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**4th IAS Conference on
HIV Pathogenesis, Treatment and Prevention
Late Breaker Track A and C
International AIDS Society and
Australasian Society for HIV Medicine
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FRANCOIS DABIS, M.D., PH.D.: Good Afternoon. This is the Latebreaker Session for Tracks A and C. My name is Francois Dabis from the University of Bordeaux in France and I will be co-chairing this session with Peggy Johnston from the NIH. We have a tight schedule for the next hour before the closing ceremony, so without any further ado we will start the program. There's a slight change. The two first presentations will be reversed, and the first presentation will be on the Effectiveness of Cellulose Sulfate Gel for Prevention of HIV: Results for the Phase III Trial in Nigeria, to be presented by Willard Cates.

WILLARD CATES JR., M.D., M.P.H.: Thanks Francois. I am the warm up for Lut who's coming after me. I'm talking about the cellulose sulfate Basse trial in Nigeria, funded by USAID, sponsored by Conrad, conducted by FHI. Vera Helparin was the principle investigator, and I'm simply the mouthpiece. But the real workers including Abungai, here in the audience, were our two collaborators in Nigeria. Including, when you're talking about working in hard court [misspelled?], working in the most difficult because of civil strikes in that country. The product that you'll be hearing about in the next two talks is 6-percent sodium sulfate. You can see high molecular weight polymer and does that potential contraceptive effects. It's a

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gel, like you heard in some of the previous sessions. A single-use applicator with 3.5 milliliters of gel, the usual dose that is being used. Study objectives: to look primarily at HIV effectiveness also looking at STI effectiveness. I'll be talking about the primary effectiveness as the endpoint.

A Phase III randomized, one-to-one, two-arm trial, no condom only arm, originally intended to be over 2100 women, almost 1100 in each arm, with a 12-month follow-up and then testing for the primary and secondary endpoints at each monthly follow-up visit. The timeline. It began in December 2004 with screening. But in October 2006 we actually had looked at 70-percent of enrollment completed, realized that the incidence was just too low to continue. And add many of the other issues—it was sort of a poster child for the types of challenges in a HIV prevention trial that Nancy talked about at the plenary—enrollment was stopped and we had planned to enroll in South Africa in order to improve the endpoints of the trial. And had projected a December 2008 estimated end of the study. But then came January 26, 2007 when the multi-country trial, Friday afternoon, 4:00, Henry Gablenet [misspelled?] called me and said, "We're stopping the trial because of the apparent increased risk of HIV" in saos, the sulfate arm at interim analysis of the multi-country trial that we will be talking about.

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We've spent literally 72 straight hours getting the data together, getting the data safety and monitoring board for the FHI trial together—looking at the data, presenting it to them. Found in our interim analysis, no increased risk of cellular sulfate Nigeria, at the interim analysis despite that based on interest for airing on the side of safety signals, the DSMB and FHI stopped the trial just like Conrad.

So what did we actually find? That the raw numbers were 820 in the cellular sulfate arm, 824 in the placebo, with a high loss to follow-up. We were following very difficult participants, both in Lagos and in Port Harcourt and that became a limitation of the particular study. The characteristics were a young age group, 23. I think it's probably the youngest age group of most of the microbicide trials. Relatively well educated, most had been pregnant; very few were married, had about an average of 1 sex-act a day at least at enrollment. And condom use between screening, self-reported condom use, between screening and enrollment, increased from about 60-percent to about a self-reported 90-percent.

What did we find? Here are the results section. In that first line were the 21 infections that we had available to us in January '07 when that decision was made. There were 9 events in the cellular sulfate arm, 12 in the placebo arm,

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slightly more in the placebo arm, less than 1, but no significant effect at all. The confidence interval overlaps 1, the P-value .55. when we followed up and completed this study, followed up all our participants, added 2 more infections to that group, 1 in each of the arms, still remains that the final intent-to-treat analysis was .56. And I'm showing a final, what we call, per protocol, as we've discussed we took those who were censored because they had a product interruption, mostly pregnancy out of the equation. The gap narrowed a little bit, but still stayed at no effect .86 P-value.

In conclusion, for the cellular sulfate trial in Nigeria, we did not observe any effect, one way or the other, of the gel on the risk of vaginal transmission. Thank you.

FRANCOIS DABIS, M.D., PH.D.: Thank you Willard. That saves us a couple of minutes for questions.

FEMALE SPEAKER: [Inaudible].

FRANCOIS DABIS, M.D., PH.D.: Yes, perhaps as the same product is going to be also used in the next trial, we should take questions to all together for the two speakers. So I'm now calling Lut Van Damme who is going to report on the other CS trail and on Phase III as well. Thank you.

LUT VAN DAMME, M.D., M.SC, PH.D.: Good Afternoon once again. Thank you Willard, I have now a little bit more time to go through my presentation.

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So as you know I'm going to present the results from the Conrad trial with cellular sulfate done in multiple countries. One of co-PI's is Figor Tarangi [misspelled?] and I speak, of course, on behalf of the whole team.

