

4th IAS Conference on HIV Pathogenesis, Treatment and Prevention
Plenary: Getting Ahead of the Curve:
Current Issues in Pediatric Treatment, Virology and Biomedical Prevention Science
International AIDS Society
and Australasian Society for HIV Medicine
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DIANE HAVLIR, M.D.: -of the third and final day of the IAS Conference in Sydney. My name is Diane Havlir and I'm from University of California, San Francisco, and my co-chairs for the session this morning are professor David Penington from the University of Melbourne and Minister da Silva from Sri Lanka.

It's a real pleasure to introduce our first speaker, Dr. Annette Sohn. She'll be speaking about pediatric HIV. Dr. Sohn is an assistant professor in the Division of Pediatric and Infectious Diseases at the University of California, San Francisco, and is a Vietnam country representative for the UCSF Institute of Global Health.

Annette is based in Ho Chi Minh City, where she directs a pediatric HIV research program. Her projects there have included studies of diagnostic and monitoring and testing of HIV-exposed infants, stigma and discrimination experienced when accessing prevention of mother-to-child transmission services, contraception and reproductive health outcomes of HIV-positive women. She is an active member of amFAR's TREAT Asia Steering Committee, Pediatric Network and their Pediatric HIV Observational Database working group. It's a real pleasure to introduce you, Annette, and to hear your talk.

[Applause]

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ANNETTE SOHN, M.D.: Thank you, Diane, and I would like to thank the organizers for giving me the opportunity to provide an update on pediatric antiretroviral treatment issues.

Health care providers face similar challenges in treating children as for adults, including knowing when to start treatment, the optimal drugs to start with and when to switch regimens due to treatment failure. However, there are notable exceptions in regards to treatment coverage, the antiretroviral tools available for use in children and priorities for insuring a future that includes reaching adulthood.

Of the approximately 2.3 million children with HIV around the world, 780,000 are in need of antiretroviral treatment and 115,000 are receiving it. Although there was a 50-percent increase in coverage last year, only 15-percent of pediatric treatment need is being met. This is about half of overall global treatment coverage, and in sub-Saharan Africa, where almost 90-percent of the children with HIV live, children represent 14-percent of those need ART, but only 6-percent are those who receive it.

This graph shows the estimate of the total number of children in need of antiretrovirals from low and middle-income countries. Total numbers is on the left y-axis with those needing treatment in red and those receiving treatment in

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yellow. The green diamonds referenced on the white y-axis represent the percent treatment coverage in each region. For example, in East, South and Southeast Asia, the number in need of treatment is about 64,000 and the number on treatment is 13,000, representing 21-percent treatment coverage, whereas coverage in Latin America and the Caribbean is estimated at almost 70-percent. When Africa is added to the graph, the scale increases by one log. The number of children in need of antiretrovirals in Africa is almost 700,000 with about 85,000 covered. These numbers demonstrate the ongoing need for feasible strategies to prevent mother-to-child transmission and the importance of efforts to broaden treatment coverage.

This table provides a more detailed description of a few of the cohorts of children on treatment in Africa. Data from Baylor's [inaudible] program includes five sites. The Kids Art Link [misspelled?] collaboration includes nine treatment sites and the MTCT Plus network and these MSF data include 28 sites, also primarily in Africa. Current national data for a selected number of countries in South and Southeast Asia also are noted. For the cohorts where data was available, children were older, with a median age of about 5 to 8 years. One half to three-fourths were already severely immune deficient at treatment initiation. Except for children in the Kids Art Link Sites, 40-percent were on protease inhibitors.

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Almost all of the children in the other programs were on regimens including NNRTIs, but the percentage of children already on second-line regimens are higher. They range from less than 1-percent in the MSF cohort, to 5.3 in Kids Art Link and 10-percent in the Baylor cohorts. Similarly detailed regional data for South and Southeast Asia are lacking and the Treat Asia Observational - Pediatric Observational database, launched at this conference on Monday, will help facilitate better cross-regional comparisons in the future.

The goals of treatment in children must be balanced between halting the effects of HIV disease and the long-term effects of antiretrovirals on a developing child. We know that treatment reduces mortality, restores immune function and improves quality of life, and treatment is critical to preventing HIV encephalopathy and improving neurocognitive development. But although we want to maximally suppress viral replication to avoid treatment failure, pediatric providers must consider how to make limited salvage options last into adulthood. What is the future for those children who are already on second-line regimens at the age of 5? And when we start treating in early childhood, we know we will face having to manage drug-related toxicities, such as lipodystrophy and bone density loss in adolescents.

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In deciding when to start, our criteria are based not only on clinical and laboratory markers, but also on the social environment of the child and the family. Like in adults, the goal is to achieve an optimal balance of benefits and risks, but it is the quality of the collaborative relationship between medical providers and the family that will result in treatment success and adherence.

To better understand, let's start criteria where most relevant and resource limited setting, the three C's cohort, Three C's for Kids cohort collaboration examined laboratory values and growth as predictors of mortality and 2,500 children who participated in studies in Africa and Brazil. They found that CD4 levels were the strongest predictors of mortality.

In addition, malnutrition and anemia played key roles, sometimes predicting mortality in children with higher CD4 levels. Total lymphocyte count was revealed as a weak predictor in these children, which further challenges those countries to improve laboratories capacity and access to the most

When available, the CD4 level is the primary laboratory marker, used to determine when to start treatment. In 2006, WHO revised their CD4 thresholds to define severe immune deficiency based on the metanalysis of longitudinal data of almost 4,000 children in cohort studies and randomized trials

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in the U.S. and Europe. These thresholds [Break in recording 7:12 to 7:20] -of less than or equal to 5-percent in children over a year of age. It also emphasized the preference for using CD4 percentages over total counts in children under 5. However, data on optimal CD4 thresholds in resource limited settings are still needed. Such data can help us determine how late is too late to start treatment in children.

The Kids Art Link Collaboration conducted a metanalysis of 12,000 children from clinical trials in Africa that showed increased mortality among children who are severally immune deficient when they started therapy. At six months after treatment, 7.8-percent of children with severe immune deficiency died, as compared with 1.8-percent for those who CD4 levels were above the age-related threshold.

A study presented at the conference from Thailand, proposed to use immune recovery as a marker for treatment success, using the time to achieve a CD4 of over 25-percent as the target goal. Two-thirds of children in their cohort reached the target, median a 72 weeks after starting treatment. Although the median in CD4, in this cohort, was low, the CD4 recovery potential of Thai children on NNRTI treatment was comparable to other Western cohorts at the same baseline CD4 levels. Those with baseline CD4 of less than or equal to 5-percent, were the least likely to reach the target.

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From these non-randomized studies, it is clear that response to treatment is better when children are started before they develop severe immune deficiency. In addition, we now have randomized data to support that early treatment in infancy is superior to deferring treatment.

