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**XVII International AIDS Conference
The Future of Microbicides: From Vaginal ART to PREP
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IAN MCGOWAN, M.D., PH.D.: I will be sharing my chairman's duties with Dr. Nomita Chandhiok from India. I think this is a very important meeting because it really underlies the evolution of the microbicide and the PREP fields. At one time, perhaps, they could have been seen as disparate areas of prevention research, but it is becoming increasingly apparent that both areas of research actually have a level of confluence; essentially we are giving either topical ART PREP in the sense of microbicides, or oral PREP, and a number of centers and groups are now beginning to explore in some detail the differential pharmacokinetics of delivering these drugs by either route and, indeed, the United States sponsored microbicide trial network in the VOICE study, which is Vaginal and Oral Interventions to Control the Epidemic. We will have the opportunity to directly compare oral PREP with vaginal topical PREP. And so I think this is going to be a very interesting area. And I think the speaker's who we have to educate us this afternoon will give you a very informative perspective of how the PREP landscape is evolving.

So without any further ado, I am going to introduce our first speaker, who is Dr. Guido Vanham, from Loven [misspelled?], Belgium. He studied medicine there but has spent much of his career in HIV research at the Institute of Tropical Medicine, and he will be telling us about future,

promising microbicidal products, what we can learn from the in vitro studies. Welcome, Doctor.

GUIDO VANHAM, M.D., PH.D.: Thank you. Well, first let me thank you for the invitation to be here at this very nice symposium.

So for those who are not familiar with the field, I will give a brief introduction, and then we will go into some real data that shows infected probably from in vitro models you can already predict to some extent the success or failure of future microbicides. And at the end I will give some issues, some questions, and some perspectives.

So we concentrate on vaginal HIV transmission, and a first important point is that we still do not know whether the virus is transmitted as a cell free, or as a cell associated virus. Then somehow the virus gets over the epithelial layer and it is controversial whether the Langerhans cells play a role in that event. But they are made that way somehow to usurp the epithelial layer and then can infect local and macrophages T cells, but most importantly, also the interstitial dendritic cells. And these cells tend to cluster with T cells and then become migratory clusters that will move into the regional lymph nodes, and there really start the systemic infection. So this event, the migration of dendritic cell and T cells, is very crucial early event.

Now the vital cycle is, of course, familiar to most of you, but to be really prevent it, of course, what you need is

prevention finally of cellular integration of proviral DNA. And you can act at several points; you can either disrupt the virus or create conditions in the vagina that inhibit the virus or kill the virus. And you can interfere with the ante process, with the fusion process, with the reverse transcriptase, or with the integrase.

And potential microbicides, candidate microbicides, have been developed in all these classes to keep the pH of the vagina low and hostile to the virus, some buffer gels and acid foam, have been developed and are in clinical trials still. There are the classical viral disrupters, such as nonoxynol-9, which appears to be obsolete because of inducing of inflammation and damage to the epithelium. And then a vast class of non-specific binding inhibitors has been, or is still, in trial. They are mainly poly and ions [misspelled?], and I cite here low sulfate, pro 2000, and fiber gel. So most of these products have already failed and some are still in phase III trial.

Now then we enter the more specific stages of the vital cycle, the inhibition of the binding of CD4 to GP120, and there are some small molecular compounds that have been developed, for instance, by Bristol Myers Squibb, and you can interfere with the binding to CCR5, and I give just some examples, maraviroc is the best known. Or you can inhibit in the case of dendritic cells, which bind to the dendritic cells via DC sine. Then you have the fusion inhibitors, you have reverse

transcriptase inhibitors, and those, of course, are the newer products that are going in their progress into clinical trials. You can either have the nucleosides, nucleotides, or the non-nucleosides reverse transcriptase inhibitors. And amongst the nucleotide, nucleoside, you have Truvada, which is a combination product. Amongst the non-nucleoside reverse transcriptase inhibitors, there is TMC120 and UC781, belonging to two different chemical classes of NNRTI.

And finally, the integrase inhibitors; for instance, I will show you some data on L870, 812, which is an analog of [inaudible]. And some people argue that protease inhibitors could also be used in combination to prevent systemic infection.

Now the clinical research process is a long process, it starts with the in vitro activity and toxicity evaluation and then it goes over animal models, and finally it comes into human clinical trials. Now in the case of microbicides, certainly this process could take ten years or more.

Now focusing on the in vitro models, there is a number, a large number, of in vitro models to test HIV microbicides. Going from very simple cell lines that express CD4 and CCR5, just those cells use U87 and a TM3L [misspelled?], which are very simple to use, rapid screening, or you can use infectious but non-replicative viruses, so that you do not need an L3 environment, but the main disadvantage is that the T cells are by no way representative of the in vivo primary target cells.

But it is very useful to screen large numbers of microbicide, or potential, microbicides in the first phase.

Now the second, more realistic, model is mitogen activated PBMC. This is a very standardized system, it is relatively quick and easy, but you look only at activated T cells, and those are not the cells that transmit the virus from the vagina to the systemic, to the lymph nodes. Therefore, we, and others, have developed a co-culture system of dendritic cells and T cells, which is more representative of the crucial, early stage of HIV transmission, but which is also more complex and time consuming, so you need some prescreening because otherwise you end up with an impossible task. As you will see later, screening in this system takes a month.

And in increasing complexity and increasing relevance comes when you combine dendritic cell, T cell co-cultures with an epithelial cell layer. And finally, of course, the most representative system is an explant model, but it has also some limitations, it has limited access because it has to come from therapeutic hysterectomies. The cells, or the tissue, are limited in viability and the different components have a different degree of viability, and it is never really intact, so it represents a kind of worst case scenario, but it is certainly very useful.

Now I will show you some data on simple in vitro systems and indicate that, in fact, there you can clearly see differences between microbicides or candidate microbicides that

at first are very similar. So we used the cell system as screening then the dendritic cell, T cell co-culture system, and then went on to the jewel chamber system, which includes an epithelial cell layer, and I will also show you results on the explant model, but then the results from another group because we have not done this ourselves.

Now in the true divided tests with the cell lines, it is very simple. You incubate the pseudo virus, or the single cycle pseudo virus, with the microbicides and you put on the cells and you let it go for 48 hours, and these pseudo viruses are manipulated in such a way that they express, or they integrate, into the DNA, and then express luciferase, so they have a luciferase gene, and so you can just look at the production of light as evidence of, presence or absence of, the virus.

And now I will show you some of the results of different classes of molecules, the inhibitors of the CD4 binding, which is the BMS compound, inhibitors of the CCR5, infusion inhibitor T20, PMPA, TMC120, UC781, and then the integrase inhibitor. And, as you can see in this system, all these products are highly potent with EC50 values below 100.

Now we go for the more complicated system, the co-culture model of dendritic cells and T4 cells. In this system, you can use either cell 3 replication competent virus, or cell associated virus. You incubate it with your compound, you add the dendritic cells, and afterwards you add the T cells, and

then you let it go for two weeks and you can have an additional expansion step to reveal latent infection.

Now this is the result if you compare the data from the pseudo virus test I just showed you with those and the dendritic cells, T cells. As you can see, the binding and fusion inhibitors, which were very potent in the cell line, are less potent when you use primary virus and primary cells, and primary cell three virus in this case. But when you use cell associated virus, these very potent compounds show hardly any activity.