As a very brief background, CS was tested in different models, we used different endpoints, we used different strains, and in vitro it was effective against HIV. In pre-clinical testing, using the rabbits, monkeys or explants [misspelled?], there was no indication that there was a potential for harm. My colleague, Estbalon Sellis [misspelled?] is here if you have more questions on that.

We also did several safety trials, 10 safety studies in women, 2 contraceptive trials, and not shown here, 2 male tolerance studies. Again, in the studies there was not a signal that there was going to be, or there could be problem in the Phase III trial. As Zeta also pointed out in the earlier session today, only the Phase III gives you the final result regarding safety. In one of the studies we did see an increase in SV alkali, a decrease in lactobacilli, at that time we thought not clinically relevant.

In the contraceptive trial, which was a non-comparative trial, very few events, adverse events were thought to be related to gel use.

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And then the HIV prevention trial, as I said in the early session, primary objective, looking at the effect on HIV transmission. Secondary objective the prevention of gonorrhea and chlamydia. Both were analyzed as our main analysis, the intent to treat principle. We randomized women to either the placebo or the CS arm, 50-percent CS, 50-percent placebo and Conrad worked in several countries, multiple sites. Everybody was blinded as to which arms women were randomized to. We included HIV negative women who are all 18 years or older, no upper age limit. And all had multiple partners, which was one of the entry criteria.

This is how were organized. Conrad was overall implementer and coordinator of the trial in very close collaboration with Family Health International from North Carolina. They were responsible for data analysis, data manager, monitoring. And Jan Dees [misspelled?] is here in the audience who worked with me to implement the trial through the 6 sites.

The HIV team, the Bass Group [misspelled?] was responsible for our work in the communities and behavioral signs of the trial. The Audi [misspelled?] QA department did audits in the trial. We also worked very closely with the Institute of Chopin [misspelled?] Medicine in Belgium who was

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responsible for everything that regarded the lab testing in the trial.

Women were included in 5 centers around the world. In Uganda we worked with Malaga Hospital, Florence Morandi [misspelled?] and Ben Progesi [misspelled?] that worked for Missule Aladi [misspelled?] in [inaudible]. In South Africa we worked the Medical Research Council, Rush Negovin [misspelled?] and Deta Ramji [misspelled?]. And in India we had 2 sites. One was in Shani YOGK [misspelled?], with Senator Solomon [misspelled?] and one in Bengal with Muri Sovekel [misspelled?] and Renald Washington. Although the clinics were in the north of the state Karnataka a very rural area. Willard already pointed out we used a placebo, which was specifically designed for microcide trials, to have no effect on pathogens or the vagina micro flora. Was just delivered in the same applicators, single use, delivering 3.5 a mil. They dose were to be used within 1 hour of sexual intercourse, and to use the precision of Dot Kator [misspelled?], our statistician, within initiation of intercourse.

This is how the drugs were delivered to the site. So we used 6 colors to which the women were randomized. So 3 colors were S3 were placebo. We obtained informed consent at screening and enrollment, and at enrollment the women had to reply to a questionnaire before she signed on the informed

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consent. This questionnaire was repeated at every quarter in the study, and additional information was given as deemed necessary. We had monthly clinical visits in which data were obtained regarding compliance with gel or condom, adverse events, or any problems she may have had. We tested for HIV at the screening enrollment, follow-up 1, 3, 6, 9 and the final visit. The pelvic exams were done quarterly and there we obtained endpoints for STIs. We traced participants as soon as they missed a visit. Again, this was in collaboration with the Behavior and Social Science group.

The endpoints are all driven by the lab and how did we assess those? At screening and enrollment we used the national or the local algorithm for HIV. During follow-up we took finger prick lab for the first sample, and tested on an algorithm of rapid test. We started to determine if positive feeded biline if an inconsistency occurred we use the ungula. If the woman was deemed to be HIV positive we took a second sample, this time venous so that extra testing could be run. She only got her diagnosis if the 2 samples were positive. On every final sample in the study we have done PCR testing to determine the real status of the women, except for our Bangalore site where very few women were included since they started late last year. Because the Bangalore team could not

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obtain the approval of the Indian Government to export the samples.

Gonorrhea and Chlamydia was done by SDA testing because cellular sulfate inhibits the PCR. And we had a quality control program. Most HIV zero conversions except 4 occurring in Durban, where it confirmed by the ITM.

This is a team that did a lot of work behind the scenes and which we often don't talk about, the lab team who trained, supervised and monitored the procedures of the trial.

We had planned to recruit almost 2600 women based on the fact that we assumed this gel would 50-percent effective in protecting women. A two-sided significance level of .25 an 80-percent private study because we had a second study going on. And also assume that 80-percent would complete a 12-month follow-up visit and an incidence of 4-percent in our control arm. We had planned 1 interim analysis which we presented to the independent data monitoring company halfway through the trial. And this, as you all know, happened on the 26th of January. The official closure was on the 29th because the IDMC calculated how many infections we would have when they gave us 2 days to control randomization and the statistical program. On the 27th a message went out that enrollment had to be stopped. And as Willard mentioned, we had the Nigerian IDNC also came together on the 29th. We had asked guidance from the

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IDNC that if the P-value in a direction of harm would go below .1 that they would give us a guidance. And this is a very liberal stopping rule. From the start from planning the study, we decided we were going to err on the side of safety. This product is not on the market, so proving beyond doubt that it's harmful was never ever our intention.

