitted. Recent data in IVDUs has shown a greater number of individuals being infected by multiple variants, again depending on the cohort understudy.

Another way to look at this is using a program called the highlighter program from the Los Alamos lab and this allows us to do is to compare each of the viral sequences. These

viral sequences are generated by what we call single genome amplification. Each sequence is derived from an independent virus genome and it therefore allows us to quantitate the frequency of variants within the pool of sequences that we are sampling.

We are looking here at 40 individual viral genomes in the region of envelope and what you can see is that if we compare every nucleotide to every nucleotide across this group, the majority of these sequences are actually identical in this region.

About a quarter of them have a single base change and about one-twentieth of them, five percent have just two base changes. If we model this then based on just what we would expect the error rate to be for the reverse transcriptase and model in the absence of selection how long it would be for this type of a pattern to be observed, we would anticipate it would be around 23 days. You can see that somewhere between 13 and 26, it turns out to be about 23, and that is very close to what was calculated based on the visits of this individual to the clinic.

Early on, the virus is very homogeneous and for the most part infection is initiated by a single genetic variant. Where does this virus come from then? Where does this bottleneck originate? Is it through limited heterogeneity in

the genital fluids or is it because there is limited infection of the mucosal surface and selection during viral outgrowth.

We have now looked at a total of nine transmission pairs comparing genital fluid sequences to those in the blood of the donor and to the virus that initiates infection in the recipient. This shows I guess around 120 sequences. It turns out that is the virus in the donor that is closest to the recipient. If I blow that up, what you can see is that that virus only differs in this region by three nucleotide changes, most of them silent, two out of three silent from the virus that initiates infection in the previously uninfected partner.

When we put those sequences now onto a phylogenetic tree, a surprising result is found. While we do see compartmentalization of viruses in the genital fluid, that is observed here with this cluster of viruses which represents about 50-percent of the total sequences that we are seeing in the genital fluid, the virus that actually is most related to the virus that establishes infection is not encompassed in that and neither is it in the other nine pairs that we looked at.

So it does not appear that the populations that tend to propagate within the genital fluid are the source of the virus that initiates infection. Based on this single genome application analysis of genital fluids we do see viruses that appear to be comprised of stable sub-populations or clades, some of which exhibit very limited diversity. But because the

variant that establishes infection is generally not derived from the predominant genital tract clades, it is unlikely that the limited diversity in the donor genital tract is the reason for a single genetic variant being transmitted.

I think what is most important here is that the data argue against a purely stochastic mechanism for transmission and in effect for selection of a variant with specific traits that favor systemic spread.

On initial analysis of eight heterosexual transmission pairs in our 2004 paper in comparison to viruses in the donor quasispecies, the viruses that established infection in recipients encoded envelope like a protein molecules that exhibited shorter V1-V4 regions. This was recapitulated in 10 additional subtype C transmission pairs in the paper by Rich Haaland from our lab in PLoS Pathogens last year.

They also exhibited fewer n-linked glycosylation sites in V1-V4 and this has been supported by some work that was presented this early afternoon from Ron Swanstrom where they have compared acute and chronically-infected individuals and shown that the acutely-infecting viruses have fewer glycosylation sites. Tony Fauci in his plenary talked about the possible requirement for loss of glycosylation sites for exposure of the $\alpha 4\beta 7$ binding site and mucosal trafficking.

We had hypothesized that perhaps these more compact envelopes might bind CD4 and CCR5 better. Actually, what we

find is the opposite. They are very sensitive to reductions in CCR5. You see a 34 decrease and a 54 decrease in CCR5 here 1,004 fold decrease when we drop CD4 10-fold. They are very dependent on CCR5 and CD4. That correlates well with the work we did with Ron Coleman showing that these viruses infect macrophages very inefficiently. That was also reported this morning by Ron Swanstrom.

What we can say then is that the envelope glycoproteins of founder viruses show a high dependency on CD4 and CCR5 for mediating entry into cells expressing different levels of these receptors. This is consistent with efficient replication in mucosal T cells, but very inefficient infection of macrophages and argues against a role for macrophages in this process.

The differences in glycosylation and V1-V4 lengths may reflect tropism determinants such as $\alpha 4\beta 7$ binding or a requirement for dendritic cell interactions that are currently under study.

Obviously, a work like this is dependent on a large group of individuals, particularly we are very grateful to our Rwanda Zambia HIV Research Group with leaders in both Rwanda and Zambia, and the team of individuals at Emory University, continued collaborations with the University of Alabama at Birmingham and Los Alamos Lab.

I have to thank the NIH and the Gates Foundation for funding and IAVI for their funding of our sites where the

discordant couple cohorts are maintained and followed. Thank you very much. [Applause]

ELLY KATABIRA: Any quick questions please? Microphone number three.

ERIC HUNTER: Joe.

JOE IERON: Hi Eric, Joe Ieron. Do you have any idea how stable the variant pool in the genital tract is in women or men over time? One of the questions I have is whether you get the sequences from the genital tract but obviously, it is not on the day they transmitted or most likely not on the day they transmitted. Is that diversity kind of stable or is it possible that the predominant variant is kind of changing with time?

So we have looked in three women over a period of about a month. So looking at times zero, 14 days, and 28 days. In two of those, these clades seemed to stay quite steady. In the other, there was sort of a mixture. There was continued existence of the clade viruses but in one instance there was also another clade appeared.

I think in general these are more stable than we might think, at least over a period of about a month. Certainly, the clades themselves seem to age for longer than a month. They look like they have been around for longer than a month just based on the diversity in those clades. There is always a

limitation with people you cannot actually be there at the time of transmission occurs.

ELLY KATABIRA: Microphone 3.

ALAN LANDAY: Alan Landay, Chicago. Very nice talk Eric. A quick question on whether not host immune factors might play a role within the female genital tact and also a role of cervical mucous in modulating the virus and that sort of bottleneck effect that you have spoken about.

ERIC HUNTER: I honestly can't answer that. One might anticipate that perhaps viruses would less like oscillation and perhaps less charge on the surface might be able to get through mucosa better. Ron talked a little bit about that today.

We are starting to look at innate factors in these pairs and TRIM5 to see whether we can see anything there. We are also looking at some NK cells of the host genetic factors. Hopefully over the next few years we will actually be able to sort some of this out.

ELLY KATABIRA: Any other questions? Well, if none, thank you very much. [Applause] Our next speaker is Dr. Ellen Kersh who has been a staff scientist with the laboratory branch of the Division of HIV/AIDS prevention at the CDC since 2006 where she serves as an immunologist on the preclinical evaluation team. She received her undergraduate training in Germany and holds a PhD in Biomedical Sciences from Washington University in central USA.

She has received her training with Dr. Rafi Ahmed of the Emory Vaccine Center. She is going to talk to us about understanding Oral PrEP during mucosal SHIV infection reduces viremia, preserves CD4 counts, and raises potent T-cell responses. Dr. Kersh.

ELLEN KERSH: Thank you for the invitation and for allowing us to present our work here. Our laboratory group at CDC has extensively used monkey models to demonstrate that pre-exposure prophylaxis with antiretrovirals can be very efficacious. This has formed the design of current clinical trials. Although we have shown that PrEP can be very efficacious to 100-percent, for the current study we are looking at animals that have failed PrEP and have become infected while attempting to stay uninfected while taking PrEP.

