4th IAS Conference on HIV Pathogenesis, Treatment and Prevention
Hepatitis Co-Infection – Not as Easy as A,B,C
International AIDS Society and Australasian Society for HIV Medicine
July 23, 2007

[1] kaisernetwork.org makes every effort to ensure the accuracy of written transcripts, but due to the nature of transcribing recorded material and the deadlines involved, they may contain errors or incomplete content. We apologize for any inaccuracies.

[START RECORDING]

GAIL MATHEWS M.B., CH.B., M.R.C.P.: Welcome firstly to the City of Sydney which after several weeks of rain has turned on a really glorious day for us today and secondly welcome to this one of the conferences first bridging sessions involving Hepatitis co-infection. And topic for this session today is really very timely and relevant given the recent advances in our understanding following pathogenesis viral resistance and the number potential new therapeutics agents we have for the management of Hepatitis B and Hepatitis C now.

Now there is a change to the program as stated in the original conference book. Unfortunately our first speaker has been unable to make it to Sydney but we have three excellent speakers and talks lined up still. And as a plus we may have a bit more time for questions and discussions as a result.

So I would like to hand over to my co-chair Jurgen Rockstroh who is going to introduce our first speaker.

JURGEN ROCKSTROH, M.D., M.P.H.: Thank you very much. Good afternoon. It’s really my great pleasure to announce the first speaker which is Marion Peters. Marion Peters is a professor of medicine at the University of California in San Francisco, is one of the world leading experts not only in co-infection but also in monoinfection of Hepatitis B and C. She will give a very intriguing and interesting talk on small

molecular inhibitors of Hepatitis C virus infection agents in the pipeline. Marion.

MARION PETERS, M.D.: Thank you very much, Jurgen and Gail. I would like to thank the organizers for inviting me to this beautiful country which happens to be mine. I was told that I wasn't allowed to talk about any drugs that are currently licensed for Hepatitis C and was made to give you an overview of drugs in the pipeline. Part of the problems you can see is there are a huge number of drugs in the pipeline so I'm going to really choose those that are closest in terms of Phase II/Phase III studies.

When we think about Hepatitis C you know it's a single open reading frame which produces structural and non-structural proteins, the core envelope proteins that are part of the varion and these important proteins to the right that are really critical for the production of virus using host as well. However, they are not a single line but the pathology of the proteins is such that there is a replication complex within a membranous web here including NS3 and NS4a as well as the RNA dependent DNA polymerase here. And when we talk about inhibitors we maybe just looking at one active site or inhibition of being able to produce an adequate replication complex. And I want to thank Charley Rice for the use of some of his slides.

So Hepatitis C is replicated in the cytoplasm. Its membrane associated as I have shown you. Large number of bioparticles produced per day; however they are very ill prone so individuals have a large number of quasi species. And because of this there is immense variability. This is increased with development of resistance mutations and combination therapy will be absolutely required for management of Hepatitis C especially looking at small molecules as well as perhaps multivalent vaccines.

So when we think about Hepatitis C infection and replication we have to think first of entry and uncoating of the virus with receptive mediated blinding. Then we think about translational and post translational processing which is why the soma producing a polyprotein then the production of structural proteins, assembly, membrane fusion, and secretion. So, there are a large number of opportunities to interfere with Hepatitis C replication.

Virus neutralizing antibodies have been unsuccessful as yet probably related to constant mutations. Receptor inhibition is an area of active interest however while CD81 scavenger receptor in cyclopentane as well as some other hosts proteins have been shown to be important in receptive binding and in internalization no one of these inhibition of, any one of these doesn’t decrease it’s replication but doesn’t stop it.

So, I think the story about receptor inhibition is not yet completely answered and is more research to be done.

Envelope glycan inhibition, E glycan is a very critical in envelope glycan protein E1 and E2 and they are essential for not only protein folding secretion assembly as well as antigenicity, receptor binding, and cell entry. So they will be important not only for entry into the cell but also for binding and excretion from the cell. They are in early stages of development as yet. And I think the recurring theme in my talk is many opportunities for inhibition of Hepatitis C have been failed small molecules or failed antibodies and what will happen over the next 12 months is really unclear. A couple of the drugs only failed last week or last month so if your drug has failed and I don't announce it today, I apologize.