In total, this is the final data. We screened almost 3000 women of whom half were enrolled. We had to exclude 3 from the ITT population because they were positive on PCR of the enrollment sample. So when a woman's status converts within 3 months after enrollment, we did PCR on the enrollment sample. Another 27 were excluded from the analysis because we didn't a follow-up sample for them. 10-percent were lost to follow-up and this varies widely among sites, with 20-percent in Binnion [misspelled?], 10-percent in South Africa and less than 5-percent in the other sites. 2-percent discontinued early and 88-percent had a final visit.

Based on characteristics a bit older than the Nigeria study, most women had been pregnant, few were married, an average 11 acts per week, and condom use also self-reported. Condom use increase we went through the trial.

Here are the results and please bare with me as I walk you through this slide. We presented 35 events. At interim analysis 24 were in the CS arm. P-value at that time .02. So,

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as I said, we had decided early on to err on the side of safety. When we saw those numbers we didn't doubt one second that we had to stop the trial as soon as we could. At that time, useless to say, that our condition of power, because we had talked about utility analysis, was close to zero. So it means we had no power at all to even come to an effect had there been one. And the final-intent-to-treat analysis, in which everybody who had been randomized is included, and for whom we had a follow-up HIV sample, we have 25 events in the CS arm, 16 in the placebo, not significant. So under ITT cellular sulfate has no effect, not harmful, not beneficial. We did preliminary contoured it toward to our [inaudible] analysis is still preliminary, as you may understand, we were under the time pressure and we will explore it out at [inaudible]. So the first [inaudible] analysis that we did is that we censored women at the time of their first product interruption. Which was very clearly documented in the trial. Just for those of you who are not familiar with statistics, censoring means that we stop the follow-up of that women, at that particular time, so we do not exclude her fully out of the analysis. We have 23 events in CS, 11 in placebo and this key value two-sided is .03. So we think that there is an indication towards a potential increased risk in the CS arm. There was no effect on gonorrhoea and Chlamydia.

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Here you can see the curves for the HIV infection as we went through the trial. Same conclusion, we don't think that CS is effective against HIV transmission and as said, we do think that there may be an indication for an increased risk.

Keep in mind that all [inaudible] are different. The other trials that are ongoing have had several IDNC meetings and can continue without change. We hope that the field will learn from our experience by, as I will come to in a minute. Some of the extra testing that we will be doing and hopefully we can come and identify potential markers of no effect or increased risk. And as I said in my first talk this afternoon, we do need to come to a range of prevention tools.

As you all know, we did not and do not have, the explanation of why CS failed in the clinical trial. It was a shock to all of us and I dare to say how people always ask at CONRAD.

A first hypothesis is that CS might stimulate HIV, captured through interaction with DC sign [misspelled?]. A second hypothesis is that the frequent use of CS may cause an inflammatory reaction or a local immune reaction. And a third hypothesis that we will test, is that there is after all an impact on the micro flora if the product is used enough. So to do that we will explore of course in very great detail the Phase III database from the multi-countries study. [Inaudible]

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and the panel of blinded products so different labs around the world and ask them to test them in their model and see if a sign is picked up.

During the trial we asked women to give us vaginal swabs for long-term storage and future research, and unfortunately we will use them ourselves in our study, to draw a base on the results of the lab findings to analyze those swabs. There is a monthly study going on and Christine Mark [misspelled?], a colleague of mine from CONRAD will soon start another detailed safety study in women in the United States.

This is the last vote of the team, in Frankfurt in April. I would like to thank all the study participants, our sponsors, the USID and the Bill and [inaudible] Foundation, and the whole team around the world to work with me for this trial.

FRANCOIS DABIS, M.D., PH.D.: Thank you very much Lut. We have time now for discussion, probably the 2 papers. So please go to the mic, identify yourself and ideally say to which trial your question is primarily directed, although both presenters can comment again. Please.

ARTIS MOE: Artis Moe from UCLA Los Angeles. This is actually for both trials. What happened to HIV positive women, did they get access to medications?

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LUT VAN DAMME, M.D., M.SC, PH.D.: Yes, before we started the trial, so independent of our results, it was at start up agreed to CONRAD give funding to the women sero convert during the trial were referred and had access to care. And those agreements had been made between CONRAD and the trial sites. And the trial sites contract with an existing care. And I want to point out; we did that before we started the trial.

WILLARD CATES JR., M.D., M.P.H.: Similar for the Nigerian trial in terms of linking with the PEPFAR sites, in the particular regions where the trial took place.

ABIL BABIKA: My name is Abil Babika from MRC Clinical Trials in London. My question is on protocol analysis. You said you censor the follow-up at the time when you knew they stopped using the gel. And that applied to both the placebo and active, that's right? Would there be any sense of actually only censoring those who didn't use the actual active component and comparing to those who were using the placebo? The reason I'm saying that, the on protocol analysis has excluded more events in the placebo arm than the other, and that could be by chance. But another way to look at their own protocol analysis is to only apply for the active gel.

LUT VAN DAMME, M.D., M.SC, PH.D.: That's a good suggestion; we can bring it to the statistician's attention.