In these animals we are asking the questions, what is the impact of PrEP on infection course when PrEP fails to completely prevent the infection. We have looked at their viral loads, CD4 counts, and parameters of adaptive T-cell immunity. This was guided by the question whether we can identify important parameters for analysis of human clinical trials when results become available.

For all of our macaque work we have used a realistic macaque model for HIV sexual transmission and prevention studies. We have called this the repeat low dose model. In this model, we take rhesus macaques and expose them repeatedly

to low doses of virus. We use doses that would be similar as can be found during the acute infection phase in human semen. For the current study, we have used a dose of ten tissue culture infectious doses.

This has allowed us in the schematic here, schematically shown to measure the effectiveness of prevention strategies here for PrEP. What we are measuring here is how many macaques remain uninfected while receiving several exposures to a virus called SHIV SF162P3 as shown here by the arrows. The naturally untreated control group would become infected after a few exposures while any exponential group would experience either delayed infection or complete protection.

For the current study, we are using this model to measure early infection parameters that would happen after such relatively natural infection at a natural dose. Here is a schematic of important infection parameters. You can see that after the exposures with this virus it causes a relatively robust infection with high viral loads. However, these are controlled relatively quickly. More so than human HIV infection. For that reason, we have focused the current analysis on the early parts of the infection the first few weeks after peak viremia.

Another parameter that is shown is CD4 counts. You can see that with this particular shift virus there is a decline of

CD4 counts early on but it recovers naturally quite well. Another reason why we have only looked at the early phase of this infection.

We have used PrEP to try to prevent infection but it failed. The animals that we are looking at also received PrEP during the early phase of infection. That was done for two reasons. A, we studied whether resistance would develop in such a case. Also, we wanted to continue to use a realistic model because we think in PrEP clinical trials it is possible that people, if they do become infected they will not immediately realize this. They will continue to take their drugs for a while until they are diagnosed positive.

This then was our exact study design. We had two groups of monkeys. One was the no PrEP control group. We had five monkeys in that group shown here in white. We had a PrEP infected group that was retro effectively picked from PrEP failures that came off efficacy testing. This consisted of six monkeys. On the right here, you can show that the monkeys received 14 rectal shift SF162P3 exposures once per week.

The control animals shown in white experienced infection and in green is shown the infection time points of our experimental animals. Two of the six animals received Truvada in an oral form once per week that is why we are calling it intermittent Truvada. They came off a group of six

animals where only two failed. You can see those two. Those were picked for analysis.

We had another group of four animals. They received an experimental tenofovir pro-drug called GS7340 and those you can also see here failed the PrEP regiment quickly. Their dose was also given orally just once a week. I should say that all these drug doses were similar as would be used in humans.

This then is the course of viremia in these animals. On the left you can see our control group. You can see that these animals reached peak viremia very quickly and it rose to about 10 to the eighth viral RNA copies per milliliter of blood. In contrast, the PrEP breakthrough animals experienced a reduction in peak viremia that was 100-fold. That was statistically significant as shown here.

The reduction in viremia was seen throughout the experiment. It lasted for twenty weeks post-viremia. Statistical significance however was only seen at week six, no longer at later time points. That is because this virus is naturally well controlled. Another reason why we are focusing our analysis on the early parts of the infection.

In green, here these lines represent the time of PrEP that it was continued post-peak viremia and it ranged from zero to 12 weeks. When it was discontinued, you can see that there was not a sharp rise in viremia. Viremia continued to go down in these PrEP breakthrough animals.

I should also point out that the PrEP regiment showed this viremia reducing affect in all of the animals regardless whether they received Truvada or the experimental drug. These results are consistent with what we have previously seen and published in two previous publications. Although our study groups are small here, we have more animals where we have seen this reduction in viremia. We have also analyzed drug resistance throughout this time course here and did not find it by PCRN sequencing. In addition, we looked at the MHC haplotypes in these animals and found them to be diverse. We do not believe that it was specific MHC genetics that caused this.

We then looked at CD4 counts in these animals and that is shown in this slide. At baseline, there was no difference between the two groups. However, at peak viremia, the PrEP breakthrough on animals had 5-fold higher CD4 counts, which was statistically significant. This indicated there was a better preservation of this important disease parameter in this type of infection compared to control animals. We did not see that at later time points, but again we think this is because naturally this CD4 count in this virus infection recovers quite well.

We then looked at specific T-cell functions in shift specific cells to analyze how good they were in viremia control. The first shown here in A is we looked at cytokine

production and chemokine production and gag-specific T-cells. These were stimulated in-vivo and then we looked at INFγ, IL-2, MIP-1β, or TNFα. Again at peak viremia. This was done to get an idea how functional these cells were.

It is generally believed the more multifunctional these cells are, the more cytokines they can produce together the better they are at controlling viremia. What you can see here is that in the PrEP breakthrough animals there was significantly higher numbers of T-cells, shift specific T-cells that were able to make simultaneous chemokines and cytokines.

On the bottom here is a result from ELISPOT analysis. We also looked at the epitopes specificity and the breath of the response in these animals by stimulating T-cells in-vivo with 14 peptide pools representing all the major epitopes that would be expected. That was done 11-12 weeks post peak viremia.

Here we are not showing the absolute extent of the responses, but we are looking at how many peptide pools were recognized. You can see that in PrEP breakthrough animals there was a greater number of peptide pools being recognized indicating that the T-cell response was broader and most likely also better at controlling viremia.

Lastly, I would directly address whether CD8 positive cells are important for viral control in prep breakthrough infection. It had already been shown that in control natural

shift infection in-vivo depletion of CD8 positive cells leads to an immediate rise in viremia because these cells are important in controlling viremia. We wanted to know whether that was also the case in this PrEP breakthrough infection. At 28 weeks post peak viremia we depleted CD8 positive cells. A schematic rationale is shown here. We reasoned that if after CD8 depletion we would not see a significant rise in viremia, then the control of viremia in PrEP breakthrough infection would not be due to CD8 positive cells. If we saw a rise in viremia shown on the right, then the control of viremia would be due to CD8 positive cells.

The outcome of this experiment is shown here where again in white we have control infected animals that received anti CD8 antibodies and on the right side are the PrEP breakthrough animals. On the top of the slide you can see how CD8 positive cells disappeared from the blood after such treatment when antibodies were injected. You can see it was efficient in both cases. On the right top side, there is one animal that received a mock IDG antibody as a control. On the bottom, we are looking at viremia and you can see that in both cases, there was a rise in viremia and it was to similar levels.

Our conclusion from that was that CD8 positive cells contribute significantly to viral control in both breakthrough PrEP infection and in natural infection.

To summarize our data, we found that breakthrough PrEP infection is characterized by the following parameters. We found decrease in viremia and found good preservation of CD4 positive cells early on. We found some parameters of T-cell immunity improved in breakthrough PrEP infection. Early on there were high T-cell counts. We found more and more multifunctional shift specific T-cells with greater epitope diversity. In both situations CD8 positive cells efficiently controlled viremia.