Really I think the most mature replication inhibitors, first we think of translation, anti-transcriptase nucleosides are a fine prime on untranslated region are going into Phase II studies. Ribosomes have already been studied and failed but I'm told they are making a come back. IRIS inhibitors also undergoing early stage development and moving towards Phase II and SIRNA interference is really a very active both pre-clinical and Phase I studies. I won't go into any more detail of those but rather focus on protease inhibition which is probably the most mature area at present. The NS3 is a searing

protease of N-terminal and a C-terminal is a helicase. And it's responsible for cleavage of all of the non-structural proteins in trans. And associated with it is NS4a which is actually bound to the NS3 if you remember from my topologic diagram. And this binding of NS4a to the NS3 allows for the searing protease activity providing stability and localization.

You can see in this ribbon diagram the substrate binding pocket and the NS4a outlined in blue. I have lots of pictures if you don't see that one. Here is another one. So the NS3 protein has not just the active binding sight, here the serum protease substrate binding site but multiple other sights as potential targets for antivirals.

The first small molecule was BLIN2061 which was most successful in genotype I inhibition but there was cytotoxicity in animals and it was withdrawn. The VX950 molecule is another serum protease inhibitor, a pethidine mimetic inhibitor of NS3, it's orally viral available. At present it requires eight hourly, not TID dosing. It provides a decrease of two logs in HCV RNA. Has significant side effects and you will hear that also throughout my talk, drugs that didn't fall off the cliff from side effects. They all have significant side effects but then again so does interferon and ritonavir so they are in excellent company.

The side effects of the VX950 rash being the most common and quite significant requiring dose reduction in some individuals, GI upsets nausea, diarrhea, and anemia. Well this resistance variance are selected rapidly, however after cessation of drug you see a return to wild type within about three to seven months. I will show you some data on that.

The early studies of this drug known as telaprivir demonstrated this reduction of HCV RNA and subset developed either a plateau or a break through and that was associated with development of resistance and you can see on the bottom increasing IC50s required with development of single or double mutations. So, Sorzen [misspelled?] and Stephen Joresum [misspelled?] looked at these individuals who received monotherapy with telaprivir and compared individuals who were not on drug, on placebo, with those in green who had continuous decline of three to four logs over 14 days and then slow return to baseline over another 14 days and compared those with the resistance of those who had suppressed HCV RNA with those who had a plateau, which you can see in blue, or those who had an initial decline in red but then a break through.

And if you look at the top which is the red, the initial decline and then a break through, you can see initially in a sort of ugly blue, or maybe it's a green, ugly green, it's all wild type. However, when the break through occurred a low

level resistance was seen and this persisted up to a couple of weeks after cessation of therapy. And when followed out for three to seven months there was still some resistance seen but the majority of virus there are only picked, mutations when it was more than five percent of the 80 colonies that they selected at every spot.

When individuals plateaued, in other words, they didn't get the best HCV RNA drop, they had actually high level of resistance with development of more aggressive resistance mutations. These also persisted up for another two weeks and then the clearly the majority returned to wild type was seen. With the bottom panel where there was continuous decline below the limit of detection, obviously no resistance was seen and after cessation of therapy there was a small amount of resistance which almost all disappeared at three to seven months.

These are the resistance patterns. You can see genotype 1A and 1B had slightly different IC50s which is being repeated in a number of different assays but apparently is within the limit of detection of the assay. Single amino acid changes in V36 or IR155 or T54 led to increased resistance to the drug but it's the 156 that gave the most resistance or double mutations. And if you look on the next slide you can see that the 155 and 156 at the active sight and a thought

probably to decrease binding of the drug to the active sight when you get a mutation whereas the T54 and the V36 are not at the active sight and are thought to be conformational change rather than a interaction of drug with amino acid. Therefore they actually have the strongest resistance is seen with mutations at the active sight. In gold, you can see the NS4a co-factor wrapped around the NS3.