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We do see the difference, that's why I also said it's preliminary. We will explore it further and try to better understand.

ABIL BABIKA: Yes, I mean there both not protected by randomization.

WILLARD CATES JR., M.D., M.P.H.: Yes, my thought is that it is chance and that that is the main reason. Because if the participants were truly blinded there really should not have necessarily been any particular effect. The one issue that we might talk about is the effect of potential pregnancy effect in the active arm with pregnancy being a key factor in terms of leading to the per protocol censoring. So your question is a good one, and that's what we have to look at.

DEAN HAMMER: Dean Hammer from the NIH in the US. As you mentioned, one of the hopes for this trial is that at least it will provide data for people to figure out what to do the next time. And one of the difficulties though has been that the preclinical data that you described, just a simple virology for example, mostly has not been published in the peer-reviewed literature. I'm sure it was all done for the FDI and D application. But I'm wondering if in this case it will be possible for people to have access to the IND application data.

LUT VAN DAMME, M.D., M.SC, PH.D.: Gustabo can you nod your head?

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MALE SPEAKER: As I said to Dean, in person, that the data that have not been published will be published as soon as possible. We have commitments with investigators and then everybody will be able to take a look at them.

DEAN HAMMER: I guess I'd like to just press it a little bit further, which is that usually for a publication of course, it's a select group of data that makes a sensible story. Otherwise publications would be terribly long. Where as an IND file is really long and contains everything, and I think since it's still sort of a mystery of why the increased infection was seen in the trial, that it would really be beneficial if other scientists had access to the entirety of the data. Rather than to a subset of the data that was pre-selected by the people who are presenting it.

LUT VAN DAMME, M.D., M.SC, PH.D.: Thanks for your suggestion.

FRANCOIS DABIS, M.D., PH.D.: Next.

KELLY BLANCHARD: I'm Kelly Blanchard from Ibis Reproductive Health. Two quick questions, do you have plans to do a per protocol based on reported adherence? Obviously for time I understand why that wouldn't have been done now, such it's just query. And then in terms of the more detailed analysis where we might try and piece out what it is about this product compared to other products. Are there specific things

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that you think are priorities to do first? Obviously there's a wide range of different cytokines, immune responses, all kinds of things I imagine you want to go back and look at. Is there sort of a priority list that you think will come first? I'm just curious of there are things that you think are more important than others?

LUT VAN DAMME, M.D., M.SC, PH.D.: For your first question, yes we will definitely look very closely at—and don't forget it's self-reported to us—but to adherence in the trial and if you seen any effect of adherence. What was the second question?

KELLY BLANCHARD: About which analyses you want to look at first.

LUT VAN DAMME, M.D., M.SC, PH.D.: We will do several things at the same time like the panel of blinded products has gone out; the study in the United States will start. With the regard to the clinical database adherence and bacteria of vaginas is our priorities. But we will not be working under such a time pressure as we are now, so we have more time. But that will be the primary things.

FRANCOIS DABIS, M.D., PH.D.: Yes please, last question please.

MALE SPEAKER: Yes, this is a pre-clinical, [inaudible] to Dr. Donsell [misspelled?], but in your healthy American

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participant study you're going to do, clearly you need to do some quite careful immunology. I was wondering can show if there's what kind of things you might be doing other and beyond the usual Phase I safety parameters?

DR. DONSELL: The endpoints, off the top of my head, are going to be, of course, the standard colposcopy and naked eye examination. And then a full bacteriology and then we are going to get into the immunology, but first perhaps into the tissue histo-architecture we're going to do histology. And then, as I said, getting to the immunological parameters, we are going to do cells, [inaudible] of immunological, cells immune, cells in the mucosa, both to determine the lineage and to determine the activation status. We are going to do cells in the cervical vaginal palettes, and we are doing cytokines and soluble markers. New markers like vascular [inaudible] markers and inflammatory enzymes. And then we are also investigating the anti-viral and anti-bacterial properties or [inaudible] 6 of the CDLs. So we are trying to assess the potential in vivo impact on innate and that to be immunity as has been described today by Robin.

MARGARET JOHNSTON, PH.D.: Thank you, we're going to move on and switch gears a bit here, with a presentation by Eric Arts from the Case Western Reserve University in the United States. His presentation's entitled "A Single Dose Of

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An HIV-Specific, Vaginally-Applied Microbicide, PSC-RANTES Can Select For A Drug Resistant SHIV In A Macaque Model." Eric.

ERIC ARTS, PH.D.: I hope you'll bear with me and we'll get to Nancy as soon as possible. So the title's already been mentioned. Basically I'll set the stage in suggesting that resistance to many antiretroviral drugs can emerge in HIV infected individuals with a single dose of treatment, and this is best exemplified in Nevirapine treated mother and infant pairs to prevent peri-natal transmission where we see resistance emerging with a single dose. And there's some suggestion that the emergence of resistance can occur independently in the infant and not being transmitted from the mother. And that's given by the example that different drug resistant mutations may emerge in the mother and the infant in the same pair, although there's still questions whether this is true selection. And this is really quite an example of the scenario that would be with an anti-HIV microbicide that you might get selection because of the selected pressure by the drug.