In contrast, the various reverse transcriptase inhibitors, in this case PMBA, and the non-nucleosides, they maintain the same level of activity regardless of whether you work with a primary virus or a pseudo virus, whether you work with cell associated or cell three HIV. And the integrase inhibitor, in this case, shows a kind of intermediary profile, so it keeps activity, but it loses some activity as compared with the pseudo virus system.

Now we complicate and make the system probably a little bit more relevant by adding an epithelial cell layer on top of the co-culture of dendritic cells and T cells. And you know, in experiments that I will show you, we use the cell associated virus on top of the epithelial cell layer.

Now in this experiment, I will show you some results of anti-inhibitors, but those are the anti-inhibitors that have been, or still are, in clinical trials, the PRO 2000 and

cellulose sulfate, and some related products, and then here a bunch of non-nucleosides reverse transcriptase inhibitors. And the difference is quite striking. In this system with cell associated virus, all these four anti-inhibitors have no anti-viral activity whatsoever up to a concentration of 100 mcg/mL, whereas older RRNTI, they are highly potent.

Now let us move to the explant model. Now the explant model contains anti-cervical, vaginal tissue, and mostly taken from the cervix. And that is in vivo after a few days, or even after one day, the dendritic cells and T cells start to move out of this system. And you can recuperate them and measure antiretroviral activity in these cells separately from the entire tissue. So in the next results, there will be two kinds of results, the inhibition on the cervical epithelium as a whole, and an inhibition on the migratory cells below. And so those are data from Robin Shaddock's lab, and in this column you see the effect of UC781, the NNRTI, which shows, so this is the control, these are the different concentrations of UC781. It shows some inhibition in this example against the entire intact endothelial, but the inhibition is much more pronounced when you look into the migratory cells.

In contrast, various binding inhibitors we have used here and I will not name them all because they are specific for the various process for CD4 for this design, for CCR5, and as you can see they show quite a good inhibition in the entire epithelium, but when you look at the migratory cells, the

degree of inhibition is much less. So it is consistent with the previous data in a more simple cellular, or cell suspension, system.

Now what are the issues in further development of microbicides? First of all, unfortunately, a quarter of a century into the epidemic, very essential questions about transmission are still not resolved. Is it cell three or cell associated virus that is being transmitted? And as I showed you from the results of various microbicides, this is a very important question because some microbicides active against cell three virus are not more or less active against cell associated virus.

We do not know which are the most relevant target cells and most relevant receptors to look at, and the role of seminal plasma and cervical vaginal fluid factors has been studied, but with variable results. Sometimes you find inhibition, sometimes you find enhancement. And there is certainly a role of the normal vaginal flora, of pathological bacteria and what they call vaginal practices, like vaginal hygiene measure that women take. So we have to take into account all these unknown factors to some extent and build them into our systems to have a reliable model.

And then in the second series of issues is that, of course, we absolutely want to avoid unwanted side effects. And those are not only the enhancement of infection by epithelial damage of infection, but also we should not limit future

therapeutic options by induction of resistance, cross resistance, against therapeutically used drugs.

Now to conclude which is the in vitro test that is most suitable and most predictive? Well, of course it is impossible to say today because you need a golden standard and the only possible golden standard is a successful phase III clinical trial, and up to now we do not have that. But in the meantime, we have to address as many as possible of the issues that I just named, and so the models have to reflect aspects of sexual transmission faithfully, so I think dendritic cells and T cells, together of course, the epithelial cells, should always be taken into account. The explant model is, of course, very important and we should include seminal and vaginal fluid factors when we test microbicides in vitro.

In addition, it is very important that we assure that there is activity, not only against cell three virus, but also against cell associated virus, and now more and more that we study optimal drug combinations because it is evident that there is synergism between the different classes of drugs, but the degree of synergism can be very different. We should do a thorough evaluation of toxicity, and we should already, in vitro, study the consequences of possible resistance in induction.

Well, this is the acknowledgment slide, my collaborators and all the funding that was available for the

data that I showed you, and the many data that I did not show you. Thank you.

DR. NOMITA CHANDHIOK: A very good afternoon to all of you, and thank you, Guido, for a very informative talk. We need an increasing pipeline of candidate microbicide products, and we also know that it is really the preclinical evaluation of these products which would determine which of these candidates would go into clinical trials. And, as you mentioned, at this point we really do not know which test would be the most predictive.

We will be taking questions at the end of the session and we will be using the question card for that, so if anybody would like to ask a question, please raise your hand and a card will be given to you in which the question could be written, and that would be given to the speaker's, and they will respond to that at the end of the session.

It is my great pleasure now to introduce the next speaker, Dr. Salim Abdool Karim, better known as Slim to all of us, who is an infectious disease epidemiologist and Pro Vice Chancellor at the University of KwaZulu Natal. He is Director of Capreca [misspelled?], Professor of Clinical Epidemiology at Columbia University, and Adjunct Professor of Medicine at Cornell University. He is the Chair of the WHO Scientific and Technical Advisory Crew for Reproductive Health, and also an executive member of the NIH funded Microbicides Trial Network, and a member of the Gates Foundation Global HIV Prevention

Working Group. And all of us know all the sterling work which Slim and his crew have been doing for many years.

SALIM ABDOOL-KARIM, M.D., PH.D.: Thank you very much, Nomita. I would like to start by thanking the organizers for inviting me to do this presentation. What I hope to do in the next 15 minutes or so is just to share with you the status of the antiretroviral microbicides that are currently in the field. And what I want to do is I will just briefly touch on the issue of is there still a need for microbicide? And I assume most of you are here because you already answered that question, so probably very rhetorical in this audience. And then I will touch on some of the past and current microbicide trials before going into the antiretroviral microbicides that are in clinical and preclinical development, and before ending off, touching on some of the challenges within these trials.

So just for those of you not totally familiar with the field, a microbicide is a product that can be applied to the vagina or rectum mucosa with the intention of preventing the transmission of sexually transmitted infections, including HIV. And they come in many different formulations; in fact, in the past, they used to be foams and sponges and so on, but now more or less the field has come down to a few of those formulations and mostly the formulations that are currently being studied are gels, vaginal rings, and foams.

And I wanted to say that microbicides that contain antiretroviral drugs are essentially just different

formulations of pre-exposure prophylaxis; it is just a different way of providing PREP. So whether we provide antiretrovirals to newborn children to prevent mother to child transmission, or whether we provide antiretrovirals orally, in tablet form, to IDU's to prevent them from getting infected, all of these are basically the same principle. And what I will be focusing on in this talk is just one formulation of PREP, and that is the topical formulations.

So is there still a need for microbicides? While I am sharing with you these data, which are data on the HIV prevalence in South Africa, and I want you to first be focused on the blue line. So if you look at the blue line, that is the prevalence of HIV infection in men. And if you look at the age group, 10 to 14 years, you can see that HIV prevalence in young boys is very low. If you go further on to the HIV prevalence in the 15 to 19 age group, similarly it is low. And it is only when you start getting into the mid-20's to the late 20's that you see the prevalence of HIV infection in men rising quite rapidly.

However, the situation is very different as far as women are concerned, or females. And if we look here at young girls, 10 to 14, and this comes from a community based survey in South Africa, you can see the prevalence is very low. However, when you start looking at teenage girls, you are seeing the rapid rise of infection. And, in fact, teenage girls in South Africa have prevalence rates of HIV infection

that are close to the peak so that by the time you get to girls or women in their early 20's, you are looking at very high rates of HIV infection. And, indeed, it is this difference in the age group between men and women that results in such a rapid epidemic in southern Africa.