What are the potential implications of this? In general, low viremia and good preservation of CD4 T-cells if it lasts and it lasts throughout the infection course could be interpreted as a sign of a potentially attenuated disease course. If that is found in followup studies of PrEP infected people, then one could hope that maybe PrEP failures could experience a longer disease free life, reduced need for antiretrovirals and fewer chances of virus transmission.

Our study had some limitations due to the shift virus that we used for our efficacy testing we were limited in how long we could analyze these animals. The long-term immune control remains unclear. Also, we continue to give the drug during the acute infection phase. We do not know if it was the drug exposure or the improved T-cell immunity that led to the low viremia. It is possible that both parameters were important. In our study group, sizes were small.

We think the most important question coming from this will be whether this will be observed in PrEP clinical trials and in humans in general. We would like to point out that there was a case report published in 2008 by Martin Markowitz's group that reported one individual who was on a prophylaxis ARV regiment, he experienced low viremia, and high CD4 counts.

We are hopeful that a similar phenomenon will be found in humans. We certainly would like to suggest that these parameters should be analyzed in followup studies of PrEP clinical trials.

The study was done at CDC. It is a collaboration between two teams. One of Walid Heneine and Janet McNicholl's. We acknowledge receiving antibodies from the NIH Nonhuman Primate Reagent Resource and drugs from Gilead Sciences through Jim Rooney. Thank you. [Applause]

ELLY KATABIRA: We have a few minutes for quick questions. Microphone 3.

FEMALE SPEAKER: Have you had the opportunity to measure the HIV failures to see whether the reduce of the replication of the activity in PrEP failing animals is due to lower number of infected cells or to the ability of CD8 sets to control infection.

ELLEN KERSH: We have not done that. In general, our failures, we do look at drug levels. They are not zero, but we have them under experiment.

ELLY KATABIRA: Microphone 4.

MALE SPEAKER: I may have missed the point, but did you see emergence of resistance in your animals? Those who failed PrEP.

ELLEN KERSH: Yes. That was done at every time point we got virus load. We did PCR analysis for specific resistance mutations and did not find them. At the end of the 20 weeks, we sequenced by bulk sequencing though, we sequenced the entire virus and did not find drug resistance.

I do have to say we have some animals that develop resistance, but these were specifically picked for not showing resistance. We wanted to do an immunological analysis, not for resistance.

ELLY KATABIRA: Microphone 3.

MALE SPEAKER: Nice talk. Do you know anything about mucosal responses? Either the immune response or the virologic response at the mucosal level?

ELLEN KERSH: No, we don't. We would like to know that. We have attempted to do the ELISPOT, but were not successful due to lack of cells.

ELLY KATABIRA: Microphone 7.

MALE SPEAKER: Can you speculate as to why you think you had enhanced T-cell immunity in the PrEP breakthroughs?

ELLEN KERSH: My speculation would be that it is, you have better preservation of CD4 cells and they are needed to

drive several other immune responses. That would be my explanation.

ELLY KATABIRA: Thank you very much. [Applause]

Our next speaker is Robin Shattock, who is a professor of cell and molecular infection in the Department of Cellular and Molecular Medicine at St. Georges University of London. He directs the research group working on the pathogenesis and transmission of HIV infection with a particular emphasis on the development of prevention strategies applicable to the developing world. His research group has been instrumental in isolating the estimating the animal mechanism for HIV transmission. This has led to the establishment of international collaborations aimed at preclinical identification, development, and selection of microbicide and vaccine candidates prior to four month clinical trials. He is going to talk to us about understanding the mucosal immunity and the HIV transmission the way to new prevention technologies. Please, Dr. Robin.

ROBIN SHATTOCK: Good afternoon everybody. It is very nice to be giving this talk and my talk has been made a lot more exciting of the announcement of the trial results from the CAPRISA 004 study. In a short period of time I am going to talk to you a little bit about mucosal immunity, HIV transmission, and how that may impact on the development of new prevention technologies. When we think about the mechanisms of

HIV transmission, a lot has been learned in the last few years particularly in primate models. We know that if you look at the diagrams showing the stratified epithelium, that cell-free virus can come into contact with Langerhans cells that reside in the stratified epithelium and thereby potentially establish infection.

Also, in the epithelial layer there are CD4 positive cells that are susceptible to infection and any ulceration that allows the virus direct passage to the underlying stroma will then meet a wider range of potential target cells. When we think about columnar epithelium, the picture is perhaps more complex. The virus has only a single epithelial barrier to breach. There is some evidence that transcytosis may take place on certain mucosal surfaces. Again, perhaps more controversially is the issue of infected cells from the donor that may be able to transmigrate across epithelial surfaces.

Now what we don't know in terms of PrEP and microbicides is whether individual candidate work equally against all these different mechanisms of transmission. Particularly cell-associated transmission which has been hard to model in primates. More recently, Roger Le Grand's group in Paris has established a very efficient method for showing cell-associated infection following vaginal challenge. This will been an interesting model to assess some of the PrEP and microbicide candidates.

When we think about the time that's required for an immune infection to take place, it is relatively short. We know that a limited exposure of 30 to 60 minutes is sufficient to establish infection in macaque models. We can see localized infection within a 16 to 72 hour window and then dissemination of that infectious virus to the draining lymph node. This certainly means, in terms of PrEP and microbicides, the drugs has to be in the right compartment at the right time.

What is less clear is how far for a microbicide, the drug needs to penetrate. The other issue is the turnover cells through a mucosal compartment. This means that potentially cells that have not seen drug may be come into mucosal tissue with a different frequency depending on the level of inflammation. This may have a dramatic impact on the duration of protection that a microbicide may deliver. When we think about drug penetration studies we don't know whether the drug needs to purely get into the underlying mucosal tissue or whether it needs to penetrate to draining lymph nodes. This may have important consequences going forward with other drug candidates that may have more limited drug penetration than tenofovir. Clearly these things can be modeled in primate studies.

Where are we in terms of the state of the pipeline for microbicide development? You will be well aware that this first generation concepts were successfully tested but lacked

potency. This led to the drive to assess more potent products, particularly antiretroviral based products. This was really built on the systematic testing of the biological plausibility of these different approaches in primate models. This is applied to both PrEP and microbicide development.

More recently this has become more specialized by correlating protection to drug distribution and tissue activity studies both in the nonhuman primate model and in human studies. This is probably now starting to provide a critical link between testing efficacy in primates and understanding what that might mean in terms of predicting efficacy in humans. Of course, this meeting has been historic in terms of seeing first proof of concept for an ARV-based drug of the tenofovir gel.

What we now need to rapidly move forward with is to better understand the dosing relationship between coital and noncoital approaches for microbicides. We also need to see a prioritization of new formulations that can maximize adherence over prolonged periods of use. We will probably see the increase in importance of the development combination of products, particularly combination products that could be added to tenofovir to enhance its activity.

Then finally seeing prioritization of products both in terms of their ability to have different dosing regimens and prioritization of those products into clinical trials because

clearly there is not enough capacity to try all the wide range of potential approaches in humans. When we think about ARV-based approaches, where might they act in terms of the transmission sequence of events?