Now we also know that macaques are a successful model because progesterone treated macaques are successfully infected through a vaginal route with CCR5 tropic SHIV P3, and several CCR5 inhibitors and entry inhibitors, when vaginally applied, can block a SHIV P3 infection of macaques in post-coital model.

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And this had been demonstrated by John Moore, and Ron Bessey and Michael Letterman, and many others.

But there are potential drawbacks to this model as well. And one of these is on the almost exclusive use of SHIV P3, which was developed by Cecilia Chang Meyer, which is pathogenic or at least infectious for macaques. And the relatively low diversity of SHIV P3, which is not really comparable to the diversity of the inoculating dose from a donor to a recipient. And there are so many other difficulties, there are too many to discuss for this presentation.

Now PSC-RANTES is a very probable and potent anti-HIV microbicide. It's a small 8-kilodalton protein, and it may be actually one of the most potent anti-HIV compounds that exist with sub-nominal or IC50 values. Inhibition is mediated by 2 mechanisms; one of those is competitive inhibition of the cell surface with the virus by binding to its natural receptor CCR5. But the other is equally as potent in its inhibition in that the receptor-down regulation of CCR5.

So in testing PSC-RANTES as a microbicide, first it's important to know that PSC-RANTES is not induced vaginally irritation, inflammation, and macaques. PSC-RANTES effectively down-regulates CCR5 on the cell surface of macaque PBMCs. And a trial was set up a few years ago, led by Michael Letterman in

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the macaques, where 30 female progesterone-treated recessed macaques, were treated with PSC-RANTES, and this study was effective in many ways. But one of those was that dose escalation was utilized for the vaginal application of the PSC-RANTES before the challenge. And this is the data, now just summarizing the paper by Letterman and colleagues, and that is that the high doses of PSC-RANTES, in this case, quite high but this is pretty typical of anti-HIV microbicides. It was completely protective at the 1 millimolar concentration, but as you see when you start reducing the concentrations to 330 micromole or 100 micromole you start seeing levels of infection. So what we did in this study was basically, as indicated by the arrows, we obtained the plasma samples from all of these HIV infected macaques in the presence of the PSC-RANTES and sequenced all of those to try to identify, within the full HIV envelope gene, potential mutations that may have led to potential resistance.

So this is a summary of the data. First off, it's very important to note that the virus that was infecting the macaques in the presence of PSC-RANTES was extremely homogeneous, as was the inoculating virus. And we only really found one virus or one animal infected in the presence of 100 micromolar PSC-RANTES that had any selection of any particular mutations that were stable within the macaque. And this

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particular mutation at 315 switching right next to the GPG conserved crown from a lysine to an arginine was only found in one macaque but was found initially at infection at the earliest time point and was maintained throughout the infection of that macaque. In the same macaque we sequenced within the GB41 domain and again, near a conserve motif in the C-terminal HEPTAP [misspelled?] repeat we found another mutation that was selected that was maintained throughout the infection of that macaque.

The first thing we wanted to do is to see if there was any selection within the population that existed. Did this mutation actually preexist in the inoculating SHIV-P3 virus that was used to expose these macaques. So we a hold of the original virus as it was used in these challenge. And what we're doing here is doing a low frequency polymorphism detection for these two particular mutations. We're using analogue nucleotide ligation assay. I won't go through the specifics of it. But what you can see here is of course there's a strong enrichment in this particular macaque M584 for this particular mutation 315K to R and for this particular mutation N640 to D. And of course those were found as dominant sequences in these macaques. But it's interesting to note that there was a small percentage of this particular mutation was in the inoculating dose. And so as a result, this was

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representative of 25-fold enrichment from the inoculating virus to the virus that actually infected that macaque. But interestingly enough, that particular mutation was not found to our sensitivity levels within the inoculating virus SHIV-P3 but was enriched within the infected animal in the presence of PSC. So this was at least a 250-fold enrichment.

We then took the envelopes out of this macaque and cloned it into an HIV virus. We don't have the SHIV-P3 cloning system that we could utilize so we used this system and then we characterized drug sensitivity of this envelope that's HIV based coming from those infected SHIVs. The first thing we had to do though was actually develop a cell line that expressed both human and rhesus CR5. So we developed clonal rhesus CCR5 and human CCR5. And you can see that both of them could induce calcium influx and mobilization equally so the rhesus R5 was functioning on the U87 cells and the rhesus R5 was also down regulated by PSC-RANTES. So functionally these receptors looked okay on the cell surface we could use this as a tool. Okay, so this is the data now looking at the sensitivity of the P34, which is the inoculating virus and the M584 virus, which was the one that was infected in the presence of PSC-RANTES, which had those two particular mutations. And what we see first off is that the macaques treated with the 100 micromolar PSC-RANTES was infected with a SHIV clone resistant to PSC-

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RANTES at least the envelope. And this resistance was more pronounced in cells expressing rhesus R5 than in the human CCR5 expressing cells. So both cell lines, this was a significant level of resistance, approximately 20 fold. But the important point is that we could not actually get complete inhibition of the M584 virus in the rhesus CCR5 expressing cells. And as demonstrated right here, that even at IC99 concentrations or above, that 100 nanomolar we weren't seeing complete inhibitions. So this really was basically a fully or closely fully resistant virus.