And it is shown here when you look at the way in which the HIV epidemic has grown in South Africa from being almost non-existent in the late 1980's, to a rapid rise in the early 1990's to a current plateauing that we are seeing in South Africa. But it is the young girls, it is the teenage girls, the women in their early 20's that are driving this very high prevalence in pregnant women that we see in South Africa. And there is no question that this group, for us to have any success in controlling the HIV epidemic in the context of southern Africa, to avoid this kind of rapid rise and prevalence, we have to find some way to empower these young girls and these young women to prevent HIV infection.

And this was probably best captured in a paper that was published in the early 1990's by Zena Stein in the *American Journal of Public Health*, which raised this issue about HIV prevention, the need for methods that women can use and control. And, in fact, since then the concept of microbicides has gelled, pardon the pun, and what we have seen since then is a range of different microbicide trials.

So let me just explain the color coding that I have used. When I have used red that is to indicate that the trial

showed that there was an increase in HIV infection in the active arm and the product was associated with an increased risk, or increased susceptibility, of HIV infection among those who used it. When I have used green that means the study was done and showed that the product was safe. And when I show yellow, it means that the study was stopped for futility and they stopped the study early because they could not show a definitive result one way or the other.

So if we look at the early studies of the first class of products, or surfactants [misspelled?], what we see is that the studies done in Kenya and the multi country study that was undertaken by WHO UNAIDS of the call 1492, a formulation of [inaudible], both showed increased risk of HIV infection among those who used the product.

On the other hand, the M9 full study that was done in Cameroon by FHI did not show any difference between the two study arms. The FHI Savvy study was stopped for futility.

So we have moved now beyond this class of products, and nobody is continuing to study these products in humans any longer. And currently what we are seeing are the results of the next class of products, the polymers that are emerging. So what we have had so far is we have had the results of the Conrad selenosulfate study, which showed, one of the trials showed, the Conrad trial showed, that there was an increased risk of HIV infection among those who used selenosulfate, and

those results are published this week in the *New England Journal of Medicine*.

There was a parallel study that was being undertaken by FHI which was stopped for futility. It did not show harm. The population console also recently released their results of Carraguard, which showed that the product was safe, but it did not show any efficacy. And then, more recently, you might have heard that the British MRC, in conducting the study in the microbicides development program, dropped one of their arms for futility, and that was the 2-percent to 2,000. So currently, in trials in this class, we have PRO 2000, half a percent, and buffer gel that are being studied.

And then if we look at the class of products that I will be talking about and focusing on in this presentation, it is the antiretrovirals. And here we have the tenofovir gel trial, we have the MTN, which is a crossover trial, which brings together the gel formulations and the oral formulations and combinations, as well as single agents. And then we have the IPM, also a trial which looks at different formulations, both rings and gels.

So where are we with each of these products? And I am just going to very briefly touch on these because Zeta Rosenberg, in her presentation earlier this week, covered much of this so I will just go through this quite quickly.

So tenofovir gel is a nucleotide reverse transcriptase inhibitor. It was developed by Gilead Sciences, and is

currently licensed by both Conrad and IPM for further development as the gel formulation. It is effective and it is licensed as a therapeutic drug, and in fact is very widely used in therapy. It has got a very good safety profile and has a very long half life, and it shows protection in multiple immunochallenged studies. And it has got a high barrier for drug resistance, so that is what makes it a particularly good drug to study for prophylaxis, whether in gel formulation or in oral formulation.

So why use the gel formulation? Why do we not just stay with studying tablets? I am going to share with you data that comes from Conrad, which looks at the levels of the drug in the different formulations. So if we look at the table formulations, taking tenofovir as a tablet, as 10:2 is the level that is achieved in blood plasma, and you can see that over time that level is pretty static, just illustrating the long half life of this drug in plasma.

If you take the tablet, what happens? What do we see in the genital tract? And so cervical vaginal washings from patients who have taken the oral form of tenofovir, taken tenofovir tablets, show that the levels in the vagina are fairly similar to that that you see in the plasma, they are just slightly higher at all the time points.

However, when we look at the gel formulation and we look at vaginal tissues, and these are levels of the drug within the tissues that are studied by taking biopsies at

differing time points, what we see is that the levels are substantially higher. We have now gone from levels that were 10:2 to 10:5. And so we are seeing much higher levels of tenofovir within the vaginal tissue and, again, they have a long half life. What we see in the cervical vaginal washings again is very high levels, but that is what you would expect, given that you have inserted the gel.

The key is so what happens with the gel formulation? How much of that gets into the blood? How much of it gets systemically absorbed? And what we have seen is that somewhere between a quarter to a third of the women have detectable levels of the drug in plasma when inserted as a gel. So these are the gel formulations inserted in the vagina, and looking at levels of the drug in blood. And what we see is very low levels of the drug and then, again, this long tail that you see with the tenofovir. So what we are seeing is that very little of tenofovir gets absorbed systemically when administered as a gel formulation. So what we are seeing is two very different distributions of the drug, whether you take it orally or whether you take it in the gel formulation in the vagina.

So what has happened with tenofovir? Where are we with its clinical development pathway? I thought I would just include this just to make the point that tenofovir was first shown to protect in a macaw challenge study in 1995, and it took us all of 11 years before we got it entering into a human trial for efficacy. Well, by 2006, we had already more than 20

macaw challenge studies with tenofovir and, in fact, every week I hear about a new trial that is being done with tenofovir or with tenofovir combined with FTC.

Well, since the beginning of 2006, in fact, in late 2005, we had the HPTN that started studying tenofovir during a phase I safety study and PK that was led by Ken Mayer. We have subsequently had a phase II study of differing dosing strategies led by Shannon Hilliard that was done at India and the United States. We will have the data that have just emerged from the Mayo Tolerance Study that was undertaken by Conrad, and then the Conrad gel PK study, which is the data I just shared with you, was completed earlier this year and presented at the microbicides 2008 conference in New Delhi. And in 2007, we had the first of the tenofovir gel efficacy trials that were initiated, and that trial is expected to be completed somewhere in 2010.

But it is not enough for us just to proceed with that, we have to look at a whole range of other issues relating to safety. And these are very specific issues in order to expand the use of tenofovir. And so the MTM initiated a phase I safety study in pregnancy, and also a phase I study looking at the pharmacokinetics of both the gel and the tablet in the MTM 001 study led by Craig. And most recently, and I will be sharing a little bit more detail with you, the MTM VOICE study.

So where are we with the current phase III and the current phase IIb tenofovir gel trial? Well, we have got the

Capreco [misspelled?] 004 trial, which is a randomized, double blinded test of concept trial, and we have 845 participants enrolled. The retention at this point is 91-percent, and there has been good adherence to the gel. I think we have had some challenges within the studies in terms of co-enrollment, but we have reached about a quarter of the endpoints and this study is still scheduled to be completed on time.

Let me just spend a minute or so on the MTM VOICE trial because it is a critical trial in that it compares two different formulations. So the VOICE trial is about vaginal and oral interventions, so it is looking at tenofovir and Truvada in oral formulation, and tenofovir in the gel formulation. And the 4,200 participants that we anticipate enrolling in the study will be randomized into one of five arms, three of them taking oral formulation and two taking gel formulation. So people will be randomized to taking Truvada, which is a combination of tenofovir and FTC and you will hear more about that from Tim when he speaks next; Viread, which is the tenofovir oral formulation; placebo tablet; or they will be taking tenofovir or placebo gel.