In the Share study in South Africa, infants, with what are considered to be high CD4 levels were randomized to start treatment at between 6 to 12 weeks of age, or defer until reaching CD4 criteria. During an interim analysis, investigators found a 75-percent reduction in mortality for the early treatment group and the child's data safety and monitoring board closed the deferral arm of the study. Further details will be presented during the Track B Late breaker session this afternoon.

The Predict study, currently enrolling in Cambodia and Thailand, is a randomized trial of children with moderate immune failure. We'll examine how starting treatment in this middle CD4 range compares with waiting until they reach severe immune deficiency. These studies will provide much needed evidence for determining optimal age and CD4 thresholds to start antiretroviral treatment in resource-limited settings.

The question of what antiretrovirals to start with is largely answered by drug availability and local and national guidelines. Of the standard WHO first-line regimen option for

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children, the most common combination is that of D4T, 3TC and Neviraprine. In December of last year, WHO and UNICEF published a pediatric HIV management guideline that formalized the recommendation to use AZT rather than D4T in children without anemia. This recommendation was based in part on data such as that presented at this conference from Thailand where 57 percent of children taking D4T in a cohort in Shag Ma [misspelled?] had lipodystrophy at 144 weeks of treatment.

But it is really the limited range of available antiretrovirals that drives what drugs are used in children. Liquid suspensions that allow for exact milligram per kilogram dosing are expensive, hard to ship, distribute and store. Consequently, most of the world has been forced to split adult tablets into child size pieces. This has been an effective alternative, as shown by recent MSF and Thia studies demonstrating increased survival and biologic suppression. However, splitting tablets into anything less than half risks under- or overdosing. That means that children who require smaller sized pieces, may be under dosed, leading to inadequate drug levels and risk of treatment failure. There also are the added barriers of how having to cut unscored tablets and then administer bitter medicines to children who may be unable to swallow pills.

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What advocates for pediatric HIV treatment have been arguing, is the need to develop generic and easy to use pediatric formulations. As of last month, this is the list of first-line generic, antiretrovirals in a pediatric dosing range that have been tentatively approved by the U.S. Food and Drug Administration and are pre-qualified by the WHO. The table shows each drug option by concentration, company and approval status. FDA special tentative approval category makes these drugs eligible for purchase by the U.S. PERFAR program. Of the 51 first-line generic options that the FDA has approved, 13 are in a pediatric dosing range. Of the 85 WHO pre-qualified options, 18 are in a pediatric dosing range. None of these are pediatric fixed dose combinations of either two or three drugs. Fixed dosed combinations have been the key to simplifying antiretroviral therapy around the world, at yet. Only recently have pediatric FDC's become available. There currently are four pediatric FDCs under WHO review, all of the same regimen of D4T, 3Ct and Neviraprine. They reflect the higher Nevirapine to NRTI ratios necessary to achieve adequate drug levels in children. There are two lower dose versions that can be used in infants, as small as 3 kilograms, and two higher-dose versions.

As you can see, in the photo of one of the Ranbaxy Products under review, there are scored, making them easier to

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cut and they are disbursable, meaning they quickly dissolve in water. After negotiations by the Clinton HIV/AIDS Initiative last fall, the price for treating a child with pediatric FDCs was lowered to \$60 U.S. dollars per child per year, or less.

Pharmacokinetic studies of some of these drugs have been conducted in Zambia as part of the Chapus 1 [misspelled?] trial. This substudy completed data collection last month, but an interim analyses present at Kroy [misspelled?] demonstrated excellent results. Sixty-four children at a median age of 7 years, most with moderate to severe malnutrition, were given standardized doses of the pedium [misspelled?] FDC's made by Sipla [misspelled?]. Investigators found that D4T and 3CT drug levels were therapeutic and comparable to adults and more importantly, Nevirapine levels were consistently therapeutic across all weight ranges. Only 7-percent of children had sub-therapeutic trough levels, compared with the groups previous studies using adult FDCs where up to 21-percent of children had sub-therapeutic troughs and there was no difference in drug clearance across the age and weight groups.

This graph describes some of the Trappist [misspelled?] 1 results, with time after intake on the x-axis and mean nevirapine levels on the y-axis. You can see that that mean levels of all of the weight ranges over time were above the

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cutoff and none of the children had to stop treatment due to toxicity.

Although these data are encouraging, we still need more pediatric antiretroviral formulations. WHO has an abstract at this conference, which is a, sort of, wish list for what we should be advocating for. Children clearly need a wider range of drugs and dual and triple combinations.

To put this into perspective, remember that the first FDC was a dual combination of AZT and 3CT, known as Combo gear [misspelled?], which was approved by the U.S. FDA in 1997. It is 10 years later and we are still waiting for approval of our first pediatric FDC.

Drug formulations are important aspects of providing pediatric treatment, because providers and families need these drugs to be easier to deliver to children. Scoring dissolvable tablets, or packaging granules into sachets to give to infants may seem like obvious solutions to simplifying ARV delivery, but they have seldom or never been product features.

Complicating our selection of first-line regimens is the growing number of studies revealing baseline resistance in children. This table shows data from three studies in the U.S., where children had standard gyno-type [misspelled?] testing before starting therapy. The yellow columns represent cohort of infants, infected during two periods of time in New

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York State. Within two years, the rate of having any resistant mutations went from 12- to 19-percent, an increase of 58-percent. The far white column, represents data from a multisite cohort of the US PACTG1030 study. Twenty-four-percent of these children had pre-therapy resistance mutations. For all three studies, perinatal antiretroviral exposure was not necessarily associated with the mutations that were indentified. Although these studies involved small numbers of children, this trend toward higher rates of resistance lead to the recommendation to obtain routine pre-therapy gyno-tying in the 2006 U.S. pediatric ARV therapy guidelines. This concern over baseline and early resistance has been raised in other countries as well. The Bots Wa Msai Trial [misspelled?] followed 30 infants. Half of whom received perinatal, single dose nevirapine and half who received placebo. All were started on first-line therapy with nevirapine at a median age of 8 ½ months. After six months of therapy, 77-percent of those in the nevirapine group had virologic failure, compared with 9-percent of those who had received placebo.

Another study presented at this conference, from Argentina, looked at the early mergence of resistance among a group of children on either nevirapine or nelfinavir. Nienty-percent of these children had no perinatal ARV exposure. By median of five and a half months of therapy, 70-percent had

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already developed at least one primary resistance mutation of those listed at the bottom of the table of the slide.

These data emphasize the importance of understanding how resistance is emerging in children, in order for us to maximize regimen durability and prevent treatment failure. When treatment failure does happen, regimens need to be switched, but how we define failure depends on available laboratory resources. Although we have clinical and CD4 related switch guidelines, we are far less experienced on how to use viral loads to make the decision of when to switch. PenPac 1 [misspelled?] is an ongoing collaboration between the European Penta [misspelled?] network and the U.S. PACTG, that will look at NNRTI versus PI regimens and switch strategies based on viral load.