Entry inhibitors, particularly CCR5 antagonists like maraviroc, have an important role to play in terms of the initial attachment and fusion of virus to those target cells. Those drugs that can impact on reverse transcription can negate or block infection of those initial founder cells. Then perhaps drugs that work later in the infection cycle may critically block the broadcasting of the initial first site of infection, either within the localized tissue or to draining lymph nodes.

Each of these approaches could work on their own, but are likely be more potent if they were considered in combination approaches to protection. When we think about delivering them as microbicides, we need to be critically focused on where they need to be delivered in terms of their penetration and likewise in terms of PrEP whether the choice of drugs actually get to the mucosal surfaces efficiently that require protection.

What it does open up for the first time in terms of both microbicides and PrEP interventions is a whole armory of potential drugs that have been developed for therapy. This means that rather than working with very early concept

candidates that may have many years before access into clinical trials, there is a wide range of products now that can be rapidly moved into the clinic. In terms of both PrEP and microbicides it's critically built on a wide range of studies in monkey models. In fact, there are over 20 studies now showing biological plausibility for different candidates both in PrEP and as microbicides. Tenofovir gel will not become the benchmark for those studies. New drugs may have to prove that they are equivalent or better in terms of protection and those studies now need to be linked with PK to relate the level of drug for the level of protection.

In terms of development, more and more emphasis is being placed on PK/PD assessment. Perhaps the work on CAPRISA, particularly by Angela Kashuba's group, has been a very positive highlight in pioneering that type of approach. Let's spend a moment just to think about what we mean in terms of analysis of PK and PD. Analysis of PK or pharmacokinetics is really essentially looking to see if the drug is at the right site, at the right time, when it is needed to provide protection. That is certainly being measured in parallel studies both in the nonhuman primate studies and in humans.

Perhaps more importantly, people are not focusing on looking at drug activity or pharmacodynamics and taking biopsy samples, both in nonhuman primates and also in human clinical studies, challenging them in the laboratory, assessing whether

they are protected from infection and how that relates to drug dosing. These are now seen as critical tools for product development. Application of those tools or further application to tenofovir gel may be critical in determining optimal dosing regimens and defining the right dosing between coitally dependent and independent approaches.

Increasingly, people are focused in terms of trying to deliver microbicides with sustained delivery approaches. This would both reduce the compliance burden and may give more consistency in terms of the level of drug and protection. Of course, what that means is the drug has to be in the right place at the right time and critical parameters that still need to be evaluated is the turnover of target cells entering and exiting mucosal tissue during potential exposure, both in steady state and in inflammatory conditions, and also the potential entry of infected donor cells.

This slide shows different dosing regimens in terms of how they might work across multiple episodes of intercourse. The downward bars, labeled A, B and C, show three acts of intercourse. What you can see with the blue line is modeling the dose drug levels of a single dose of drug given directly before intercourse with the panel A. What you see next to it, shown in red, is the type of drug levels that you're likely to see with a once daily approach. Of course, what is critical

there is that there will be potent protection both at maximal drug exposure but also at the trough between daily dosing.

Shown in the dotted line is the approach that might be achieved with steady-state release from an intravaginal ring. Now we don't know which of these approaches work, and perhaps what is more complicated is understanding what works in the CAPRISA trial where there was a requirement for a dose to be applied anytime in 12 hours before intercourse or anytime in the 12 hours post intercourse. What we don't know is whether the best protection was achieved when the two doses were far apart, as shown by this modeling, or if the two doses were very close to sexual intercourse, showing a different type of modeling. Now this is just showing the type of modeling in terms of free drug rather than the accumulation of intracellular drug which again would show different kinetics.

What it does argue for is that it requires more clinical trials to look at the strengths and weaknesses of different dosing options. What are the biological questions that come out of CAPRISA 004? The first is 54-percent protection was seen in those that had greater than 80-percent compliance. Now if we assume for a moment that that 80-percent compliance really is a real figure, and ignore the fact that often compliance can be perhaps misleading, why was it not 80-percent protection. What are the potential reasons that could have meant that it was less than higher than 50-percent?

One possibility is that more drug could have been better. A higher dose of tenofovir might have given a higher level of protection. Alternatively, adding different drugs to tenofovir may increase the breadth and depth of protection and in future studies could show a higher level of protection.

The second issue is the spacing of the dosing. We don't know whether there are a subsection of individuals that either had closely spaced dosing or widely spaced dosing that were better protected. Then clearly better adherence might improve the efficacy of a product if the 80-percent reported compliance was not correct. The field will rapidly be looking at better dosing forms.

Target cell turnover, whether those individuals that were less well protected, individuals that had higher levels of preexisting inflammation. Are there potential differences in efficacy between cell-free and cell-associated virus? If tenofovir gel only prevented cell-free virus, for example, we would still have a job in terms of coming out with a second candidate to block cell-associated virus.

The next issue is that of resistance. The risk of resistance in ARV base microbicides are hypothetical. Risks may differ between products, so although not seen in the CAPRISA trial that does not mean going to be seen with other products. Topically applied products may be able to block resistant viruses because they will be applied at much higher

doses than orally applied products. There is some evidence that resistance viruses may have reduced fitness for transmission.

In the clinic, we have often argued that the risk benefit ratio is likely to be low in a clinical trial and really the CAPRISA trial validates that in terms of no observed resistance in those individuals that were infected, but that was a small number of individuals and is too early to assess whether it may have any impact on eventual treatment.

Finally, the ARV approach is likely to be implemented as a prescription only approach and resistance will be continued to be monitored with wider introduction and if identified could be tackled by development combination products. This means as we move forward, there is a clear call for product prioritization. Prioritization in terms of the mechanism of activity rather than taking many products from the same class of drug into trials. This stage of development more advanced products should be further ahead than less advanced products. There appropriateness for combination studies. The ability to define PK/PD studies and the ability for those products to be delivered in multiple dosage forms. This is the prevention landscape as it looks now. Clearly, we hope that the CAPRISA results will lead to an acceleration of more clinical studies to optimize microbicide strategies.

This is a map or a diagram showing potential mechanisms of action of microbicides. What you can see, if you look at immunological protection that might be elicited by vaccines, both are focused on mucosal environments. So now is the time to start considering whether they can be combined, both in terms of whether there is potential synergy but also to ensure that a microbicide does not reduce the potency of immune responses that might be elicited by vaccines.

How might they work together? Combining microbicides or PrEPs with a vaccine may deliver even better protection. The microbicide could potentially provide a window of protection when the vaccine is being delivered if it requires some mucosal activation. It could reduce the infectious challenge and so a vaccine might be able to be more potent if there is less virus. Also protection of an individual exposed to virus may boost an immune response. We have already seen in nonhuman primates some evidence of that. It may lead to broadening of the mucosal immune response and it may convert a high risk challenge to a low risk challenge. The type of challenge in which we saw some success with vaccines in the RV14 Thailand study.