The next thing I want to touch on is daviripine, TMC120, which is a non-nucleoside transcriptase inhibitor, which was developed by Evotec and licensed to IPM. It was initially developed as a therapeutic enzyme, it is very potent as an antiretroviral, it has low toxicity, it is easily manufactured, and so it is a very appropriate drug to develop.

It is also being developed by IPM as a combination with maraviroc. Its clinical pathway that we have seen to date is we have seen the gel PK study, the safety studies that were undertaken by IPM, the Mayo Tolerance studies, and then we have seen the ring development studies that have been initiated, and the ring safety and PK studies, leading to what is going to be the efficacy study, the phase III trial that is being developed by IPM as it stands.

And just to touch on some aspects of their trial, it is an adaptive trial design with multiple arms comparing both the ring formulation and the gel formulation. There is a strong focus on safety within the trial, it early looks for futility, it is a study being powered for licensure, and they are looking at many innovative strategies to improve adherence within the trial using smart applicators and, again, using ring formulations which only need to be inserted once a month. And they anticipate that this study will start somewhere in 2010.

The third antiretroviral drug, which is in an advanced stage of clinical development, is UC781, which is licensed to Conrad. It is a non-nucleoside reverse transcriptase inhibitor with potent anti HIV activity. Again, low toxicity, and it is being developed by Conrad, also as a combination with tenofovir. It has been through phase I safety studies, Mayo tolerance studies, and vaginal PK studies, and rectal studies, and is now proceeding along that pathway.

I am going to very quickly touch on Mervon 150 [misspelled?], which is licensed to the Population Council; they are developing it as a combination of Mervon 150 with Carraguard, and they anticipate starting their phase I safety trials later this year or early next year. So this is just completing its preclinical studies and is entering into human trials.

Maraviroc, which you have heard quite a bit about, a CCR5 inhibitor, is licensed to IPM, and is being developed as a gel and as a ring, and is being developed as a combination product with dapivadine [misspelled?] and maraviroc, and is currently in preclinical assessment.

There are many other products and compounds in the pipeline by ITP, I am not going to touch on them and I want to just end by touching on very quickly some of the challenges in antiretroviral microbicide trials. You have heard already about the challenges of how do we select the best products and how do we select for us to move forward given that we do not have a validated animal model. And so we are using a whole range of different assays, we are using a range of different criteria in order to pull together a complete picture to identify those products which are most suitable to move forward.

And we are learning that they are readily developing, there is a whole range of newly developing assays that are coming forward. One of those which is interesting is the ex

vivo challenge model where the gel is put into a human being and then a bar-c [misspelled?] is taken and then the bar-c is challenged with HIV, so getting very much trying to simulate what would happen in the real world situation.

We have no surrogate markers for safety or protection at this point, and so we have to look at endpoint as the only gold standard we have for safety and efficacy. We have many challenges in adherence, if you have done a microbicide trial you know this problem, and we know, for example, trying to achieve over a long period is a major challenge. I mean, just look at what is achieved in the daily acyclovir study where they had only 34.5-percent of the participants had detectable acyclovir 12 months.

The potential for drug resistance we have already heard about and there are many design challenges which have been adequately covered in the ION report, which I will not touch on.

So just by way of concluding there are there adverse transcriptase inhibitor antiretrovirals, tenofovir, dapivadine, and UC781 that are well along in the clinical development pathway. The first antiretroviral microbicide effectiveness trial is under way and we have two further larger effectiveness trials, the VOICE trial and the IPM trial, which we anticipate will start within the next two years.

We have new formulations in the way of rings, and we have two other antiretrovirals that are also well along in

terms of their preclinical development and will be entering pretty soon into human trials, and lastly we have got many combinations that are being developed. You heard about UC-781 and tenofovir, there is a whole range of combinations that are being developed.

I would like to end by just thanking the many people who contributed data to this presentation. Thank you very much.

DR. NOMITA CHANDHIOK: Thanks, Slim. I think we all agree that there is still a need for microbicides and we have all moved to an era of antiretroviral containing topical products, and the many challenges in this field have been highlighted by him. It is not an easy field, it is a learning process, and I think we have learned from every clinical trial that has been completed.

We move to our next speaker and I would like to introduce Dr. Timothy Mastro, whose training is in internal medicine, epidemiology, and public health. He is Senior Director of Research at Family Health International, and has worked on HIV prevention programs, science clinical trials, and research at the US CDC for 20 years. His work has included clinical trials of antiretroviral agents for preexposure prophylaxis and the prevention of mother to child transmission, microbicides and HIV vaccines. Dr. Mastro's training, as I said, is in this field and we look forward to his presentation.

TIMOTHY MASTRO, M.D.: Great, thank you very much for that introduction. It is really a privilege to be here today to present all these trials on behalf of the investigators, the institutions doing them.

There is a very strong rationale for the use of antiretrovirals for the prevention of HIV infection. ARV's are very effective in preventing mother to child transmission, they are effective for post exposure prophylaxis for HIV, and they are very compelling animal models in mechanics showing the prevention of an HIV-like transmission using antiretrovirals. The available ARV's that we have appear to be safe, we have tens of thousands of person's year's experience with these drugs, and the available ARV's we will talk about today can be used once a day. All of the studies I will be talking about today use really two chemicals and free formulations; tenofovir, or TDF with the brand name Viread; Emtricitabine, or FTC. When you combine these two drugs together the brand name is Truvada and all these drugs are made by Gilead Sciences.

These are data from [inaudible] laboratory at the Centers for Disease Control which shows some of the background work that really informed the human clinical trials. This is a multiple challenge, a rectal challenge model from the mechanics at CDC showing here that the control animals shown in red very quickly get infected after 14 weeks of challenge with the shiv virus. Now the higher the line, the more protection, so when you give oral tenofovir you can protect some of the animals for

a few weeks, but eventually three out of four get infected. If you use FTC subcutaneously you do a little bit better, if you combine two drugs together orally you do even better, and if you give high dose FTC and tenofovir subcutaneously you protect all six of these monkeys with 14 challenges, so perfect protection in this small sample of monkeys.

There has been one completed human clinical trial to date. This is the FHI West Africa phase II PREP trial with support from the Bill and Melinda Gates Foundation. This is a randomized control trial of tenofovir 300 mg a day and placebo. Altogether, 936 women were enrolled in Ghana, Cameroon, and Nigeria. Only the Ghana site went to completion; the study was stopped early in Cameroon and Nigeria, conducted from 2004 through '06. The positive outcomes of this study were that there was no evidence of increased clinical or laboratory adverse effects. There was no evidence of risk compensation, that is women on study did not have an increase in risky behavior, however there was inadequate power to assess the efficacy of this due to the smaller sample than intended. Their rate of infections in the trial, two in the tenofovir arm, six in the placebo arm, but due to the small numbers, this was not significant.

So for the rest of the talk I am going to summarize seven trials. There are five ongoing trials, the three CDC trials, iPrEx and Partners [misspelled?], and there are two

trials that are in planning that should be starting relatively soon, FHI's fem PREP trial, and the VOICE trial from the MTM.

Moving forward, the first CDC trial is a smaller extended safety trial of tenofovir to assess clinical laboratory and behavioral safety, adherence, and acceptability. Again, it is a randomized, double blind, placebo controlled trial with tenofovir daily versus placebo daily. Individuals are on study drug for 24 months with DSMB safety reviews every six months.