Whether or not we have access to laboratory testing to guide, any decision to start or change ARV regimens is made with ultimate goal, to plan out a lifetime of therapy. Sequencing regimens into adulthood requires careful consideration and as much clinical and laboratory data as possible. Data from the US pediatric spectrum of disease study emphasized the serious consequences of multiple regimen switches. Researchers examined the triple therapy regimens in over 15,000 children and found that in 1997, 4-percent were already on their third or greater regimen. In 2001, that

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increased to 17-percent. Of grave concern was that the regimens durability, as defined as the length of time the child's taking a given regimen, started out at a median of 13 months for the first line and then fell to seven months for the third line regimen. In some of our countries, we do not even have a second-line regimen to offer.

So, with few switch options, our focus should be on limiting resistance to delay treatment failure. Of the many approaches to delay failure, I will discuss three specific areas, including the need to support adherence in the home, using the best and most potent available drugs and regimens and the importance of anticipating resistance on a global level.

Even the most efficient medical care will falter if the adherence in the home is poor. In addition to simplifying ARV delivery, we can work towards mobilizing resources, to improve the social stability of families living with HIV. Providers and donor should recognize the impact that poverty, death, orphan hood, stigma and violence, have on a families ability to care for their children. Comprehensive pediatric care and treatment programs can address these issues in parallel with clinical interventions. As our population ages, we can encourage caretakers to begin disclosure discussions with their children, as soon as this is culturally and developmentally appropriate. There were presentations at this conference from

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Thailand, Uganda and Hong Kong, emphasizing how disclosure can improve adherence in the home by involving children in the decision making process. This is especially relevant as children transmission into adolescents and is expected to take on more responsibility for their care.

Another priority is to provide the best drugs for children, which may not necessarily be the same as the best drugs for adults. Data from non-randomized studies, have questioned the potency of nevirapine in children. In a cohort in Thailand, 64-percent of those on nevirapine had virologic suppression at 72 weeks, as compared with 91-percent of the children on Efavirenz.

In a study in Uganda, the use of nevirapine predicted virologic failure at 12 months of treatment. This concern over the relative potency of nevirapine also was noted in a comparison between NNRTI's and PI's in South Africa. In that study, half of the children were on NNRTIs, of whom 90-percent were receiving nevirapine. Forty-three-percent of the children on NNRTIs had virologic suppression, compared to 60-percent of the children on a PI-based regimen at 24 months of follow up. The immediate impact of these differences remains unclear. Although there was a small CD4 benefit, when using efavirenz in the Thai cohort, there was no difference in CD4 between NNRTI and PI-based regimens in the South African cohort.

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Randomized data in children are needed, but these studies have raised concerns over the long-term impact of virologic failure on resistance.

We have the opportunity to delay treatment failure by actively participating the resistance we know is immerging. This includes providing greater access to laboratory monitoring to optimize selection of future regimens and switch decisions whenever feasible. This translates into CD4 testing, then viral load and later to gyno-typing. With each progressive test, comes greater expense and requirements for sophisticated laboratory capacity. Dry blood-spot samples have been successfully studied for both viral load and genotyping, and would allow more sites access to these tests and reduce overall costs. Much of the genotyping being done in resource-limited setting is still through ongoing clinical trials. These and other research studies are critical to helping us learn how to obtain better treatment outcomes in children. Knowing how long it takes to develop and approve generic first-line pediatric formulations, we should be broading second- and thrid-line antiretroviral availablity.

In summarizing some of the current issues in pediatric treatment, we first remember that although the gap is narrowing, only 15-percent of the children who need antiretrovirals are receiving them. To expand treatment

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coverage, we require more and better generic pediatric antiretrovirals that are both potent and limit long-term side effects. We are learning how best to use these drugs through research, seeking to identify optimal times to initiate treatment and switch regimens. If we hope to be able to offer our HIV positive children a lifetime into adulthood, we must delay treatment failure. This includes recognizing that social support for a child's family is as much a priority as the clinical interventions that we offer.

I would like to acknowledge the many people who generously contributed their experience, data and time to this presentation, in particular, Gina Watch [misspelled?] and Taoni [misspelled?] of Thailand, and Lyn Mothoson [misspelled?] of the U.S. Thank you very much.

[Applause]

DAVID PENINGTON, A.C., M.A., D.M., B.Ch., F.R.C.P., F.R.A.C.P., F.R.C.P.A.: I'm David Penington and it's my pleasure to introduce Dr. Ben Berkhout to address us on "Outpacing HIV: Viral fitness, escape routes and resistance patterns." Ben Berkhout commenced his career at Leiden University, where his PhD was in the field of RNA studies with RNA bacteriophages and then he went to Harvard Medical School at Dana Farber Cancer Institute and spent three years of

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postdoctoral studies immersed in the molecular biology of the immune system, which a continuing fascination with RNA.

The next stage in his career was two years character forming and highly productive at the National Institute of Health, studying HIV1. Still, with a great fascination for RNA, before returning to an appointment at the University of Amsterdam in Holland, from which he has continued to be an extraordinarily productive investigator in the field of experimental virology but still pursuing the imaginations of RNA systems, both in viral evolution and the interaction with the immune system. It's with great pleasure that I invite Ben to now address us.

[Applause]

BEN BERKHOUT, Ph.D.: Thank you. Good morning. I would like to thank the organizers, in particular, Sharon Lewen [misspelled?] and Damien Pursell [misspelled?] for inviting me. I'm called to present quite a few subjects, but the common sense, in case you start wondering about it, the common sense will be virus evolution. I hope to convince you that the study of HIV1 evolution is fascinating, sometimes quite puzzling, but many times rewarding and providing new insight into complex mechanisms. In a sense, our research in Amsterdam is not [inaudible] driven, but driven by the virus. We just try to

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follow the virus on this evolution path and try to understand what it's actually doing.

Let's start with some of the basics. As we all know, and you can find in the textbooks, HIV1 has enormous genetic flexibility, although, I guess immediately, you have to bring death into perspective. It certainly has a high mutation rate and we usually blame the RT, the reverse [inaudible] enzyme for that. That's probably correct, although in some cases, I think we cannot exclude the possibility that RNA perliminates too, [inaudible] enzyme that makes new viral transcripts is actually also introducing RNA genomes, this point mutation that end up in the [inaudible]. Then you have this restriction factor Ablebec [misspelled?]. Of course, we have learned that the restriction factor, it gives G2A hypermutation, and by doing so, it activates the virus, but there may be situations that actually the G2A introduced by Ablebec also help a little bit in the evolution of HIV1. Of course, this virus has a relatively high replication rate, again, many RNA viruses will do it similarly or even more quick and it has a fairly large population size. Of course, the special thing about HIV1 in data aspect, it's joined by Hepatitis C virus, is that it's causing a persistent infection, such that you really get the time to build a significant [inaudible].