Then finally, vaccines may be good news for a microbicide if there is intermittent compliance as it may prevent breakthrough virus and resistance evolution. As we look forward for new prevention options, we already now have

proof of concept for the first ARV-based microbicide. We hope that we will see some more promise with PrEP in the near future. We have partially effective vaccines by combining them together. We may start to have a wider impact on the incidence of HIV before eventually, hopefully, one day developing a highly effective vaccine. Multiple combinations of prevention options are probably our best hope for having a major impact on HIV incidence.

Thank you for your attention. [Applause]

ELLY KATABIRA: Because of time we have one question. Anyone? Fortunately none. I will now hand over to my co-chair Tim Master.

TIM MASTRO: Thank you very much Elly. My name is Tim Mastro. I am with FHI in North Carolina. At this bridging session on HIV transmission, we've heard about virology and immunology of transmission, mucosal immunity, and animal models. The next three presenters will talk about the pharmacology clinical trials and community participation. So our next speaker is Dr. Kristine Patterson who is a North Carolina native, and North Carolina neighbor of mine. She's an associate professor in infectious diseases at the school of medicine at the University of North Carolina at Chapel Hill. She is part of the large team at UNC that is exploring many parts of HIV transmission. Her particular interest is HIV in women and the pharmacology of the female genital tract. Kris

will tell us about exposure at mucosal tissues to PrEP with Truvada.

KRISTINE PATTERSON: Thank you. It is an honor to be here today and I greatly appreciate Robin for setting the stage for this talk. We've heard throughout the conference the importance of antiretrovirals in preventing the transmission of HIV and we await the results of the current PrEP studies evaluating daily dosing of tenofovir with and without FTC. We've also heard the many reasons why daily dosing prohibitive. As such, clinical trials evaluating the episodic oral dosing are being planned. However, there are no pharmacokinetic data evaluating the duration of drug exposure in these vulnerable mucosal tissue following single doses of tenofovir and FTC. Moreover the intracellular phosphorylated or active component of these antiretroviral have not been measured in mucosal tissues. Characterizing the duration of exposure in mucosal tissue is important to guide these episodic dosing trials.

The primary objective of this study was to characterize the extracellular and the intracellular concentrations of both tenofovir and emtricitabine or FTC in multiple biological compartments in both men and women following a single dose of the fixed dose combination pill Truvada. Our secondary objective was to analyze the decay characteristics or the half-life of these same compounds in the same compartments. Due to

time constraints, the half-lifes will be shown on these slides but will not be discussed during the presentation.

This is a single site open label trial that subjects were healthy, HIV negative, men and women who underwent a comprehensive sexually transmitted disease evaluation prior to enrollment. Subjects were required to abstain from sexual activity during this study and were also asked to continue with their contraception. All subjects received a single observed dose of Truvada. Our pharmacokinetic sampling is as follows. All subjects had paired blood plasma and PBMCs obtained on days one, two, five, seven, ten and 14 days post dose. Women also contributed cervicovaginal fluid at those same time points.

Women underwent a single cervical biopsy and a vaginal tissue biopsy at two time points and men had 10 to 15 rectal tissue samples taken at two time points. Subjects were randomized to undergo their tissue biopsies at either one in five days post dose, two in seven, or ten in 14. Samples were analyzed using validated methods using liquid chromatography and mass spectometry. The lower level of quantification for the parent compound was 0.1 nanograms per mL and for the phosphorylated compound was 2 to 10 pentonals.

Composite non-compartmental PK analysis were performed and summary statistics were described using SAS. Now the ANC ratios, or the penetration ratios, are calculated by dividing the zero to 14 day AUC of the matrix divided by the zero to 14

day AUC for the blood plasma. There were seven women and eight men enrolled. They were essentially identical in age, body mass index and race. There were no adverse events.

Just to orient you, all of the slides will be set up in this configuration. Tenofovir will be on the left, FTC will be on the right, time will be on the X axis and will be in days, concentration will be on the Y axis and that's a log scale. Blood and PBMC concentrations will be in red. The individual matrix will be identified here with that matrix's AUC next to it. When we do calculate an AUC, it will be presented next and then the half-life is here just for completeness.

Using very sensitive mass spectometry methodology, we could quantitate the tenofovir and FTC in the blood plasma up to 14 days post dose. Now in this figure the blue line represents rectal tissue concentrations. In the concentrations of tenofovir in rectal tissue remains greater than blood plasma at all time points and penetrates rectal tissue extremely well providing a rectal tissue to blood plasma concentration of 33. Now emtricitabine also maintains concentrations above blood plasma up to 14 days and also penetrates rectal tissue but not quite as well as tenofovir and has an AUC ratio of 4.3

These bar graphs show the concentrations of tenofovir diphosphate and emtricitabine triphosphate in PBMCs in red and rectal tissue in blue. In both PBMCs and rectal tissue, tenofovir diphosphate can be detected up to 14 days. FTC

triphosphate can be quantitated up to 10 days in PBMCs but only two days in rectal tissue. Now we did not calculate AUC ratios for the phosphorylated compounds and that was because unlike the PBMCs, the rectal biopsies are homogenates and not composed of a pure cell population. Therefore the exposure in the tissue are most likely underestimating what is present in the rectal lymphocytes due to a dilutional effect.

Now let's move to the female genital tract. In the purple line shows the concentrations in cervicovaginal fluid in which both tenofovir and emtricitabine maintain concentrations higher than blood plasma at all time points. The greater penetration of FTC in cervicovaginal fluid, as compared with tenofovir, is consistent with our previous measurements. These figures show the concentrations in cervical tissues shown in green and vaginal tissue in pink. The concentrations of tenofovir and FTC are similar to or higher than blood plasma in both the vaginal tissue and cervical tissue.

Now looking at each drug separately, tenofovir is not quantifiable in cervical tissue beyond day seven and the overall exposure of tenofovir in cervical tissue, however, exceeds that of vaginal tissue by nearly tenfold. FTC is quantifiable in cervical tissue and vaginal tissue only up to day 10. FTC penetrates the cervical tissue six fold that of vaginal tissue. Overall, FTC has greater penetration than tenofovir in cervical tissue and vaginal tissue.

Now these bar graphs demonstrate the phosphorylated compounds in cervical tissue in green and vaginal tissue in purple. Tenofovir diphosphate is quantifiable up to 14 days in both vaginal tissue and cervical tissue. Now there are some earlier time points with unquantifiable data in the tissue and that is probably because these are composite date, they are composite profiles. FTC triphosphate was only quantifiable for two days in vaginal tissue and one day in cervical tissue.

So in this table we tried to summarize the important characteristics of the study. After a single dose of Truvada, tenofovir, tenofovir diphosphate, and FTC could be detected in all matrices for 14 days with the exception of cervical tissue in which tenofovir was only detectable up to seven days. FTC triphosphate was detected for less time in these matrices, especially in tissue. This is most likely due to the challenges of measuring the phosphorylated compounds in tissue.

HPTN 066 has been specifically designed to directly measure tenofovir diphosphate and FTC triphosphate in mucosal lymphocytes. The highest exposure for tenofovir was in rectal tissue and the highest exposure for FTC was in cervicovaginal fluid, cervical tissue and vaginal tissue.