This is just showing the low level of resistance with single mutations and higher level of resistance with either double mutation or the A156b, shown in two different assays. When they looked at relative fitness of these variants you can see on the bottom right is wild type which has no resistance and excellent fitness and as you develop a single mutation you increase resistance but decrease fitness. However, production of gnome mutations appear to increase the fitness back towards wild type. And they used this by looking at the IC50 as well as HCV RNA viral load.

So this is an interim analysis of combination therapy. Obviously monotherapy was associated with rapid high level of resistance. So John McCastleman [misspelled?] at Easel in May presented this data of four different groups who either received interferon and ritonavir as the standard of care or in triple therapy interferon, ritonavir, and telaprivir for 12 weeks followed by 36 weeks of combination interferon/ritonavir

or triple therapy followed by 12 weeks or in a small group just triple therapy. Most of the individuals, these are not co-infected in most of these studies I will to you today are not. Had high viral load and ten to 12—percent were African American. Traditionally, very difficult patients to treat because decreased sensitivity to interferon and ritonavir.

Well, what they showed was rapid virologic response is loss of HCV RNA at four weeks and whether it's less than ten international units or less than 30, you can see triple therapy markedly improved your rapid virologic response compared to interferon and ritonavir alone. And we think of 12 weeks as decision point whether to stop therapy or continue. And those who had a negative EVR so no HCV RNA detectable at 12 weeks, much higher on triple therapy than jewel therapy however, break through was the still the preview of the small molecule and much higher than in the pegylated interferon and ritonavir arm.

It occurred in all but one patient in the first four weeks, side effects were also higher in the triple therapy than interferon and ritonavir. When they looked at group D alone, that is the group who only receive 12 weeks of triple therapy, these were 20 patients, three of whom didn't receive any drug, one withdrew consent one week, 16 of them had undetectable HCV RNA during therapy, three was stopped because of adverse events and though all three relapsed, 13 completed 12 weeks of

therapy, nine of them met a rapid virologic response, that is negative HCV RNA at four weeks. And they stopped therapy at 12 weeks and six of them had undetectable HCV RNA 20 weeks post therapy. And of most interest, two of the six were actually African American, three of them relapsed.

This is a very small group whether they will have a sustained virologic response the time of EASEL didn't give them another month to give us those data but it's exciting but so many patients actually appeared to clear virus with just, or suppress virus with just 12 weeks of therapy whether you will give clearance is yet to be found but an exciting finding with triple therapy showing the value of the small molecule but still the reliance on interferon and ritonavir to decrease resistance. They, Sazasum [misspelled?] also looked at other proteinase inhibitors and showed both in vivo and in vitro resistance seen to the NS3.

So go back to my little topological picture to remind you that NS3 is intimately associated with NS4a and that leads us to another prototype molecule, an achilian molecule an ACE urease urea which is an NS4a antagonists that inhibits binding of the NS4a to NS3 protease and therefore inhibits activity, prevents the formation of this replication complex. The Phase I was halted a couple of months ago for renal toxicity but they have other drugs in the pipeline. And of great interest they

present at EASEL that no cross-resistant with protease or polymerase inhibitors.

So what about polymerase inhibition? And it's for B, is RNA dependent DNA polymerase that has the classic finger, palm, finger, thumb with aloetic sights of inhibition as well as the active sight. And there are also both nucleosides of the active sight or non-nucleoside opportunities for antivirals. Flucytosine [misspelled?] which went off last week but I'm still going to show the slide was one of it's, the prototype drug and it showed a modest decrease in HCV RNA on it's own but more profound in associated with interferon and ritonavir, a recurring theme I'm sure you are hearing.

Both Identics and Roche had nucleoside analogs. The one I showed you was flucytosine on the left, on the right, the Roche drug gives a decrease of about 1.2 logs, but with interferon and ritonavir it increases to 3.7. Again side effects, GI upset, or anemia. There is another drug which is a non-nucleoside polymerase inhibitor. Resistance associated however on it's own a modest decrease in HCV RNA but in combination with interferon and ritonavir not only a more profound decrease in HCV RNA but also no resistance over two weeks.