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Now let's start with a seemingly very simple evolution scheme that is a direct resistance against the nucleus side analog 3C. Because for that, only need one amino acid change and it always turns out to be the same amemia acid change. The methicillin at position 184 is mutated into a fayline [misspelled?]. Of course, we know that if actually, you look carefully in patients, and if you look early, that you will sometimes see transiently another various methicillin 184 in isolation and we will come back to that in some of the later slides. We know why, in this case, you always see this one unique amino acid substitution, because what you need is a very precise and specific substitution that can prevent the incorporation of the 3DC leukocyte analog.

If you zoom in the catalytic core of the reverse transcriptase enzyme here in the palm region, the structure of a natural building block, DC3P and let us compare it with the direct 3DC and it has a different conformation, as you can see here. The ribos ring is sticking out and over here, the yellow, you see the space filling [inaudible]. What the virus is going, it's changing a methicil into a phallin [misspelled?] and as shown here, clearly, there's [inaudible] between the phallin and this leukocyte analog, and of course [inaudible] is a natural leukocyte. You can imagine that this is a very precise happening if you have a substitution with a very small

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amino acids, it will cause resistance if you put in an amino acid that's too thick, you will start to interfere with incorporation of the natural building block.

So, let's move to AZT. The situation is very much more complex, you end up with 4 or 5 or perhaps even more mutations and if you look in the individual patients, you will see that the order of appearance of some of these mutations will differ a little bit. Five of amino acid substitutions that you select here or there may differ a little bit and that already makes it very complex. But, there's probably much more to it, because these mutations are the ones that we still see in most of the patients and are in the list of the IAS mutations that we have to screen for. But, actually, if you go into databases, you will see that there will be much more. Until recently, it's that we looked at the known resistance positions in the RT enzyme and we looked for alternative amino acid changes. And we got some very big surprises.

According to the IES list at position 75, you have to look for their substitution, phallin to isolation. If you see something else, it's usually not [inaudible], but in fact, in our Amsterdam database, and it thus confirms by looking at the Stanford database, we saw quite a lot of methicillin substitutions. So, what we did is, we put the number of isolations that we saw 100-percent and you actually see that we

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see six times more methicillin. Both in our database and in Stanford. So I think it's about time to update the database.

Also, our alternative substitutions that are quite significant. This is just another example but we have seen similar things at 50 positions. Here, usually you see a [inaudible] acid change that changes to [inaudible] other amino acids are actually quite present. So it's likely that there are many more with the resistance-associated mutations and some recent publications from other groups using database mining have shown that, indeed, these mutations are probably there. They may directly add to the direct resistance, or they maybe compensatory mutations.

Let's move to a more [inaudible] therapy and a theme that was already introduced yesterday by [inaudible], so I won't do that, but I would like to use the RNA interference therapy protocol, because again, because we got some interesting viruses escape routes. Here, you see the RNA [inaudible] that you can program to actually start affecting the HIV RNA genome and if that occurs, you are slicing your HIV1 RNA. Now, it's a very nice method, it's beautifully sequence-specific but that, at the same time, of course, you'll create trouble because if it is very sequence-specific, the virus will probably start to change its sequence and you may

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expect that that will occur exactly in the sequences that are targeted.

Let's see how the virus escapes in this case, from an inhibitor that's looking at protease sequences in the preliminary [inaudible]. Over here, we looked at 28 escape cultures and you will see that in this target sequence, the mutations can be found almost everywhere. Yeah? Actually, if you look carefully, you'll see this [inaudible] mutation and then two positions, no mutation. Here's a very popular mutation that we see eight times, again two positions nothing, and so on. The people who are trained in molecular biology will immediately recognize that these are probably silent mutations that are selected. So the virus becomes resistant by mutating the target, but it doesn't want to mutate the protease enzyme. Because the numbers are quite striking, 25 out of the 28 cultures that we analyzed, have a silent codal change.

Here, we did similar studies with other inhibitors, infibural inhibitors because if you use them combined, you will actually not get any escape anymore, at least [inaudible] results obtained four different infibural inhibitors. As you can see, mutations appear everywhere. There seems to be a hot spot here, around position 8, not around here at position 18. What's also striking is that that both ends of the target sequence are actually not mutated. Probably because they do

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not provide a high level of resistance because they do not significantly destabilize the duplex between the targets and the SIRNA.

If you do many evolution studies, and this is actually the reason why we do so many, you sometimes come across exotic, puzzling mutations. This one certainly is most puzzling because here, you actually have the target sequence, the gray box, and then, all of a sudden, we saw a mutation that appeared seven nucleotides upstream - not in the target, but upstream. That didn't make sense. But you already heard that my topic is RNA structures, so RNA structures will actually give you the explanation. What the fibers are trying to do, it's mutating the G into an A, but doing so, it breaks a [inaudible] pair, if this happens, structure is destabilized and the RNA switches into another conformation as illustrated here. The target sequence is here and especially this [inaudible] end of the target sequence over here is [inaudible] and here, it's totally free. We know that [inaudible] is really the business end, that's where the SIRNA starts to bind and apparently that's possible here, but over here, the structure makes the RNA genome insensitive resistant to RNA [inaudible].

Okay, thus far we have been talking about [inaudible] evaluation. We have focused on selection, drug resistance, the RNAI resistance, what we usually ignore is the first and

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important step of evolution that many of these variants, they have to be made. And that's not all equal. The second step of evolution is to select for the variants which improve fitness. This is basically the Darwinian evolution, even though he did not know anything about genes, but so he had the concept perfectly right.

The mutational vise of HIV1 is very decisive in many evolution scenarios and, of course, it also forms a successful HAART because if you combine [inaudible] you, the fibers will be required to come up with four, five, six, seven drug-resistant mutations at once and that's - apparently, it cannot do.

What that means for the differences in mutations, well, there are some very well-known rules, and simple ones. Of course, a single mutation is much for easy than a double mutation and so on. This already means that sometimes, certain amino acid substitutions are not within reach of the virus. For instance, AZT, this is a mutation that we frequently see, but it's a very difficult one and that makes that virus will not show this mutation early on AZT therapy. The fibers really have to work on it for many, many months to come up with this, what we call a double transition transversions in this [inaudible].

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The second rule is that transitions pure in changes, are more frequently seen than transversions, that's also sort of a general rule in molecular biology.

And then there's one special mutation that always sticks out and we still don't really have a clue what's going on and that is the G2A mutation. It's always number one in drug resistance, in RNAI resistance [inaudible]. We expect it and as I said, we still don't know, but this is a special property of the reverse, it gives us time to introduce a lot of G2A's or perhaps, as we suggested recently in an AIDS commentary, or perhaps this may be a role of the ablebec enzyme because the signature of that enzyme is to change Gs into As.