In summary, the preferential antiretroviral penetration seen in this study of tenofovir and FTC supports quantification of other antiretrovirals in all mucosal tissues as part of early development strategies for oral PrEP. Additionally, the

differential drug terminal elimination or the tail emphasizes consideration for combination therapy, especially in episodic dosing.

I would like to thank the study volunteers who literally gave a piece of themselves to allow this study to proceed, Gilead who funded this project through an investigator initiated program, the UNCC clinical pharmacology and analytical chemistry lab who spent two years developing these assays, the NIH and clinical translational research center at UNC. [Applause]

TIM MASTRO: Thank you very much for that very elegant presentation. We have time for a few questions. Microphone number 6.

TIM FARLEY: Tim Farley from WHO. Thank you very much for that presentation. I just wanted to get an idea of what you understand by episodic dosing? As far as I can understand from here that at least you got some tissue levels, and very high tissue levels, for at least several days, maybe not going out to seven and 14 days in the tissues. How does that match with what you understand by what you call episodic dosing?

KRISTINE PATTERSON: Episodic dosing could be once a week and if that's the case then you're not going to maintain coverage, at least based on this data, in the vaginal tissue or cervical tissue. Then that would mean that once a week is not going to be sufficient. If episodic dosing is pre coital and

post coital that may be a different story. Episodic dosing means a lot of different things and that's a really good point, but in order to determine in what episodic dosing is most efficacious or most protective we need to understand how long the drugs are going to be present not only in their parent compound but more importantly in their active component in the vulnerable tissues.

TIM FARLEY: I fully agree and I think for one has to qualify by exactly what one means by an episodic dosing when we are saying this. Thank you very much.

TIM MASTRO: We will go back to microphone 7.

WARD KATES: Hi Ward Kates from FAHI also a neighbor of Tim's, like Kris. Kris together with the data that Angela presented on Tuesday, it might seem that oral tenofovir would be less effective than vaginal tenofovir in the vagina but oral FTC would be more efficacious in the rectum. Opening up, possibly that combination method that would be topical tenofovir and oral FTC. Any comments?

KRISTINE PATTERSON: That could certainly be a strategy but would need to be investigated further.

It depends on – for women, if you're just giving them one component in one vulnerable tissue, if that tail is not covered with a secondary agent that may actually promote resistance in that specific compartment so you may be able to protect them rectally but not vaginally. For men I think we

have a lot more work to do regarding topical gels in the rectum.

TIM MASTRO: We'll take two more questions. We'll go to microphone number 4; and then we'll do 3.

JEAN MICHEL MOLINA: Jean Michel Molina from Paris. I was wondering how soon after oral dosing you were able to detect tenofovir in rectal tissue?

KRISTINE PATTERSON: Our first sampling point was 24 hours and we could detect tenofovir at that time point.

TIM MASTRO: Thank you. Microphone number 3?

MARK MILANO: Mark Milano from New York. That was kind of my question too. I think it's going to be critical to know not days but hours. If you can take this an hour or two before sex is that being planned? And lastly I really wanted to thank you for including the rectal tissue. I think it's absolutely critical that development of a rectum microbicide parallels that of a vaginal; anything less would be foolish at best and homophobic at worst, so thank you for that.

TIM MASTRO: Great. Thank you for that comment and thank you Chris for a wonderful presentation. [Applause] We will all remember our week in Vienna for the results of the CAPRISA 004 Study, so consequently I can think of no better person to present the next presentation then Salim Abdool Karim who along with his wife, Quarraisha, were the lead

investigators on the CAPRIA Study, truly a landmark in this epidemic.

Salim is a clinical infectious disease epidemiologist whose research interests are in microbicides and vaccines to prevent HIV infection, as well as implementing antiretroviral therapy in resource constrained countries. He has faculty positions at the University of KwaZulu-Natal as well as Columbia University in New York, and he's Director of CAPRISA, the Center forth AIDS Program of Research in South Africa, one of the leading research institutions in the world and the place that gave us CAPRISA 04. Salim?

SALIM ABDOOL KARIM: Thank you very much Tim. Let me just start by saying that this talk can't be half as good because I'm missing my wife doing the first part. So I'm just going to do the second part and I'm going to talk about current and planned HIV prevention trials and give you a bit about the landscape of what is going on in term of PrEP and microbicides.

I'll briefly touch on why the interest in PrEP, because I think you've already heard quite a lot about that, and give you a brief tour of some of the historical aspects of PrEP research before going into what's current and what's planned and what are some of the challenges.

I think that all of us are aware that PrEP is an experimental HIV prevention strategy that uses antiretrovirals; agents prior to exposure to prevent HIV acquisition and now

even that's changing and we might want to look at it only after exposure in what we call at least PEP.

PrEP for HIV prevention builds on the concept that medications can be used by healthy people to prevent other infections. We use Mefloquine prophylaxis for malaria, INH prophylaxis for tuberculosis and several others. We know certainly from mathematical modeling that it has a huge potential impact between 2.7 and 3.2 million new HIV infections could be averted in Southern Africa in 10 years by targeting PrEP which, if 90 percent effective, to those at highest behavioral risk and that comes from the ABAS paper.

Why the interest in PrEP? I think first and foremost is the strong biological plausibility. We know that antiretrovirals work by affecting viral replication and we know certainly from numerous animal challenge studies from as early as 1995 that we can use antiretrovirals to prevent SIV infection in monkey models.

We've also known from the successes of the post exposure prophylaxis for needle stick exposure, from observational data, and lastly and probably more strongly, from a completely different route of transmission. From the prevention of mother-to-child transmission we have proof of concept via that root that antiretrovirals and prevent HIV infections.

Much of the work you've seen and you've heard about all stems from this particular study that was done very early in 1995 where monkeys are challenged with tenofovir and it showed a high level of protection. This really stimulated a whole series of research investigating tenofovir both for therapy and for prevention.

That also led to a series of studies particularly from groups such as that of the CDC, which looked at tenofovir in the gel formulation in macaque and again these studies have shown pretty high levels of protection from using tenofovir gel in the vaginal compartment of monkeys as well as the rectal compartment in monkeys.

Well it hasn't all been smooth sailing. For those of you who were there in Bangkok in 2004, some six years ago, you'll recall the trauma that we witnessed there. That these initial efforts to test PrEP were thwarted by the efforts of activists who stopped the studies that were being done in Cambodia, and certainly led to several of the studies in Africa also being changed in terms of sites and some even stopping.

These effort that were undertaken to stop these trials, we now have to look back and say at what cost? How many infections could have been prevented if those trials were allowed to go ahead. We also have had other challenges. It is not only the activists. We've also had studies that have been

stopped due to low HIV incidence in study populations in several countries in Africa.

We've also had challenges in Southern Africa due to the high mobility of study populations and low HIV incidence in non mobile populations. So we've had challenges at a political level, we've had challenges at the level of trying to identify the appropriate study populations and we've had challenges in study conduct.

What's going on right now as I speak to you? These are the five trials that are currently underway. The first of those is the CDC Bangkok Tenofovir Trial which is studying tenofovir in the tablet form among intervenous drug abusers in Thailand.