The other thing we did was we looked at another CCR5 antagonist TAK-779. And again, this particular virus that was infecting these macaques in the presence of PSC-RANTES was again cross resistant to TAK-779. But this was only found in the rhesus CCR5 expressing cell lines and not in the human CD4 CCR5 cell lines. And then finally we also looked at T20. Now this virus also has a mutation in the conserve C-terminal heptat repeat. And interestingly enough, this virus became hypersensitive to T20. And this is possibly due to that mutation.

And then as a very final slide, I'll just make a mention that we also looked at the replicate of fitness of this virus to see if these mutations was impairing the fitness of this potential virus that was infecting these macaques. And

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what I can say for now is that by comparing the fitness of these two viruses, the inoculating P3 and the one that infected this macaque in the presence of PSC-RANTES. We did not see a fitness differences or definitely not a fitness decrease. And we're starting to investigate whether actually there was a fitness increase in the rhesus CCR5 expressing cell lines.

So the take home question is, if we can select for PSC-RANTES which has been cloned from a SHIV-P3 inoculum in even one out of ten macaques vaginally exposed and treated with PSC-RANTES, will this happen if we treat humans with vaginal microbicides for the inoculating virus could be more diverse by orders of magnitude. And that's just one last point I'll leave you with. And thank you for your attention [applause].

MARGARET JOHNSTON, PH.D.: Thank you.

ERIC ARTS, PH.D.: Oh sorry, just one last point. All these studies were done by Dawn Moore [misspelled?], who just graduated with her Ph.D., so I have to thank her for that [applause].

MARGARET JOHNSTON, PH.D.: We have time for a few questions. If you have any, please come to the microphone.

MALE SPEAKER 1: So I'm not a SHIV macaque person, but you've presented very eloquences of experiments. I don't know if it's even feasible, can you do conventional serial presage [misspelled?] that would use to pull out resistance mutation in

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an HIV more conventional system? In other words, can you demonstrate this evolution of PSC-RANTES specific mutation in other systems?

ERIC ARTS, PH.D.: That's a very interesting question. Actually a number of us have tried in the field to select for resistance to PSC-RANTES and have been largely unsuccessful. And it might be associated with the ability of the drug to down regulate the receptor. And it also may be related to the initial starting virus and the context as to where you're trying to develop the resistant mutations. So we really have nothing to compare to except for the fact that there is accumulation of V3 mutations that exist for resistance to other CCR5 antagonists or CCR5 blockers. But we don't know if these would be the ones that would be selected in, let's say, human trials for example.

MALE SPEAKER 1: And just a quick follow on question, have you had the opportunity to look at some of the RANTES analogs I know others are developing in terms of whether this is a broad spectrum loss of sensitivity?

ERIC ARTS, PH.D.: We're just doing that now. I can say this, we've done a lot of analyses on what we term intrinsic resistance. So in light of the fact that we can't select for resistance, we have found clinical islets that are resistant to PSC-RANTES. And when their resistant to PSC-

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RANTES, they're usually resistant to the other end terminal modified RANTES molecules and usually share some cross resistance to TAK-779 and some of the CCR5 antagonists.

MALE SPEAKER 1: Thanks.

MARGARET JOHNSTON, PH.D.: Two more questions then we'll move on.

MALE SPEAKER 2: Nice work. I think I missed the significance of that GP41, I think, mutation. Is that a compensator earlier? I mean, I'm sure there's a simple answer.

ERIC ARTS, PH.D.: Well, yes, it's a good question. Other mutations have been characterized in association with differential CCR5 utilization in the GP41 domain. The interesting thing about this one is it appears to be additive in resistance. I didn't show the data but we have actually chopped up the envelope just looking at sensitivity with the GP120 domain from the SHIV P3, or this infected macaque and the GP41. And both of them appear to contribute to resistance. And interestingly, the same region where this mutation actually occurs has been shown to affect the rate of fusion. So how that might play into sensitivity is for example the T20 will be interesting.

MALE SPEAKER 2: But is it thought that the binding of RANTES or other molecules to CCR5, does that affect the fusion process? I thought they were kind of sort of separate.

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ERIC ARTS, PH.D.: Well –

MALE SPEAKER 2: Maybe it does.

ERIC ARTS, PH.D.: What we've found recently is that if you increase the fusion rate, you can often reduce sensitivity, well just the whole entry process. If you increase that entry process, the rate of that, you can reduce the sensitivity to a lot of the entry inhibitors at different steps. But that's not a guaranteed rule either.

MARGARET JOHNSTON, PH.D.: Brief question.

LINDA BAUM: We learned from, Linda Baum [misspelled?] from Chicago.

ERIC ARTS, PH.D.: Yes.

LINDA BAUM: We learned from systemic treatment with antiretrovirals that with one drug you get resistant variants and with multiple drugs you don't. Are you thinking along those lines in terms of the microbicides as well?