Let's go back to this very simple starting point, the change from methicillin to phallin in 3DC resistance. As I told you, there actually is, in patients, there is sometimes an attention form of isolation, and I think we totally understand this sort of mixed pattern. First you see the isolation change and the later on the phallin. It has to do with these two steps of virus evolution. Because, if you look at the [inaudible], this methicillin to isolation, the codal change is a G to A, that's the number one that that's why [inaudible] you see this initially in some of the patients, but then, this is a little bit more difficult, lots, lots more difficult. It's an A2D, but actually, this enzyme and this virus is doing much

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better, its [inaudible] than the isolation variance. So, the generation of the mutant prefers isolation, but if you then compare the two viruses or the two RT enzymes, you'll see that phallin [inaudible] is the better solution. Here, I just plotted some of our old data where we score for the RT enzyme activity of the [inaudible], the phallin, as you see, is a little bit lower so a lower fitness of the fibers is expected and that can also be measured, but isolation is even further down in activity.

In the lap, you can, of course, play around with these types of evolution schemes and we try to highlight this step number one, so not a selection, but it's the generation of the mutants, and you can do so if you start to dilute your sample during the selection of drug resistance. You do that in a 96 [inaudible] and the key issue here is that once a drug resistant mutant is selected, it will be in isolation, it will be in both of these realms, but it will not have to compete with any of the other variance. Yes. So limiting solution selection of drug resistance.

If you do this experiment, indeed, you get the respected answer that you see many more isolation because of G to A and phallin change, so that's nice, that confirms our idea. Actually there was a big surprise if you do this, because all of the sudden, you see a third variance,

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[inaudible] and we have never seen that in patients and actually we have never seen it in bulk evolution cultures either.

Now, it turns out that if you go down here, that threonine, again, is a relatively simple change, it's the middle position mutating to a C, but that is still more difficult in isolation, so we don't see it that frequently, why don't we see it in nature? Well, that is because this represents a very poor RT enzyme and a very unfit virus. It can only pop up in this clonal selection scheme because it doesn't have to compete with any of the other variances.

Okay, we know of many more complex evolutions scenarios for instance in the selection of protease drug resistance mutations and I sort of plotted this five step process, where, of course, first you're going to inhibit the [inaudible] virus, then you'll see the appearance of protease, drug resistance mutations and some of them actually, they'll provide a partial loss of enzyme function and partial loss of viral fitness, then we see the second way of mutations. Compensatory changes in the protease enzyme to be stored and [inaudible] function, and if that is not good enough, the fibers will think about changing the cleavage side of the protease, the cleavage side that you will find in the [inaudible]. Then, if that is not enough, mutations have been [inaudible] in the frame shift

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signal that coordinates the expression of the proteases protein and apparently, more protease can be made, in case you still, still the virus is left with a wimpy preliminary enzyme.

It seems that the evolution capacity is endless and [inaudible] will be that you end up with a very fit and a very highly resistant virus. If the question is asked, can we drive HIV1 to reduce fitness and low [inaudible], I would say that is very likely.

Let's move to another very surprising evolution scheme that we came across in a study we did together with [inaudible] on one of the first patients that was treated with [inaudible], this is a relatively old story, but, very surprising. This is a patient that failed on IT and protease inhibitors and [inaudible] his initial success on D20 therapy, so we see a drop in the viral loads and then over the months coming, you see that there's an increase in the viral loads suggesting that the virus has become resistant. We did some initial sequencing and we saw interesting mutations, but it was very complex, so we decided to do clonal sequencing of different time points from samples of this patients and we ended up, actually before we could make the complete picture, we ended up doing [inaudible] sequences. This sort of summarizes what's happening in this patient.

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This is the pre-therapy sample; you see one box that means it's a homogeneous population of viral type virus. Then, upon therapy, and these are the time blocks, you see that a total population moves to the variation as a mutation here in the, what we call the gift [misspelled?] motif and that is the known position in HIV1 that's causing resistance to D20. The virus is not happy yet because the same code has changed again to change the [inaudible] into a [inaudible]. About the same time the fiber resist found a different evolution route, again looking at the same amino acid position, but a different codal change, in which an LNL is introduced and then things go fast because this mutant seems to win, but only after being combined with a second mutation in the HIV1, we will have another loop here for HIV1 and HIV2 [inaudible]. Here we have a double mutant, the purple box here, and that starts to dominate the populations, and what you would expect is that here, over here coming from left to right, there should be an increase in [inaudible] resistance and that's actually what we measured.

So, this far, it's not so surprising. There was one other point that made us quite interested in the samples, because when the patient stops therapy, we saw a very abrupt and sudden disappearance of this double mutant. Later on, we understood this phenomenon because when we made all these different mutants and then double mutants and we tested them in

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the laboratory, it turned out that all these mutants are resistant including the double one, the double mutant is actually dependent on the 220 peptides. It is resistant, but it cannot replicate anymore in the absence of the peptides. That, of course, explains why it disappeared immediately once therapy was stopped.

So, how does that work, dependence? Well, of course, we thought about that a little bit and this is sort of the most simple scheme of what happening in the envelope. You see the conformational change that drives membrane fusion and as you probably all know, T20 is a mimic of HR2 and therefore, it already binds to HR1 and it builds defense this conformational change.

Resistance to T20 is also fairly easy. We have seen, and many of our studies have seen those before, the mutations in HR1, that cause resistance, and what happening is that you actually are mutating the binding site 2020 so that it cannot efficaciously bind in here and therefore the fibers can still undercoat the conformational change in the presence of the [inaudible].

How does dependence work? That, of course, is the key question. Well, we did a little of experiments and, of course, some thinking, and this is our current model, and I must say we have done additional experiments and it still holds. The

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[inaudible] dependence is seen as a double mutant because as a mutation in HR1 and HR2, if you make this [inaudible] protein, we see it is highly hyperfusogenic, it's highly active and it seems that it [inaudible] too early and that's why the fibers itself is dead because this is not an [inaudible], this is one time reaction. If you start with this scenario, you perhaps can imagine that now, with these mutants, the inhibitor comes to the rescue. The inhibitor is doing the same thing as it was doing here, it tries to bind in there and to prevent the conformational switch. That, in this scenario is very beneficial. Here's it's inhibitory, over here, it becomes beneficial and that's why we think this variant can only survive in the presents of T20.

Okay, if you talk about HIV1 evolution, we also should mention the topic of recantation. It's [inaudible] for us and you keep missing of genotypes, of course we know that this is happening in nature because we see all these [inaudible] genomes and that actually means that somewhere, people should have been dually infected, super infected, or co-infected with two viral strains. The key question that we have to answer then is, how can you detect these super infections? For that, I know have a very simple solution, that's based on the [inaudible] of HIV genotyping assay.

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This is some data from the Amsterdam database that we have. Basically what we do is the sequences that look back because you have a lot of these degenerates IUB basecoats, you don't see G, A, C or T, but you see, for instance, an R, [inaudible]. In the past we used to throw these bad sequences in the garbage can, now we look more carefully at them. If you see a lot of these IUB, IBU, I don't know what it is, IUB I guess, if you see a lot of these codes, for instance, more than 34 and you are sequencing 330 codets or about 1,000 necleocytes, then we found out that in these samples, 43 percent do infections. If you come to higher IUB coats, actually the percentage of finding dual infections even increases. If you go to the samples with the highest IUB codes, you will most likely find coinfection with different subtypes. Although dual infections are still there, I think this is a very beautiful and easy [inaudible] genotyping, easy methods to use.