Enrolled into this trial are 400 HIV negative men who have sex with men in Atlanta, San Francisco, and Boston. To assess whether or not one's behavior changes prior to the time of initiating study drug, for 200 of the participants, they are enrolled for follow up, but then they are not randomized for drug for nine more months, so there is a run-in period of nine months of observation of behavior to compare with the behavior after they are on drug. There is very close monitoring of seroconverters to see if there is the emergence of drug resistance. Adverse events are monitored and managed. This study started in 2005, it took longer to enroll than intended, it was completed just one year ago and with two years of follow up, this study will be completed next year. It is, again, fully enrolled.

The second CDC study is the Bangkok tenofovir study. Shown on this map are the BMA drug treatment clinics where injection drug users are enrolled, and I must say having lived

in Bangkok for a long time, Mexico City is the only place that makes Bangkok feel like a small town.

These 17 drug treatment clinics where these IV users were enrolled were also the same clinics where the VaxGen HIV vaccine trial was conducted from 1999 to 2003, and this is a good example of research infrastructure being developed in the past being used for current studies.

The BTS study is a phase II/III randomized trial. Now going forward, many of these studies have the same elements, they are all randomized, placebo controlled trials so I will not say that about each one, but I will point out the unique characteristics that make each study special and different.

Now this study population is HIV negative drug users enrolled in treatment clinics, age 20 to 60. The original sample size for this study was 1,600; however, due to an observed lower of HIV incidents that had been observed earlier, the sample size is now up to 2,400. All participants will be followed for one year after the completion of enrollment. Participants may choose to receive their drug with daily observed therapy. Many of them are on methadone and maintenance, or methadone treatment, and they come to the clinic daily. Or they can come in monthly and get a 30 day supply of drug. As of last month, the study was 90-percent enrolled and there was an event driven review, so it is unclear exactly when we will reach the endpoints of this trial.

These objectives are similar for all the trials we will be talking about. Really the purpose here is to determine whether or not tenofovir prevents infection, is it safe, and then we will evaluate risk behaviors, adherence, alteration of disease progression, and seroconverters, and an important issue, whether or not resistance develops in those that become infected on trial.

The third CDC trial is the Botswana TDF-2 trial being conducted in Gaborone and Francistown; again, a similar trial design to the tie trial. This trial also started in 2005, however, initially it was a single agent, it was a tenofovir trial, but after the enrollment of a small number of participants, a decision was made to convert this from a tenofovir trial to a Truvada trial thinking that two drugs are better than one based on the data from the McCac [misspelled?] studies. So this was converted to the TDF-2 trial, it started in February 2007. The original population was going to be 1,200 heterosexual men and women, aged 18 to 29, but again another challenge, the enrollment has been slower than expected to date, HIV incidence is slower than estimated, so the investigator's are now planning to expand the age range to 39 and expand the sample size up to as high as 2,000 through the addition of an additional site or two.

The next study is the iPrEx study sponsored by NIH with support from the Gates Foundation and drugs donated by Gilead.

This study is unique and as an efficacy trial, it is enrolling

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3,000 high risk MSM, randomized 1:1 daily oral prep or placebo, and the prep drug here is the two drug combination, Truvada. The participants will be followed for these things here, which are similar for many of the studies; however, I will point out a couple of special things. Both of these drugs are nucleoside reverse transcriptase inhibitors, which is also active against hepatitis B virus. Some individuals that are hepatitis B surface antigen positive are at risk for having hepatitis B flares. If you start drugs for hepatitis B treatment, then you stop the drugs, it is possible that people will get a resurgence of their hepatitis activity, and that is an important consideration that has to be monitored in these prep trials.

Of course, people will be monitored for their adherence and, if infected, viral load and immune characteristics and CD4 count and, importantly, drug resistance will be assessed.

This map shows the iPrEx studies, the PI is in the room with us, Bob Grant, currently enrolling in two sites in North America and four sites in Peru and Ecuador, and study sites in Brazil, Cape Town, and Chan Meyer in the preparatory phase.

Bob Grant was kindly updating the slides this week and after three years of preparation, iPrEx is enrolling as planned and there are now more than 1,000 MSM enrolled in the iPrEx study.

Now it should be obvious why MSM are important for the PREP agenda. MSM bear a major burden of the epidemic,

certainly in the America's and some parts of Asia, and there is a growing understanding of the burden that MSM plays, that gay men play, or men who have sex with men play in the African epidemic, as well.

It is important to assess efficacy for rectal exposure. It is quite possible that due to the higher transmission efficiency of rectal transmission versus vaginal transmission that the dynamics could be different and that a possible different tissue penetration of the virus and drug will be an important thing to monitor. And iPrEx is the only efficacy trial of PREP among MSM.

The next trial is the Partner PREP study. This is a study being conducted by Kellerman and colleagues from the University of Washington. The unique characteristic here is this is a study being conducted in discordant couples, and it builds on the infrastructure built by Connie and her team for herpes simplex virus impression studies. So this is a parallel comparison, a three arm study of tenofovir alone, Truvada combination, and placebo.

Now when one considers the widespread implementation of PREP, HIV discordant couples would be a high priority group to focus on. Studies from Africa suggest that more than half of new HIV transmission events may occur in stable couples. This is a three arm trial comparing tenofovir, Truvada, and placebo. And the unique characteristics of enrolling couples in a study is not only will the HIV negative individual, who will be

randomized in the study, be enrolled, but their HIV positive partners will also be enrolled in the study and followed longitudinally. So this will allow us to assess the efficacy of PREP in those individuals that have high viral load and low viral load, thinking that the partners are likely the source of infection, but of course they might not be the source of infection in all cases.

Drug levels of the study drug will be assessed in both the participant, as well as the partner to assess whether or not the study participant who is on drug shares the antiretroviral, the study agent, with their partner. There will be assessment of baseline resistance in the positive individual and longitudinal resistance to see if there is a change in that resistance pattern, and to monitor whether or not there is transmitted or required resistance in the seroconverters, so couples really provide a great opportunity to learn a lot about HIV transmission and resistance.

This is a study design, eight sites in Kenya and Uganda. We will conduct this study, the 339 discordant couples, the HIV positive partners will have a CD4 count greater than 250 so they will be not on antiretroviral treatment for their own disease, they will be randomized to the three arms we talked about. They will receive best preventive services, and I would say also that is a common element in all the prevention trials we are talking about, all of these trials will provide prevention services to the best of local

standards, followed for two to three years, the endpoint of infection and safety.

Now with a sample size of 3,900 in the three arms, an estimation of incidence just over 3-percent in the placebo arm, there is an estimation of 191 endpoints. This would give the study 80-percent power to detect 60-percent efficacy in each arm against placebo and to rule out an efficacy of less than 30-percent.

The important thing here in the timeline for this study is that it has started. There are two sites that have activated in Uganda in just May and June, so this study has been enrolling now. The six additional sites expect to be activated by the end of 2008, and with the target enrollment taking two years, and we expect to have results for this study in 2011.

There are two planned trials we will talk about, FHI's FEM-PrEP study; again, a randomized trial. The sponsor is FHI with funding from USAID and the Gates Foundation. Six sites currently planned in South Africa, Malawi, Kenya, and Tanzania will enroll 3,900 women at high risk for HIV infection. A strong component of this trial is an integrated, interdisciplinary approach. Lut Van Damme is the PI of the clinical aspects of the study and Amy Corneli is PI of the social, behavioral, and community aspects. And there is a great deal of effort going into understanding the sociobehavioral aspects of the individuals that will be

enrolled and community engagement, both in preparatory and the ongoing conduct of the study. And the study will also have a pilot intervention development, so that if the study product is effective it will be possible to roll it out on a pilot phase programmatically. Again, the study drug here is Truvada versus placebo. People will be on drug for 52 weeks, and people whose seroconvert will be followed for an additional 52 weeks.