ERIC ARTS, PH.D.: Yes, I think, and Dawn Moore can probably speak to that more, because they've done some combination work and other as well, that probably a combination of anti-HIV microbicides is probably going to be more effective. I think you can really look at the model that exists in peritoneal transmission and the blockage or the hope of blockage with Nevirapine and the high rates of really selection of Nevirapine resistant mutation out of the quasi

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species. Sometimes it becomes dominant but sometimes it just becomes more frequent. And I don't see any reason why that wouldn't occur in this type of system as well with a microbicide in the HIV specific drug.

MARGARET JOHNSTON, PH.D.: Thank you, Eric [applause]. Our last speaker for this afternoon is Nancy Padian from the University of California San Francisco. Her talk is on the diaphragm and lubricant gel for prevention of HIV acquisition in Southern African women, results of a randomized control trial.

NANCY PADIAN, PH.D.: Thanks. I know we don't have a lot of time here so I wanted to let you know that this has been published in Lancet so you can scrutinize at your leisure. But I'll give you a brief overview right now, some of which I already went over in the plenary. Lots of co-authors and quite a few of them are here. So maybe when we have the discussion, they'll be able to contribute. By the way, people, it stands for methods for improving reproductive health in Africa, wonder where the diaphragm fits in.

Our primary aim was to examine the effectiveness in the diaphragm and gel from preventing HIV acquisition. It was an open label, randomized control trial. We expected an HIV incidence of about four-percent. It was powered to detect 33-percent reduction in HIV infection. Our expected adherence was

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about 80-percent. And our study duration was three years. This was design. And so you have HIV negative, sexually active, non-pregnant women who were randomized to either diaphragm and it was actually a lube plus condoms versus male condoms alone. All women received risk reduction counseling, free male condoms, diagnosis and treatment of curable STIs, and all were followed quarterly for 12 to 24 months depending upon when they enrolled in this study. Our sites, we had three sites and five clinics, one was University of Zimbabwe UCSF in Harare [misspelled?] where we had two clinics where we enrolled about half of the women, the peritoneal HIV research unit in Swetone [misspelled?], where we had over a thousand, and the MRC in Durban [misspelled?], where we had about 1,500 women.

Our study products were the male condom and then it was the Ortho ALL-FLEX Diaphragm. One of the reasons we choose, well I mean it – an appeal of the diaphragm is not only is it female controlled but we already had good data that it can be used discreetly. You don't have to let your male partner know that you're using it. It's reusable. And that it was already available. It was already approved. The lubricant that we chose was Replens lubricant, which is an over the counter vaginal moisturizer. It's not a contraceptive. It facilitates diaphragm – we wanted something that sort of simulated the normal use of the diaphragm, we could not use Nonoxynol-9 for

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reasons that I'm sure you're aware, that it would facilitate a diaphragm insertion and lubricated the vaginal walls.

Our inclusion criteria were women between 18 and 49 years old. They had to have been sexually active greater than or equal to four sexual acts per month, as I said before, HIV-negative, chlamydia, gonorrhea, and pregnancy test negative, that they were willing to follow the protocol in an informed consent, they had a healthy cervix, and they were able to insert and remove the diaphragm correct, which is something that we assessed before randomization. Recruitment occurred from well baby clinics, community groups, word or mouth, community outreach. Our screening to enrollment ratio was about 2.2 to one. Retention, defined as percent returned for closing visit, 93-percent of the women completed their scheduled closing visit. Two-percent had early withdrawals. And we had about five-percent lost to follow-up.

Baseline characteristics, there were no significant differences by group. Seventy-seven-percent were 18 to 34. Seventy-percent lived together. More than half were married. More than half had some earned income in the last year. Forty-four-percent had at least some high school education. Current contraceptive use, which we also made available during the study if women wanted, 36-percent were on the pill, 25-percent were used injectables, 20-percent used mainly condoms, 13-

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percent other, and six-percent were on some sort of long term of permanent method. Baseline sexual and risk history, mean lifetime numbers of sexual partners was two. Mean age at first intercourse was 18 years. Sixteen-percent had tested positive for gonorrhea, Chlamydia, trich, and 59-percent had tested positive for HSV-2. Fifty-nine-percent said that their partner was not circumcised. Twenty-nine-percent had more than one indicator of high risk behavior. We came up with accumulative indicator of high risk behavior that had to do with whether you had unprotected sex, whether you had exchanged sex for money or material goods. This is all detailed in the Lancet paper. And we modeled this on the hormonal contraceptive study, which also came up with an indicator variable rather than look at each one individually. And 69-percent had more than one indicator of a high risk partner. Likewise, we had accumulative risk indicator for partners.

Results from our intent to treat analysis, which I showed this morning, which is on about 5,000 women, our overall incidence rate across the study was 4.0. The hazard was flat 1.0, obviously not significant. And when you look at the results by site, although the incidence rates differ, you still see the flat effect. So it was not driven – there weren't site specific differences in the overall measure of effect.