Let me address one more topic before I end, and this is a basic science topic. It has to do with splicing of HIV1 and do you always have these complex slides because HIV1 makes 40 different spliced [inaudible] and it's not just that but ratio for all of them needs to be coordinated. So splicing is really asking for a lot of complex calculation and we know that the HIV1 was [inaudible], was spiced [inaudible]. All these splice

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have in common is the use of the splice donor sequence over here. I think we have some new information on the important of [inaudible] structure moleculating this motif. We came across this and then we made some mutations that are shown here, where we here, look at the [inaudible] type spliced owner sequence, it's situated on top of this structure, and these, these stabilize, these happen to change just a little bit [inaudible], we saw that the virus is fooling that. That's nice, but for us usually, then the experiment only starts because if you have a death virus, we try to select for escape mutants and they paint a very interesting picture.

They come in two categories. The first category is shown on top. We see that the fibers select for mutations, additional mutations in this [inaudible] structure to open up the thing again, so it doesn't like the [inaudible] to be closed. The second category is even more intriguing because it sticks to the mutant [inaudible] but it selects one point mutation in [inaudible] sequences. Initially we didn't understand what was happening and now it turns out that a new spliced donor site is recreated over here. This one is probably not accessible anymore due to the structure and the virus creates a new one and we proved that [inaudible]. We actually think that in the [inaudible] type virus RNA structure

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may also play an important role in down [inaudible] the activity of the spliced donor sights.

I'm going to skip my vaccine part of the talk and I'd just like to thank the co-workers in the fields, in this picture according to [inaudible] and I thank you for your attention.

[Applause]

NIMAL SIRIPALA DA SILVA: My colleagues and the speakers at the head table, [inaudible], ladies and gentlemen, I have been [inaudible] with the pleasant task of introducing the last speaker of this morning's session, Dr. Nancy Padian. She will speak to us on biomedical HIV prevention, [inaudible] challenges and [inaudible] directions. Dr. Nancy Padian is director of international programs at UCSF AIDS Research Institute, co-director of UCSF Center for Reproductive Health Research and Policy and a professor in UCSF's Department of Obstetrics, Gynecology and Reproductive Sciences.

Dr. Padian has spent the last 17 years developing and directing a range of domestic and international research and intervention projects on sexually transmitted infections, HIV and contraception in high-risk populations.

In 1994, she co-founded the UZ-UCSF Collaborative Research Program in Women's Health in Zimbabwe. Her current research focuses largely on developing and evaluating the

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efficacy of female-controlled physical and barrier contraceptive methods that might decrease susceptibility to HIV and other sexually transmitted infections and alternative strategies for fostering young women's economic independence , thus reducing their susceptibility to HIV, STIs and unwanted pregnancies.

In Zimbabwe, she recently completed a study on the risk associated with use of intravaginal preparations and will also test microbicides as part of HIVNET international prevention network. Dr. Nancy, the floor is yours.

NANCY PADIAN, Ph.D.: Thank you, Minister, than you conference organizers, special thank you to Getter Range [misspelled?] and John Kaldor for inviting me today, and thank you to those people who opted to stay for this rather than the 3D version of Harry Potter.

Today, I am going to be reviewing challenges and status having to do with HIV prevention. What I'm going to do specifically is begin with a very brief overview of particular biomedical HIV prevention and specifically HSV2 suppression, prep, microbisides and female-inititated barrier methods. An issue came up in another prevention session about the lack of focus on IDU, which I think is a valid point, but this talk was put together really, with the focus on hyperendemic countries in Southern Africa. Then, after that review, I will talk a

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little bit about the results from my trial on cervical barriers, the methods for improving reproductive health in Africa and use those results as illustrative of methodological challenges faced by many prevention trials and then hopefully, I'll give you some recommendations that I have thought of for future prevention research.

This is just a nicer slide showing you again what I'll be talking about. Prep, microbicides, cervical barriers, that's my study, a brief talk about the female condoms and HSV2 suppression.

Beginning with HSV2, there are three trials, although the one at the bottom, the Welcome Trust 1, with 821 individuals done in Tanzania, Debbie Watson-Jones just presented the results of that yesterday, but the others are ongoing. One, Partners in Prevention, with other, this is a discordance couples study with over 3,000 couples being done in a variety of countries. The results are due to be out in 2008 or '09. The ongoing 039 which is funded by National Institutes of Allergy and Infectious Diseases at NIH, over 3,000 women, and I think the result, I'm not sure about this, are due to come out later this year, or shortly thereafter.

There have been many sessions pre-exposure prophylaxis here at this conference and I'm sure that, virtually, everyone in this audience knows more about it than I do, but there are,

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I should say that I limited these slides to larger trials, not phase one, there's many things in the pipeline. So these slides are not absolutely complete. But, there are four ongoing large studies looking at different antiretrovirals as pre-exposure prophylaxis, somebody asked me this yesterday, when are the first results coming out, and the one with male and female injection drug users with about 2,000 people, I believe the results are due out in 2008 in Thailand, looking at Truvada with men and women, over 1,000 people in Botswana, a year later, the Topavir study in men who have sex with men, again in 2009 and the NIH-funded study, looking at Truvada, also with men who have sex with men, due out in Peru and Ecuador in 2010.

Moving onto microbicides, and I'm only going to touch on this very briefly because later today there's a whole session, I think, on microbicides with Lutz Van Damm [misspelled?] from Conrad FHI is going to be reviewing these trials in far more detail, and again, I've limited this to the larger trials. There's ongoing and IH HPTI035 looking at buffer gel and Pro 2000, the mechanism of action there is you've got one that's an acid buffer and an entry inhibitor with over 3,000, in a variety of countries, mainly in Southern Africa, the results due out in 2009. The Conrad USAID funded [inaudible] trial being done in South Africa with 1-percent

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terniafer gel, as I'm sure you know, the second generation of microbicides and you will hear more about this later, really focus on use of specific viral targets and antiretrovirals, it's sort of vaginal prep. I believe that they've just begun enrolling with about 1,000 people in the [inaudible] study, run by the MDP, also looking at Pro2000 with two different dosage, almost 10,000 people in a variety of countries, results due out in 2009 and the Pop Counsel USA ID Gates funded Caragard [misspelled?] study with about 6,000 people and I think the results for this study will actually be coming out this November. I think.

Moving onto barriers, there are no trials that I know of going on with the female condom, but I think it's important when we think about these bi-medical preventions, to keep this on your radar screen. It's been around for a while, but what I really wanted to point out is that there is some novel new designs. There's the reality, the new condom, by the Female Health Condom, they're second generations, it's made out of a synthetic nitril product, Path has a nice female condom that sort of dissolves when you put it in as a causal. It's waiting for FDA clearance, and then you have the Ready [inaudible] latex condom, female condom, and I don't know, and I certainly could be wrong, but I don't think there are specific trials

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funded for HIV or expected, but I think this is something that we can't neglect.