Similar objectives for FEM-PrEP are the other trials in safety and efficacy. I will draw our attention to a couple of items here. Tenofovir has been associated with a decrease in bone density, so people are assessed for that potential adverse effect, and with 3,900 women in Africa being enrolled, there will undoubtedly be a number of pregnancies. Now to enroll in the study, women need to agree to be on an effective mode of contraception, but as we know from previous PREP trials, previous microbicide trials, and many prevention trials, pregnancy is a common outcome and it is an important consideration here. And this study will no doubt give us a great deal of information on the incidence of pregnancy in these situations. We will learn about adherence and behavior on the time of seroconversion as well, and monitor for risk disinhibition.

The important things in the FEM-PrEP timeline is the study was approved by FHI's Prevention of Human Subjects Committee in March of this year, the IND has been submitted, and training has started in Kenya last month. It is hoped that

the first screening will take place in the fourth quarter of 2008, and if all goes well, the primary and secondary objectives will be reached in 2011 and 2012.

The VOICE Study wins the award for the prettiest picture of all the studies, and this is the Vaginal and Oral Intervention to Control the Epidemic study that Slim talked a bit about, conducted by the Microbicides Trial Network with support from NIH. The objectives of this study are to determine whether or not tenofovir gel 1-percent, oral tenofovir, or oral Truvada prevents HIV infection and whether or not they are safe in women at risk for STD, sexually transmitted HIV.

Slim has showed you this already, but just to go over it that women will be on the product an average of 21 months with 840 in each of the five arms. Oral Truvada, oral tenofovir, or oral placebo, and tenofovir gel, and placebo gel, so a very powerful design that will allow us to look at both a topical use of tenofovir and an oral use of the agents.

The hypothesis behind VOICE is that there is at least a 25-percent difference in effectiveness comparing each of the agents against placebo, tenofovir gel, tenofovir oral, and Truvada, and that there is no difference in safety. VOICE sites are wrapping up in South Africa, Malawi, Uganda, Zambia, and Zimbabwe.

This is the timeline as of a couple of months ago. The study is going through approvals, IRB approvals are being

obtained. Accrual will start either late this year or early 2009, and we expect completion in 2011 or 2012.

So just in summary, these are the three CDC studies; a US study will definitely be done in 2009. Thailand and Botswana are having problems with both accrual of clients and low incidence, so there is a question mark next to the 2009 to 2010 completion date of these studies. The iPrEx is up and running, we hope to have results in 2010. Partners PrEP is just getting started with discordant couples, a large study, hopefully we will have results in 2011, and the two planned studies will enroll more than 8,000 women in Africa into this clinical trial, and we hope if these studies go well we will have data on these products by 2012 as well.

So when you put all these seven trials together, nearly 20,000 individuals will be randomized in these clinical trials which will give us a very robust evaluation of tenofovir, Truvada, and oral tenofovir and gel tenofovir, for 11,000 African women, 3,000 African men, 3,000 MSM from around the world, and 2,400 IDU's in Bangkok.

So the next steps, it will be important to understand if intermittent PrEP is feasible and effective, taking the drug intermittently around the time when one might be exposed is probably more feasible for many people in the world. There are a lot of the new ARB's and new combinations of ARV's, potentially new formulations in addition to oral and gels, potentially an injectable agent with a long half life, vaginal

ring formulation. It will be important to understand how PrEP works for adolescents, and there is a myriad of problematic implementation issues that are beginning to be worked on now, certainly at CDC and other normative bodies.

So I would like to end by acknowledging all the collaborators who were kind enough to share their slides, their wisdom, and special thanks to Mitch Warren and AVAC who has done a great job in actually promoting the PrEP agenda, disseminating information. Thank you very much for your attention.

IAN MCGOWAN, M.D., PH.D.: Thanks very much, Tim. If I can just remind everyone that if you have burning questions, you need to put your hand up and fill out your question card, and we will do our best to try and address those questions given the time we have left.

So I think at this point in the symposium, the audience is pretty clear that whether we give oral or topical PREP, we have a number of problems; identifying populations and rolling them, keeping them on study, monitoring for safety, and tying that logistical list we should now probably add resistance. Will resistance occur in the setting of these studies, how are we going to measure it, how big a problem might it be or not?

So to help us with this rather complex problem, I am delighted to introduce one of my colleagues from the University of Pittsburgh, John Mellors, who is a Professor of Medicine and Chief of the Division of Infectious Diseases. He is also the

Executive Director of our HIV/AIDS program, the Virology Core for the MTM and the Microbicide Trial Network, and as we all know, his areas of expertise are in HIV infection, antiretroviral therapy, particularly the issues surrounding drug resistance. So, John, tell us, should we be worried or not?

JOHN MELLORS, M.D.: Well, all I can say, and I hope I can convince everybody in 15 minutes or so, is that showing efficacy in a clinical trial is really just the first step in trying to achieve a public health benefit. I would like to thank Ian and the organizers for giving me an incredibly challenging topic to cover in a short time.

So first, let me dispense with the issue of oral versus topical PREP, and in this context in this talk, I am talking about antiretroviral PREP, the use of specific HIV inhibitors, for instance the nucleoside analog, RT inhibitors, or the NNRTI's that you have heard about.

So in terms of topical, there are concerns that there is less systemic exposure, but greater mucosal exposure. There is a sense, and if I took a show of hands, that there would be less efficacy for a topical preparation, but I encourage you after this session to go see Waleed Hanani [misspelled?] from CDC present a study done by Irving Preck on tenofovir FTC gel. And I will not preempt Waleed's punch line, but the efficacy rivals that of anything given orally. Less toxicity and possibly less resistance, there is also a bias that there will

be less adherence, but who knows, we could get an added benefit, it could improve sex if it is the right formulation, and that might actually improve adherence.

In terms of oral, there are certainly greater systemic, but it looks like less mucosal exposure. That might or might not translate into greater efficacy; it could result in greater toxicity and possibly more resistance. There is a bias, again, that there will be better adherence for one pill once a day, but not if it is less tolerable and we are getting systemic exposure to the drug.

So I think it is far to say that there is equipoise as to whether an oral or topical ARV PREP will be better, and that is why we do clinical trials. And those of you in the audience who think you know the answer to this, please let us do the studies.

So some key population issues; first, the effectiveness of PREP, the percent protection, and will it be adhered to? Will there be sexual disinhibition, either on or off PREP in either susceptible or those who are already infected. Will there be erosion of other preventive strategies, such as migration away from condoms, or less motivation for circumcision? These are bona fide concerns.

What about the toxicity of PREP? We really are talking about a very high bar here. We are talking about giving indefinite therapy to healthy users, both male and female who may be pregnant or breastfeeding, and there is a bit of a

dilemma here because the regulatory agencies are very reluctant to let us study these agents in pregnant or breastfeeding women.

A huge concern is will we be able to provide access to PREP, reaching at-risk groups, and the cost involved. And the cost is combated by the need to monitor HIV sero status before, during, and after. HBV coinfection, you heard what Tim said about starting and stopping tenofovir, tenofovir FTC, and also monitoring for pregnancy. And, finally, drug resistance and its impact on prevalence and antiretroviral therapy efficacy, this is the question I am asked about most often.