Another drug which I put in here because it's associated, the RNA dependent DNA polymerase actually binds

cyclophilin and cyclosporin is being shown in transplant patients to decrease HCV RNA. DB025 is a cyclophilin inhibitor. It decreased here you can see on the left HCV RNA over a two week course of therapy, that actually showed quite a slow return towards baseline and was shown with more than just genotype 1. Once again there were side effects here, the elevated bilirubin and decrease platelets but it brings a point of the host inhibitor, small molecule inhibiting the host protein that will decrease HCV RNA.

And other host targets viral assembly inhibitors such as Celgosivir which is an alpha galactase inhibitor and is now being studied in being able to prevent virus assembly and exertion. Vatuxamab [misspelled?] I know nothing about but it's anti-phosphatidal cerin monoclonal antibody and it apparently will be studied in co-infected individuals. There have been some studies looking at CPGs to stimulate the innate immune system. Drugs studied so far have lost and fallen into disrepute but they may come back. Aisle 7 studies looking at aisle 7 antibodies might become drugs of importance. They are really in early stages at the present time.

So I think we think of the issues for new Hepatitis C therapies. We have development of single small molecule inhibitors but you can see that they really expanding across the spectrum of viral replication. So combination therapy is a

possibility. There are multiple different classes of agents with complimentary properties and no cross resistance shown as yet. So the combination of more effective therapy will be really this combination of drugs that utilize different strategies for viral inhibition.

As I have shown you many of the prototypes are withdrawn for either poor efficacy or side effects and I think this is a recurring theme in Hepatitis C and I don't have to remind this audience. They saw this with HIV as well. Of disappointment to many of you in the audience is the fact this synergy with the majority of these small molecules and they increase interferon sensitivity. So that interferon and ritonavir will probably stay the main stay treatment for a few years yet. The studies I didn't show you showed that optimal response required the maintenance of a good trough concentration that has been shown very nicely with two of the protease inhibitors. I also mentioned that some of the drugs are given eight hourly, that's a big problem. It's not TID so whether one can optimize formulation to allow for an easier drug use, I don't know about you but I can't take a drug every eight hours, I have enough trouble taking it every day.

What about resistance, big problem. Common, distinct for class, clearly decreased in combination with interferon and ritonavir so Hepatitis C resistant testing will be required in

the clinical setting for these molecules. And the old ADA80 which we use with interferon and ritonavir will not be sufficient adherence probably be greater than 95—percent required.

I wanted to thank Charley Rice, Michelle Porsbluski [misspelled?], and Chip Schooley for the use of some of their slides. And to remind you that I have really shown you a glimpse of where small molecules have clearly played a part moving into Phase III studies but there are many, many different sights in both pre-clinical and Phase I studies and this talk will probably be 60—percent wrong next year. Thank you very much. [Applause]

JURGEN ROCKSTROH, M.D., M.P.H.: Well thank you Marion for a great overview. I think we will have to invite you back if things change that quickly. If we have questions from the floor please go to the microphones, identify yourself and ask a question. Until the first person walks up to the microphone, let me ask you one thing. With regard to the trough levels and the fact that some of these newer drugs are the protease inhibitors are being medicalize by the cytochrome system you can envision that there might be drug interactions with HIV compounds. So do you think that when you would envision that if it be, you know a drug would develop for monoinfection would there be extra hurdles in co-infection you would envision?

MARION PETERS, M.D.: I think that's an excellent point. It clearly PK studies will be essential for the major HIV players in association with Hepatitis C drugs. And we actually at the ATCG with Rob Murphy have written a show protocol to allow us to drop in drug x and study all the major classes to look for drug/drug interactions. This will also be a major problem post transplant.

JURGEN ROCKSTROH, M.D., M.P.H.: Alright from the middle.

NICK ELLIS: Nick Ellis from Dallas Texas. Just a question about listing the vertex compound, has that even entered as most, as you mentioned all your studies were in monoinfected patients, most of our patients that are protease inhibitors now that co-infected are lucid with ritonavir. Any data, any PK data boosting of the vertex drug with ritonavir?

MARION PETERS, M.D.: I think they are looking at it. I have no data. I have no, I only presented what is published and that's one article and what is in abstract form which was the majority of the data and I have no access to any PK data but obviously boosting one would hope would help that problem, with looking ahead they would have everybody on it right now and they don't.