The second is the IPREX Trial which is just in the process of wrapping up its data collection and that study looks at Truvada and enrolled 2,500 men who have sex with men in several countries throughout the world including the Andean region in South America, Brazil, the US, South Africa and Thailand.

We also have the partners PrEP study which is testing both Viread or tenofovir tablets and Truvada in discordant couples in Africa. The Voice Study, or known as the Microbicide Trial Network 003 study is a five arm trial comparing tenofovir gel to placebo gel, and simultaneously comparing tenofovir tablets, Truvada tablets to placebo

tablets, and this study intends to enroll 5,000 women in several countries in southern Africa. At this stage it is well underway having enrolled around 1,000 women already.

Finally but not least it's the study known as FEM-PrEP which is studying Truvada and it intends to enroll 3,900 women in several countries in Southern Africa. This is the current pipeline of studies that are currently underway and you can see from this table that we have studies in intervenous drug users, in men who have sex with men, in discordant couples, in men and in women.

If we look at how those feature within the broader pipeline of studies, you've already seen – I've showed you briefly – the FHI West African study which was complete quite a while ago which gave us some very early evidence that looked at the challenges in conducting these studies; some of the issues relating to adherence, incidence rate and we have within that study seroconverters that have also been investigated.

Subsequent to that, the study that was undertaken and presented at this conference by the Centers for Disease Control and Prevention looking at the safety of Truvada and tenofovir in the United States.

The Bangkok Study, we patiently await to see those results, and I want to go now further down to the IPREX Study, which as you have just heard is intends reporting some time towards the latter part of this early next year.

The CAPRISA 004 Study which has just been completed, and the remaining studies as you see them going forward; we should be expecting to see those results from 2012 onwards.

Just briefly to give you a quick synopsis of what came out of the CAPRISA 004 Study so we have a sense of what some of the benchmarks could be in terms of the levels of effectiveness we have already seen and what we want to aim for as better than what we found in the CAPRISA 004 trial.

So just to summarize those findings. There were no substantive safety concerns. There was no tenofovir resistance that was observed. It was found to be safe in the Hepatitis B virus infected women, although there were only 34 of them, 17 and a half of them on the actual tenofovir gel. We found no evidence of risk compensation, of behavioral disinhibition and we found a 51 percent reduction in HSV-2 infection.

With regards to HIV, the overall protection was 39 percent but after a single year of gel use a 50 percent reduction in HIV was observed and a 54 percent overall effectiveness was observed in those women who used the gel most consistently. If one looks at how that features in relation to other studies, in particular to relation to Inhibitor-012 [misspelled] the nevirapine study which showed 41 percent protection in prevention of mother-to-child transmission. Tenofovir showed a 39 percent level of protection, going up to 54 percent in a high adherence group. Just like the way in

which tenofovir – the way in which nevirapine in the Inhibitor-012 study served as a starting point for the future development and improvement in efficacy. We would hope that the upcoming studies will be doing something along those lines in looking at how can we do better because 39 percent is simply not good enough if we want to really impact on this epidemic at the level we'd like to see.

So what are the trials now being planned? And the pipeline at this point for effectiveness trials is very much one that will be driven by the way in which the news of the CAPRISA results are taken forward. These are very preliminary at this stage. The first is the IPM-009 study of the Dapivirine ring a much awaited trial. We'd really like to see the first studies of the ring formulation and we look forward to seeing that because unlike the gel formulations, the ring offers a new approach to addressing one of the biggest challenges in these trials and that is adherence. By being a ring that is present over the long term, of over a month or two, we may be able to see higher levels of efficacy from the ring formulation.

The MBP-302 study, which is a protocol that was developed a few months ago in anticipation of the CAPRISA 004 results, looking at how we might move forward with a four arm study, that's now being reactivated and plans are afoot to try and move that forward studying tenofovir gel.

Then lastly a South African tenofovir gel trial as a confirmatory study for the CAPRISA 004 trial is currently in the planning phases. We're certainly starting to see a little bit more movement in terms of enhancing the pipeline for topical PrEP.

Let me just end off with some of the key challenges in the future implementation of PrEP and how they might impact on study design. I think the first question we have to begin with – after all that's our credo, first do no harm. We have to ensure that these antiretroviral drugs that we're giving are safe enough to give to healthy people.

There we have different formulations. We have different doses. We have different approaches and in all of those we have to ensure that we measure safety accurately and to the best of our ability so that we are able to do those comparisons to assess safety going forwards.

Will those who get infected have HIV that is resistant to PrEP antiretrovirals and will this affect the subsequent care and choice of antiretroviral treatment? Will healthy people be willing to take medications every day or at the time of sex for long periods of time? Is this an affordable and practical HIV prevention strategy for scale-up if it is efficacious given the nature of what we're talking about in relation to having a regular HIV test and these being prescription medications.

Will there be behavioral disinhibition or risk compensation in the long term? Will we see some movement away from proven technologies towards lower efficacy technologies? All of these are some of the challenges and all of these are factors we need to build into the design of the PrEP and microbicide trials.

So I'd like to conclude with saying that we very patiently await the upcoming results from the oral and topical PrEP studies. We have five studies in that pipeline. We anticipate now with much eagerness those results, and in fact I would go so far as to say we feel much more positive that we will see efficacy from those trials.

The current trials of tenofovir gel or the tablets, or the Truvada tablets, very pleasingly we've seen different routes of transmission and different groups being studied so that we're seeing this diversity and this tapestry involving intervenous drug users, discordant couples, young women, MSM. So we're seeing all of these different target groups or vulnerable groups or groups that are really at higher risk. All of these are now part and parcel of this research agenda. And if effective implementation programs will require extensive community education to promote PrEP with integrated use with other prevention strategies including strategies like circumcision and condom use and behavior change.

The provision of PrEP will require integration into existing HIV prevention and healthcare services. This is not going to be the kind of thing we can put into a little bowl and stick it into a local shabine [misspelled?] as we would do with condoms. This is going to have to be something initially that is part and parcel of the medical service. We need long term follow up and surveillance in sentinel groups to monitor adverse events, adherence, resistance and to better appreciate what the impact is on subsequent achievement.

Let me end off very briefly with some future questions. We need to continually ask ourselves which drug, which combination of drugs, which formulations, which dosing strategies and all of these we need to better understand how they might impact on adherence, efficacy and safety. And do long acting formulations such as vaginal rings or slow-release bolas, dosing impact on adherence and drug resistance. Our combination is going to be better.

Lastly and probably the most complex political issue is should an antiretroviral or class of antiretrovirals be set aside for PrEP. Thank you. [Applause]

TIM MASTRO: Thank you very much Salim for that very, very thoughtful presentation. We'll take a couple of quick questions. Microphone number 3?

MALE SPEAKER: I feel I have to respond to your accusation that activists wrongly closed down the early PEP

trials. Activists did not close those trials down; the government closed the trials down after people with HIV from both inside and outside the countries found serious ethical concerns in those studies. There was inadequate safer sex counseling. There was no provision for medical care for those who seroconverted. There was inadequate consultation with the PEP communities before the trials were done when they were being planned. It would have been great to have the answers sooner. I think they would have come at a serious cost to the people in those trials and I hope that we've learned our lesson from those trials and are planning better ones now. [Applause]

TIM MASTRO: We'll go to microphone number 2.