So how do we gain insight? Well, we ask for an expert opinion. The problem with that is everybody knows an expert and everybody has an opinion. And that usually does not lead to much insight. We generate data through clinical trials, but the major issue is the primary endpoint is efficacy, not effectiveness in the population. Population studies can be undertaken, these are difficult and costly. Both take time. You heard from Tim nicely the timeline for the various trials before we have data, so the current state is what I would describe as a data free zone in human beings.

So what to do? Well, mathematical and computational modeling can represent the dynamics of transmission and the impact of interventions. It can identify key drivers of outcomes, rather than the actual outcome, and this is important for gearing up for public health intervention measures. You

can do modeling much quicker than you can do any clinical trial, and you can do it while you are waiting for data, and once you get data you can refine the model.

The cautions are simple. Although complex in structure, models tend to oversimplify the human race. And garbage in, garbage out; if you have bad assumptions in your model, you are going to get a bad and unreliable output. So what I would like to tell you about is two models; one on the population impact of PREP and one on the impact of PREP on resistance and vice versa.

This was published in PLoS One [misspelled?] last year by Omi Abas [misspelled?] and Roy Anderson, Roy looking very young and smart, and the same with Omi, I did not show my picture for obvious reasons. And the methods were to use a dynamic mathematical model with coupled non-linear differential equations at about 1,000, with numerical analysis through simulation, a model population stratified by age, gender, sexual activity, stage of HIV infection whether they were on or off PREP, and HIV drug susceptibility in a very crude way initially. The model parameters were chosen to simulate a mature HIV epidemic in sub-Saharan Africa. Sensitivity analyses standard were used to determine the relative influence of PREP related input parameters on prevention, and scenario analyses used to assess the differential potential impact of PREP. And this just shows that the model simulates nicely an epidemic in its evolution compared with data on HIV

prevalence in Zambia and in natal clinics, and I could not help to recognize it looked very similar to the data that Slim showed about the South Africa epidemic.

So with a sensitivity analysis we generate something called a partial rank correlation coefficient. It ranges between minus one and plus one, one being the most efficacious for preventing new infections, and minus one the most hindrance for preventing new infections. And four things popped out, but three very strongly; obviously no surprises here, the effectiveness of PREP, the coverage of the population, and whether people discontinue it or not are the major drivers of the proportion of new infections that are prevented.

So using that information, we derived different scenarios, both optimistic, neutral, and pessimistic, and different strategies, whether to target by activity, age, or just the general population, and examine the impact of sexual inhibition. And here are just the parameters for the optimistic, neutral, and pessimistic scenarios, I do not have time to go through each, but you get an idea, 90-percent, 60-percent, 30-percent effectiveness, coverage 75, 50, 25, and so forth, time to ramping up, prep discontinuation rate, and increase in risk behavior.

And with no sexual inhibition, the optimistic scenario non-targeted, targeted by age or targeted by activity shows 74, 45, and 28-percent reduction in cumulative new infections over ten years, which in southern sub-Saharan Africa would translate

into millions of infections prevented. With the neutral scenario, we see less effectiveness, and with the pessimistic, little.

Now the important thing here is that with 100-percent increase in sexual activity or disinhibition, we see very striking erosion of the neutral pessimistic scenarios with less of an effect on the optimistic and largely different by the high efficacy of PREP in that scenario.

So the conclusions from this initial work are PREP could have a major public health impact by targeting it to groups with the highest sexual activity, minimizing sexual disinhibition, and having a high effectiveness, and the best scenario would be 90-percent. I do not know if we will achieve that with the current agents. The benefits could be lost, though, by sexual disinhibition, high PREP discontinuation, especially with lower PREP effectiveness. And the key variables identified in this model will likely determine the actual impact of PREP at the population level.

So let me move on to the resistance model. You see Roy is a little bit older and Omi looks a little more haggard and I will explain why, but first, some reasonable resistance assumptions. The reasonable assumptions are that in an individual who is put on oral antiretroviral prep with undiagnosed HIV infection will develop resistance. I think that is a safe assumption from our studies and of treatment naïve individuals. Resistance is likely to persist on PREP,

but it will decline off PREP quickly for some mutants, and with tenofovir and FTC, 65 viral and 184, the major culprits, and there is data in treatment experienced individuals that these decline rapidly because of the fitness effect on the reverse transcriptase. It is also reasonable to assume that individuals will become infected on antiretroviral PREP, will likely develop resistance unless the PREP is stopped. Resistance will persist on PREP and may decline more slowly off PREP, particularly if no wild type virus is transmitted. And this is a key, unanswered question in the field.

So the detailed drug resistance modeling we did involve 22 HIV drug susceptibility states, including both transmitted and acquired resistance and modeled inadvertent PREP use in previously infected individuals and in those who become infected on PREP, and modeled variable persistence of resistance. And this is just a flow diagram of the new model structure. The modeling methods analysis and output whether there was a stratified model population would attract over 5,000 different states. We used the standard sensitivity on scenario analyses, and the key model outputs were change and cumulative new infections and prevalence of drug resistance and incidence of transmitted resistance; I will not show you that in the interest of time.

And out of a whole series of variables, the key drivers of prevalence of resistance according to partial rank correlation coefficients were PREP coverage of the population,

the more drug pressure, the more likely there is to be resistance; persistence of acquired resistance, how long it persists in an individual that it is selected in; increase in risk behavior; and the fraction that develop acquired resistance when inadvertently put on PREP; use effectiveness of PREP, which is a function of efficacy times adherence was a negative predictor of resistance. What is that telling us? That is telling us that if you block infection, you do not get resistance. If you take the medicines and you have high use effectiveness, you do not get infection, and that translates into the mantra no infection, no resistance.

And we went through various scenarios, I do not have time again to describe the input parameters for the optimistic, neutral, or pessimistic scenarios, but one of the key factors is the PREP discontinuation rate is very high, and the inadvertent PREP use duration and the proportion within inadvertent PREP use is very high in this kind of doomsday, pessimistic scenario.

And when we look first at the efficacy of PREP without any drug resistance, so drug resistance can occur in this model, we see very similar results to what I showed you before. But strikingly when we add in drug resistance in the optimistic scenario where there is less use of inadvertent PREP, the efficacy, or effectiveness of PREP, is not reduced in the optimistic scenario, but it is really hampered, tampered down

to nil in the pessimistic and lowered significantly in the neutral scenario.

And when we look at the prevalence of drug resistance by these different scenarios, and this does not include the complication of concomitant antiretroviral therapy in part of the population, we see this terrifying prevalence of resistance of close to 50-percent regardless of strategy in the pessimistic scenario, but in optimistic and neutral scenarios, modest prevalence.

And when we do an analysis of the key parameters influencing prevalence of resistance by sensitivity interpretation, we see that the risk, excuse me; and this is the proportion of that high level of resistance explained in the different scenarios, the rate of inadvertent PREP use in users with pre-PREP infection and the duration of that use are major contributors. The rate of emergence of acquired resistance is also a driver, and less so the duration of inadvertent PREP use and emergence of resistance in those who developed infection on PREP. So these are the top three.

So, in conclusion then, inadvertent ARV PREP use will drive the population prevalence of drug resistance. The rate of its misuse and the duration are critical. Previously infected are probably more likely to drive high prevalence than those infected on PREP, but that is a bit speculative.