I'm going to skip the cervical barriers because I will be talking about my own study in that context.

All of these studies, there's been well-documented challenges with doing prevention research. In particular, in some situations, there's been lower than anticipated HIV, high pregnancy rates, which have caused people to have to go off study, there's been challenges maintaining high retention rates, and difficulty, this has been a major theme at this conference, of sub optimal adherence. Again Lutz Van Damm, in her talk later today, I think will be talking about these in more detail, so I'm not going to focus on them.

As far as challenges with my own study, although I will talk a bit about adherence, I do want to just say one thing that came up in a session yesterday, and whether you can apply this standard of meeting adherence to treatment, something like 95-percent, 98-percent to prevention and I just think that it's very important as we view for as a challenge for prevention, that people taking a drug when they are sick is very different from convincing someone to do something when they're well. I think it's a much greater challenge for prevention, than it is for treatment.

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Moving to my study. Methods for improving reproductive health in Africa and, as I said, I'm just going to very briefly give you results, we have a late-breaker at 4:30 today and an article recently out in Lancet that go over much more detail, so I'm really focusing on this to discuss methodological challenges.

This was a study where we enrolled HIV-negative, sexually active, non pregnant women, who were randomized to a diaphragm and a lubricant plus condoms versus male condoms alone. But, all women, all of them in both groups received risk reduction counseling, free condoms, diagnosis and treatment of curable STIs, also voluntary counseling and testing. Women were followed quarterly for 12 to 24 months, depending upon when they enrolled in the study.

These are our main results. We had almost 5,000 women, if it was done in three sites. [inaudible] overall, the incidence rates for all sites was 4 and our relative hazard was flat. 1.05 and if you look at site specific hazards, although the incidence rates differ depending on the site, the results are pretty consistent, that we did not see an effect of being in the diaphragm arm.

What I'm going to talk to you today about, is why that might have been the case and what are some of the methodological challenges.

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Specifically, the challenges that I'll be focusing on are the difficulties of assessing multi-component interventions. That we're looking at the effect of these new technologies in the context of a highly effective, but maybe not sustainable prevention package. I'll come back to that. Also, we had a particular challenge of having an open-label design and I said I'm not going to focus in depth on adherence, but I am going to talk more about measurement of it and more generally, measurement of unobservable behavior that's a critical part of all these trials.

These, let me just start with the Caveat. These circles are really not to scale relative to what you did in the control and intervention arm, but I used them to make this point. As I said, we have a new technology, in our case it was the diaphragm in addition to an effective preventative package, condoms, STI treatment, counseling, versus, this same effective prevention package in the control arm. What you see is that when you are looking at an intervention, that you're [inaudible] has a modest effect, which is what we thought would be the case with the diaphragm, it's in fact, very challenging. Because, the yellow, would be our, sort of, ability to look at the diaphragm alone, because it's both part of the infective prevention package, its part of the intervention and then we're

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comparing that in the control. It's very challenging to detect an independent effect at a modest level prevention technology.

Then you might say, well, if that's the case and you have this effective prevention strategy, STI treatment, counseling, condoms, should we not just promote that. I think that that's a fair issue and an important questions, and so, what we have to think about is, is that sustainable beyond a course of this study and if yes, whose going to fund that. So, as one example of that, this was a Ron Roddy [misspelled?] study, the first author was Wong, can these sustainable high levels of condoms, is it sustainable beyond the duration of the study. They surveyed participants that were enrolled in their [inaudible] study in Cameron and 14 months after participation and you'll see, during the study the percent of acts with condoms was 82 to 84 percent, after the study, 57-percent. The percent of patients reporting consistent condom use, during the study 64- to 67-percent, after the study, 35 percent. Now, did the condom use drop or was it attributable to a change in reporting? Maybe they felt more comfortable, they might have over reported during the study and feel more honest after, or some variation. The point is we do need to think about sustaining these packages when the trials are over.

As I said before, we had the particular challenge of being an open-label design. People clearly knew that if they

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were in the diaphragm arm and if they weren't. An issue here is that the study arm can influence behavior and increase the possibility between diaphragms and condoms which I'll talk about in a minute.

I do think it's important though, not to simply throw out open-label designs. There are diffidently times when they need to be used. In our case, there was no placebo, so does that mean you can't touch something unless there's a placebo? We had no alternative. In many of the microbicide trials, there is a condom on the arms and I think it's important because it distinguishes the effect of the placebo from that of the study product. If the placebo had a protective effect, you would know, was it, say, the GOOP principal, you could separate that from the biological efficacy of the product itself, and also you could control for behavior change that might change with product use because you would know behavior without.

Nevertheless, in our study, as I said, we did have differential condom use and this is the proportion of women reporting condom use at last sex. This is the control arm and this is the diaphragm arm. As you can see, there are higher rates of condom use in the control arm. However, I need to [inaudible] a misconception that's actually, this meeting people have used this slide as being [inaudible] of behavioral disinhibition and as I understand that, that is actually not

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what we saw, because what's not on this slide, and I should have changed this slide if I knew that this misconception would occur, is that people, in both arms substantially increased their condom use from entry into this study, to the study itself, so there should be a line at the, from screening to here that goes from about 30-percent to almost 70-percent, more or less, but it was just that after people were randomized, they continued to increase in the condom arm and they even went up in the intervention arm, but everyone's condom use increased from entry during this study, it just increased more in the control arm. I should also say that in spite of this, there was no difference in HIV rates between the arms, so even though we did have differential condom use, there was no increased risk.

Why could there be reasons for differential use?

Therapeutic misconceptions, we were very clear, or tried to be clear that the diaphragm was of unknown efficacy, but it is possible and we are now analyzing some of these data, that in spite of that, people want something to work so badly that they might have misconstrued the fact that it might have been protective rather than it was unknown and we were testing this.

We also have some data indicating that the partner, male partner, may have been more likely to refuse a condom if he knew his partner was using a diaphragm, and also, there's

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the possibility as we analyze some of our quantitative data, that using two products in your vagina, might be less appealing than one, so the idea of having to have a condom when you already have a diaphragm from the prospective of the women may also be less appealing. I am getting back to how difficult it is to detect in effect of these modest level interventions. Steve Sabasce [misspelled?] and my group, a statistician did some post trial simulations, using the levels of diaphragm and condom use that we actually saw in the a study on the diaphragm arm and in the intervention arm, that's about 70-percent diaphragm use and I should say that when we designed this, we had [inaudible] that that would be over 80-percent. It was somewhat less than what we had projected. This is overall condom use in the intervention arm, 85 in the control; our power to detect a 33-percent reduction in incidence was only less than 25-percent and we underestimated how much condom use there would be and the adherence to the diaphragm and we thought that this was powered at 80-percent. Again, this is very challenging to detect and affect.