GUILIA: Hi I'm Guilia from Paris. I'd like to know if you already have any information on what type of population, between the MSM and the sero-discordant etcetera. Who would be more willing to take PrEP on a regular basis? Do we have any information on that yet?

SALIM ABDOOL KARIM: I think we have some information but it's difficult because much of it is driven by feasibility studies in trial populations which might not be replicable or might not be broadly applicable to a wider population.

I think what's becoming clearer to us is that we need to understand the target population that we want to focus on. We need to understand their behavior. We need to understand what impacts on their adherence and we need to better

understand what is most likely to give us the highest impact. I think it's in that context that all these populations are being studied. I think that once these results start coming out and we are able to get better impressions of adherence particularly from levels of drug in the tissues and in the blood, we'll get a better sense of the levels of adherence we were able to achieve in these different populations.

TIM MASTRO: The last question, number 5 please.

MARCEL DELGADO: Thank you. I'm Marcel Delgado [misspelled?] from Spain. I'd like to comment seeing your opinion about a fact. Many of my patients who have sex with men usually use condom with rectal relations; however, they usually never use it with oral sex. So I think that it would be interesting to see levels of those antiretrovirals who have been commented today in the saliva and in the pharyngeal tissue because sometimes, and there are interesting reports from certain groups saying that the mouth recognizes the entrance of the virus and that people uninfected can develop antibodies and different kinds of reactions against the virus in people not infected. So I think that would be interesting to have a good level for exposure of prophylaxis in the saliva for so many patients who have that kind of sexual relations. Thank you.

SALIM ABDOOL KARIM: I think we want to try and move towards getting samples that would give us the quickest and clearest understanding of what these drug levels are and I

think the description you provide is certainly one important avenue moving forward. Thank you.

TIM MASTRO: Salim thank you very much. [Applause] So in this bridging session, our next presentation will talk about community preparedness. We have a change in the program and it's my pleasure to introduce our last speaker of the day Dr. Florence Temu, who is Deputy Country Director of AMREF, the African Medical and Research Foundation based in Mwanza, Tanzania and Dr. Florence will present on prevention research advocacy. Dr. Temu.

FLORENCE TEMU: Thank you. I'm going to do this presentation on behalf of my colleague, Charles Shagi who is unable to show up in Vienna. He is actually the winner of the 2010 Omololu Falobi Award.

The context behind this is the microbicide development program MBP 301 which was a [inaudible] double blind, placebo control phase III clinical trial which took place 2005 [inaudible] proceeded by two year [inaudible] study and was funded by DFID and UK Medical Research Counsel.

It took place in four countries, Tanzania being one of those, and it engaged a number of European and African Research Institutions and I'm with the African Medical Research Foundation and the National Institute for Medical Research, London School of Tropical Medicine and Hygiene where among those. In this presentation I'll change the gears a little bit

and we'll see the other side of science where communities are actually involved.

This took place in Mwanza, which is in the northern part of Tanzania and around 1,146 women who were recruited as an occupational cohort. These were women working the bar, hotels and guest houses and other related venues. The results of this study came out end of last year and is a valid point in the Web site as shown.

The [inaudible] system, this was established as one of the main [inaudible] for the trial as important as the clinical components and the other components of a clinical trial as most of us know, but that is [inaudible] laboratory companies. The aim of this system was to enable effective communication to their trial participants and other matrix including healthcare providers, local government leaders and other stakeholders. And it went through a number of preparations and during [inaudible] study and also during the trial. The same community contributed in naming the program and this program had a Swahili [inaudible] Swahili words.

What this means a woman is sad about your own life and it cannot [inaudible] female controlled decision making. So somehow it's kind of involved them deciding on the naming and also conduct of the program.

As I said it was in Wanza, this is not a part, and a participant were enrolled for 52 weeks as a full time trial.

But what's special are used as multiple but also there are other groups known as advisory groups and one of those was [inaudible] which the very participants of the study were presented and also some of them were leaders of the group and it improved a lot in terms of follow up and uptake.

This is one of the mapping that came out from this [inaudible] approaches where they actually map the areas where the clinical trial would take place. Where mobile clinics in the community set ups. This gave us advantage of the community, participants and the study objective of the trial and also the expected results and they are ready for any outcome of the results of the study. Also they've got opportunity to access other services beyond just clinical trial. I've tried to mention a few.

I'd like to share with you a video snapshot of one of the sessions recommended [inaudible] and you will happen to see Shaggy, who was the community liaison officer for the program.

[Video played]

Sorry I'll have to cut that short. That's one of the sessions where some of the responses were [inaudible] but the meaning behind – participants were involved in it and [inaudible] potential fears, concerns and stigma that they face from the community and this enabled them to discuss up to the level of solutions. So it was highly participatory and they

use highly friendly methods like [inaudible] Tanzania so they used that one to describe the program.

So in short, [inaudible] is a quite important component in clinical trial involving the prospective participant of the trials and then this one is [inaudible] influence on the retention and the participants remain proud of the [inaudible] as they came out. Thank you. I acknowledge all who participated here and also like to recognize the presence of – I wonder if they're still around, Richard Heis [misspelled?] and [inaudible], Dr. Kenya and [inaudible] who are all part of the study. Thank you so much. [Applause]

TIM MASTRO: Thank you very much Dr. Tamu for that important aspect of the work that we do involving the community. We'll have time for one last question of the day. Microphone number 4?

LAURIE MILLER: Hi my name is Laurie Miller I'm from AVAC and I'm actually here to let you guys know this is related to the presentation that we just heard and also the comment that the gentleman made about he hoped that the trials are conducted in a better way with participating with communities more positively. UNAIDS and AVAC today are releasing the Good Participatory Practice Guideline, the Second Edition. The first edition was released in response to the controversies around the PrEP trials and today we're releasing the second edition draft for public comment. These guidelines

specifically talk about how trial entities, trial funders, sponsors, and research teams should be working collaboratively with communities on how to engage in the trials together so hopefully this document will be able to prevent some of the controversies that we've heard and also help other researchers to be able to do the kind of excellent research that was done by MDP-301 thanks. [Applause]

TIM MASTRO: We will have one last question and then we'll close it up. Thank you. Microphone number 3.

FEMALE SPEAKER: It is just a proposal; just a comment. I would like to say that maybe we should as women working particularly in the communities, as you may [inaudible] could be better to say – to speak about several different [inaudible]. Consider that the world's discordance maybe civil discordance – if that's the right word – do you think that discordance is the best word to speak about among couples? I think that as everyone knows, there is only differences between men and women, not discordance. So [inaudible] different. I think it's really respectful for the couples.

TIM MASTRO: Thank you very much Dr. Tamu for your presentation. Well thank you all very much. It's been a long day. This was a terrific session. It brought together virology, immunology, clinical trials, pharmacology, the view for the future for the next clinical trials and the importance of community involvement, so it was really a great bridging

session to bring together the full spectrum of science that's going to be required to develop new prevention modalities. Thank you all very much. Dr. Katabira thank you for co-chairing and thanks to all of our speakers. Thanks very much.

[END RECORDING]