So as an early warning, PREP roll out must include routine testing of recipients sero status. If we do not and we

give PREP indiscriminately to those that are infected, we will have a much higher prevalence of resistance.

PREP effectiveness is still maintained at low resistance prevalence and resistance from antiretroviral therapy is likely to reduce PREP effectiveness and vice versa. We have not modeled that yet; we will all be very old by the time that model is ready to present. An overlap between PREP and ART agents are obviously suboptimal because of cross resistance, and I would be happy to end and answer any questions. Thank you very much.

IAN MCGOWAN, M.D., PH.D.: John, thanks very much for that comprehensive overview. We have a few minutes for questions and so I will try and field some with Nomita. Perhaps we can begin with one interesting question from the audience addressed to Dr. Vanham. This issue of how do we identify best in class to move into clinical testing because, as it has been pointed out in the absence of proof of concept of a drug ever working, how do we actually validate any assays? So how do we actually move forward in that context?

DR. GUIDO VANHAM, M.D., PH.D.: Yes, well there is only an empirical answer to that, that is to say that I showed you different various models, so I think you need to test the drugs, or compare the drugs in the same class, in various models. And I think this is especially important to look at cell free and cell associated to compare activity against cell free and cell associated virus, as long as we do not know

whether cell associated, I mean, there is a lot of evidence that cell associated virus can be transmitted, you should make sure that your candidate is active against cell associated virus.

IAN MCGOWAN, M.D., PH.D.: Thank you; another question, which I will address to Slim, just to be controversial. What about anal sex in your efficacy studies of jails? And, Tim, can maybe chip in if he wants to, too, because clearly if you are assessing a vaginal product, you might protect the vagina, but you will not do much for the rectum. So how do you rationalize that?

SALIM ABDOOL-KARIM, M.D., PH.D.: Right from the initial microbicide gel studies that were undertaken, anal sex has always been quite an important issue in that the net effect of rectal anal sex is that it would reduce the likelihood of showing efficacy. So the strategies that have been undertaken is to look at your inclusion criteria, and to focus your cohorts, or your study participants mainly on those who are not engaging in anal sex. And for those who are, to have them go through counseling so that they reduce the amount of anal sex, or avoid anal sex, for the duration of the study period.

I think the big issue is that none of these products that have been tested have been through all of the requisite safety procedures for us to recommend their use in the rectum. And so all of the vaginal gel studies that have been done, have been done on the basis that we would allow for a certain amount

of anal sex to dampen the overall effect and so that the sample size calculations that are done to show efficacy take that into account.

By the way, the same issue applies to pregnancy. We anticipate that once anybody, any woman within a microbicide trial becomes pregnant, they have to go on product hold, and so any infections that occur in pregnancy are occurring off product. And so the net effect of pregnancy is to dampen the effect and reduce the likelihood of showing a difference across the arms. And the way in which we deal with that is simply by increasing the sample size and trying to show an effect over and above that dampening effect of anal sex and pregnancy.

IAN MCGOWAN, M.D., PH.D.: So now we are going to move to quick questions and even quicker answers, just like Jeopardy. So, Nomita?

DR. NOMITA CHANDHIK: Yes. Several questions for Tim; what is going to happen with people that change their partners during the follow up of the cohort of discordant couples in the PREP studies? Just a quick answer to that, Tim.

TIMOTHY MASTRO, M.D.: That is an excellent question, and as I suggested in the study, enrolling the partners allows you to identify one potential source of infection in those that are HIV negative. Of course, there are other potential exposures for those individuals. I do not know if any of the investigator's are in the room. I actually am not sure what

the plan is for that study if partnerships change during the course of the study.

IAN MCGOWAN, M.D., PH.D.: Quick question for John Mellors; this is a critical one, I think. What is going to be the optimal way in terms of frequency and technique, to ensure that our population is remaining HIV negative? So some of the audience had asked should we use antigen antibody tests, should we use molecular techniques. How often should we use them?

JOHN MELLORS, M.D.: I think a molecular test is not feasible because of cost and availability. I think it would be sero testing and it would be screening with rapid tests as we do in our VCT's. The frequency of testing on PREP is something that we need to think about. You know, and it would require capacity building and there are some feasibility issues. I think that in the luxury of the clinical trial environment, we are testing monthly. That is unrealistic for the general population.

DR. NOMITA CHANDHIK: This is a question for the full panel and it says that if we get the positive result from iPrEx and other studies in MSM first, how important will it be to continue and complete the PREP studies in women before bodies like the WHO and UNAIDS give guidelines on PREP, considering that MSM's and women are different populations with different modes of transmission. So how will the positive results from PREP studies and MSM impact studies in women, in the PREP studies in women?

TIMOTHY MASTRO, M.D.: I can take a try at that. That is a very important issue and now that we will soon have six or seven ongoing trials, the impact of the results from one clinical trial and the other clinical trials will take a lot of careful consideration. We did have the experience recently of the South African circumcision studies showing clear efficacy there. There were two other ongoing studies in Kenya and Uganda and there was a clear decision that one trial really would not be compelling enough to have normative bodies make circumcision recommendations, so those two other studies were carried to completion.

I think given the finding of efficacy in one mode of transmission, a similar process might go forward. I think you would have to consult with a variety of experts in the area and determine whether or not you still have equipoise in the ongoing studies to continue a placebo arm in those. And I think that would be influenced by the degree of efficacy in the trial, issues related to adherence and comparison of where the other trials are in the course of the trials. Very nearing the end it is much easier to say let us carry this trial to the end and see if this different mode of transmission is different.

IAN MCGOWAN, M.D., PH.D.: Thank you, Tim. The next question was what about pregnancy? And I think a number of speaker's have alluded to this, so maybe I will answer that one. You know, we cannot enforce to regulation guidelines at the moment continue exposure to drug for individuals that

become pregnant on study, and so the approach we take generally in the networks is we optimize family planning contraceptive advice and try and ensure that women do not become pregnant, but if they do, they have to come off. However, the microbicide trial network, at least, is beginning to start looking at safety in pregnant women of tenofovir, which is just at the start of the study in the women who are about to have an elective cesarean section as a beginning to see whether or not we cannot create a framework where we can dose through pregnancy because this is a hugely vulnerable period in women's lives to the acquisition of HIV. But for the moment in trials, they have to come off of drug.

DR. NOMITA CHANDHIK: This is also addressed to the panel. Seeing that several factors were mentioned as considerations of models to be used, but what do we know about menstrual factors? About menstrual factors, are they also being considered?

SALIM ABDOOL-KARIM, M.D., PH.D.: Well, I think it is more difficult to model that in vitro, at least.

IAN MCGOWAN, M.D., PH.D.: It is a question, perhaps, asking whether menstrual fluid, blood, whatever, might inactivate the products, the microbicides. I guess we know that the polyamines, those sort of things, can impact efficacy, but I think with the antiretrovirals, I do not see that would be a problem.

SALIM ABDOOL-KARIM, M.D., PH.D.: No, in fact, most data indicate that the antiretrovirals remain active no matter whether you add seminal plasma or add something else.

IAN MCGOWAN, M.D., PH.D.: Okay, I think in the interest of time, we are at about a minute before 4 o'clock. We should probably wrap up this session. I would like to thank you all for coming, I would like to thank our distinguished panel for some great presentations, some excellent questions. This story will continue, but thank you.

[END RECORDING]