Another issue that we encountered, which is measurement of self report. Here I am telling you there's differential condom use, there's problem in adherence, but where people being honest about that, can we really measure that. Another challenge in all of these trials. It's clearly not a problem

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for vaccines or circumcisions or directly observed treatment, but right now, there is no directly observed prevention, a biological marker for condoms or test products, although there are things that are being looked into. We need novel methods of self report, in electronic diaries, or some computers, we had thought about a computer chip that you could put in a diaphragm that would register heat when it was in your body, but it's not only important, these issues of measure are not only important for adherence, but also because you have to measure confounders and in effect modifiers in all of these microbicide trials and other trials, you're going to want to do know, did people use condoms or not. You're going to want to know something about their sexual behavior, where they at risk, or not? Very important, not just for adherence.

So, what is our bottom line? Our bottom line in our study is in context of a comprehensive HIV package offered to all participants. We found no additional protective benefit against HIV infection from providing the diaphragm plus lubricant in the intervention arm. I also think it's important to see what we couldn't assess. Is this the death now for cervical barriers? We could not assess whether the cervix was the most vulnerable. We could only look at whether the people, if people didn't use the diaphragm, then you couldn't even assess that hypothesis. Keep in mind that one of the reasons

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we did this study is because Bob Bailey [misspelled?] did such a lovely talk yesterday, there are actually some commonalities between the cervix and the foreskin in terms of target cells and epithelium. We also couldn't assess whether a diaphragm was as good as a condom. We also couldn't assess whether other cervical barriers might have worked better. If we had a quarterly independence cervical barrier, or one that you could just leave in or that was smaller or something like that. We could not address that.

Finally, we don't know whether a barrier, cervical barrier would be more effective or promising when used with the microbicide.

What are some of the future direction that I think we can address some of these challenges. One is behavioral research in the context of biomedical interventions, new study designs and new research directions. This has come up in many sessions that I've attended and that is, the reality is there is not biomedical and interventions without behavior, they are integrally bond up together. In fact, you have to be able to understand adherence in order to look at efficacy and if you have an efficacious method, then your adherence would probably increase, so you have to be able to address these simultaneously, it's then just a question of effectiveness.

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Even to be able to evaluate the efficacy in a phase three trial, you have to consider behavior.

In my opinion, the need for basic behavioral research is as important as it is to develop new biomedical technologies. I say that, I think it needs to be done separately with great thought, not simply in a hurried way, tacked onto an existing protocol, but we really need to take stock of how we look at these issues. From my perspective, the two most important behavioral aspects, but there are lots more, are adherence, which I just talked about, and I think we need to understand the motivation, the context, when are women using diaphragms, is there partner there, and then Liz Montgomery from my group, gave a nice presentation on male partner involvement. Do we need to involve them or is that the worst thing you can do, and so on.

As I said before, I think an essential challenge in all of these trials is the measurement of self reported behavior. Not only for adherence, if we had directly observed prevention, as the international partners in microbicide is looking for that in a microbicide, but short of that, we need to deal with these adherence and measurement issues. Not just for adherence, but also just for confounding and in effect, modification. I think we need to think more deeply. What is the value added for individuals not to say what you want to

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hear. If people are participating in this study, they're getting many benefits. I think for myself, if I were participating in a study, and getting something verging on health care, would I tell the interviewer what I didn't want him to hear? How can we get around social desirability bias? There are other methodology work, like the order, the type of questions, and I think we need new innovative, confidential and anonymous tools for measurement.

Some future directions that we thought about. One is, could you do, before a study, a condom study. Sometimes you might call this run in where you actually do a condom intervention and then randomize people who are only non condom users, who have difficulty using condoms. Of course, you would continue to promote male condoms, but you're starting out know you are selecting people that are challenged. Or, would you only select people that are good adherers, although, this assumes that you can measure either one of these. There are also interesting post randomization strategies called adaptive designs. I know less about these where you can actually begin your study, look legitimately at your data, at our outcomes and the tweak your data, making directions to your power after you've already begun, if, for example, you find out that you could, in fact, find people who are better adherers after randomization.

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Another issue, and I sort of have a vision of people taking this slide and saying, Padian thinks that we shouldn't offer condoms to the intervention arm. That isn't, I'm not saying that, but I think we have to, at least consider our ability to detect and effect when we're doing such a great job with this effective prevention package. Again, not because that effective prevention package should be promoted in and of itself, it should, but for those women, still, who are unable to get their partner to use male condoms, we are still not able to address what they need and it's very difficult in these study designs to get that information that would be valuable for them. I think we need to just think deeply about what we do.

Also, I can't help but put in a pitch for this. This is moving, sort of, out of the realm of biomedical, this is a slide that was, sort of, my attempt, it's obviously not every intervention that there is, but the green line is money, so if you have vaccines, microbicides, barriers, you could have other things here, and then below, interventions for gender empowerment, the order goes that there's more money down the food chain, but the interesting thing I think, is that if a structural intervention, such as keeping girls in school, other kinds of strategies for empowerment would work, we could scale it up tomorrow. It's less appealing to funders and I have

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often thought, if we could just take a million dollars from here and put it here, we might see something. I think the issue is that there's really quite a bit of literature here, but it tends to be sort of, gray area literature. We need to look at these methods, evaluate them with the same order that we evaluate other biomedical preventions, if not in randomized control trials rigorously done observational studies, but it's essential that they be funded so they can be evaluated properly and I don't think we see enough of that.

What do we know. Just, this has come up many times. What we are promoting, we're holding our HIV prevention with these new methods to a very high bar. We know circumcision works, there is conflicting data, at least from the randomized controlled trials, there's other sources of data that STD treatment works to prevent HIV. Male condoms, I'm talking so much about how we should promote this, for which there are no randomized controls for HIV prevention. Our gold standard is not held to the same bar. Similarly with female condom, absence, reducing numbers of sexual partners and postponing sexual début. But, how do we decide what gets promoted?

I just want to end on a quote. Bradford Hill was one of the people, and very famous internationally know epidemiologist, did a lot of work on smoking and lung cancer and he said, "All scientific work is incomplete, whether it be

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observational or experimental. All scientific is liable to be upset or modified by advancing knowledge, that does not confer upon us the freedom to ignore the knowledge we already have or to postpone the action and that it appears to demand at a given time. We have to move forward, we have to move forward in prevention, things may change afterwards, again, I worry about the bar for HIV, the fact that we would look for a method that would be 100 percent efficacious for individuals is a bar that I don't think we hold for any other aspect of public health and we must do something. This is a raging epidemic."

I'd like to acknowledge my funders, The Bill and Melinda Gates foundation and all the numerous people, both in particular this presentation and all the mirror investigators again, will be presenting data on that later today at 4:30. This is just a montage of staff and participants from the study. Thank you.

DIANE HAVLIR, M.D.: Nancy, thank you for interesting, insightful and inspiration talk. Thanks to all of the speakers and my co-chairs and then you to the audience. This session is closed.

[END RECORDING]

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