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Sub-group analysis, same, I'm not showing them here, but because they're not really that informative. And that is that their relative hazard of HIV was stable across all of the subgroups that we had predetermined before we began the study. And that includes age, education, coital frequency, circumcision status the partner, whether they were STI positive, HSV-2 positive at baseline, the behavioral risk variable, the partner risk variable, contraceptive use, or condom use, all flat. Per protocol analysis, now we did this two ways. Our primary way that we looked at use is that, and we predetermined this before we did the study, obviously you can do this different ways, was use at last sex. And if you look at use that way, then there would be 73-percent of the women said they used at last sex at each visit. And based on that, our relative hazard and the per protocol was 0.9, still not significant. When we tweaked it another way and looked at use as all of the time since last visit, which some people might argue is a better and more realistic indicator of what they actually did, the per protocol hazard rate goes down somewhat but it's still not significant. So it's flat.

Pregnancy results, yearly incidence of first pregnancy was the same in both groups, about 13-percent. Adverse events, percent – there were no differences between severe or adverse events between the groups. The total, again, there's a lot

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more detail on this in the paper, the total number of the total events, so that would be adverse events and severe adverse events, was 61-percent. Again, no difference between groups, so the data aren't shown here by that. We didn't have a lot of adverse events in either group. And it was safe.

This came up and I'm sorry, my colleagues who work with me are going to be mad that I took out the other graph because I think that what is not indicated on this, which is what I said this morning, is that just like the CS study, we had an increase in condom use in both arms from screening to enrollment. Where things diverge was after people were actually randomized. And there we did have differential condom use by arm. And this is the control group, which reported condom use more often than the intervention group. However, this did not translate into increased risk. As you know, it was flat and I told you the subgroup analyses were also flat.

And interesting point, and I brought this up this morning as well, is that based on post trial simulations that we did with our actual level of diaphragm and condom use equivalent to what we observed in the study are adherence rate to the diaphragm, again this is self-report rife with all of those problems, was about 70-percent obviously in the intervention arm, overall 50-percent condom use in the intervention arm, 85-percent condom use in the control arm. So

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our power to detect the effect that we had thought that we had aimed for in this study was actually less than 25-percent. So we had a severe erosion of power. We could do, I think, in large part to – actually, it's not only increased condom use, but this was a complete package of an intervention with STI treatment, counseling, and also less than optimal adherence in the diaphragm arm.

So what is our bottom line? In the context of a comprehensive HIV prevention package, we found no additional protective effect against HIV infection from providing the diaphragm plus lube in the intervention arm. I showed this, this morning, I'm going to bring it up again because my colleagues pointed out that I forgot to mention I didn't delineate one item on this. This study was not designed to assess and we couldn't assess whether in fact the cervix is more vulnerable or not. I think there's still go biologic data indicating that it is. We couldn't assess whether a diaphragm was as good as a condom. I forgot to mention this, this morning, we could not assess whether a diaphragm is better than nothing, which I still think is the essential issue for women who cannot get their partner to use male condoms. We don't know whether other cervical barriers might have worked better or whether barrier would be more effective with a microbicide or vice versa.

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What are our next steps? At the ISSTD, we'll be presenting results from our secondary, some of our secondary endpoint, specifically the cervical infections, chlamydia and gonorrhea. Shortly after that, well be HSV-2 and trich. We have working on a paper more specifically looking at adherence. Statistical analyses using causal inferences to try to look at better control for condom use and look at the direct effect of diaphragms on the outcome and a paper on acceptability. The end.

As it says here, don't throw that old diaphragm away, because it can be used as a doorknob cover, no need to worry about fingerprints ever again, a bathtub stopper, a rain hat for your cat, or a small Frisbee. Thank you, and – to all of the participants obviously, the staff, these trials are hard to do, lots of people involved, and many of them are in the audience [applause].

MARGARET JOHNSTON, PH.D.: We have time for a few questions if anybody would like to come to the microphone.

MATI ZECKA: Hi. Thank you. Mati Zecka [misspelled?], Switzerland. Did you make any attempt to control statistically for the differences in condom use in the two groups?

NANCY PADIAN, PH.D.: [Laughter] Yes. That part was rejected from the paper. And that will be coming out. I think I'm – it looked definitely more protected, however, the

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confidence limit is extraordinarily wide. The bottom line is, there are too few women in that cell to really be able to make statistical sense of it. It's something that the trial as I said, we really couldn't separate it out.

MATI ZECKA: Thank you.

NANCY PADIAN, PH.D.: Sure. Wait, one other thing, there also could be significant over-reporting of all that. So this is all confounded by that too.

MARGO WHITFIELD: Thanks, Margo Whitfield [misspelled?] from Sydney. Were you surprised that with 59-percent HSV-2 that you had a main partner rate of only two lifetime sexual partners?

NANCY PADIAN, PH.D.: No. Based on other study – I mean I only know the data sort of well from Zimbabwe because that's I've worked. I mean the HSV-2 rates are astronomically high and most of my work there has been on women who are – see the women are relatively monogamous or have few partners, but their male partners have lots of partners. But in this data, these data are consistent with what we've seen in other studies that we've done there.

MARGO WHITFIELD: Thank you.

MARGARET JOHNSTON, PH.D.: Thank you, Nancy. Please join me in thanking all the speakers [applause]. The closing session will begin in five minutes. For those of you who wish

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to go next door, the exits are here and up top. And let me also say for those who wish to stay where you are, the closing session will be broadcast in this room. So you can stay here if you like.

[END RECORDING]