JIM MAGALIN: Hi, Jim Magalin from New York. Since there is no DNA, no proviral DNA what are the implications or

as resistance is concerned with recycling these drugs or putting them together with future options as they come down the line?

MARION PETERS, M.D.: Do you mean in terms of archiving resistance? Well, the only paper Serson's paper and he really, they really very carefully looked and they were conservative in only calling, written if it was greater than five percent, they only looked at 80 clones. One would assume that these resistant clones were archived. However when they used combination therapy with interferon and ritonavir they didn't get any resistance. So I had no data to answer that and I am not a resistance expert. I inherited the resistance data to give to you when Abadon [misspelled?] decided not to come to this meeting. So don't torture me too hard. [Laughter]

JIM MAGALIN: Do you know where they would be archived and where would that be persistent?

MARION PETERS, M.D.: Well that's another very interesting question because when people have actually looked at differences between HCV RNA in serum, in liver, in full blood mononuclear cells, in vaginal secretions, they showed different quasi species. So one might suspect that they may be protected environments within the host where some resistance seen but you don't see it in the serum but that's all postulate. I know some data from Andrea Kovack's where she

shows clearly different quasi species between different sights of Hepatitis C replication.

JURGEN ROCKSTROH, M.D., M.P.H.: Alright. Thank you, Marion. [Applause] So over to Gail for the next speaker.

GAIL MATHEWS M.B., CH.B., M.R.C.P.: Okay, so I would like to introduce our next speaker, Professor Stephen Locarnini. Professor Locarnini is the head of the research and molecular development at the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia. He is also the director of the World Health Organization collaborating Center for Virus Reference and Research. Stephen is one of the worlds leading Hepatitis B virologists and really has a unique insight into the complexities of Hepatitis B resistance. And I would like to invite him to give his talk today entitled “Hepatitis B Molecular Resistance Pathways”.

STEPHEN LOCARININ, B.SC.(HONS), PH.D., M.B.B.S., F.R.C.(PATH): Thank you Gail for those kind words. It’s not too far for me to come up from Melbourne so I’m always happy to sort of hop on Quantas flight and which never runs on time but I get here and have a talk about Hepatitis B. I also wish to thank the organizers for their incentive in a co-infection workshop. I think this is a really important issue and I’m glad to see infectious diseases people come in the area. I think it’s very important.

Alright so the organizers have asked me to talk about resistance pathways which has been a major area of our laboratory activities in the last couple of years. And I feel like I just set the scene for where we are. It was so gratifying to hear Marion who is a hepatologist to actually talk about combination chemotherapy because in Hepatitis B we really unfortunately have not had a lot of drugs roughly at the same time of approval to be able to do combination therapy studies. I would have to say actually the field is reluctant to do combination therapy studies until we have burned off all the drugs that we have. So I do hope that in the next 20 minutes I can sort of show you where we will be in the next three to five years if we don't start doing combination therapy studies for what little drugs we got left with efficacy studies in Hepatitis B. But that's just a personal bias.

So, with that as a background, I think in Hepatitis C I hope you don't make the same mistakes because I do promise you one thing Marion, I will not get into Hepatitis C resistance testing because we are too busy with Hepatitis B. Okay.

So, where we are in 2007, I think that we have these sort of figures, for incidence prevalence data, I updated them just last week with some data from BMS partly provided to me for the four year data on entecavir and with lamivudine we roughly run a 25—percent premium. Entecavir I think this is in

naïve patients, initially in the first year, that was actually started [inaudible] with 311 and entecavir 29—percent. These arm studies are pharmaceutical registration studies. They have limited value but these are all we have to actually gauge resistance.

I can reassure you in the real world of the service provider which is what I am, on a day by day basis that the resistance is much higher. And more importantly is that in this country, in Australia we have tenofovir approved for limiting resistance cases. So you can see it doesn't work as well. Where at least in the first year there were three studies where the ranges from north to 18—percent. The Korean study of 18—percent is quite shocking because of the fact that they normally in patients who are not naïve you have zero percent. So you can see where the problem is.

Entecavir starts out at 0.1-percent, 1.4 a month, consensus so the rate is very low. Again in naïve patients and whereas in lamivudine resistance patients as you will see as we go forward that the resistance doesn't accelerate as well with six percent in one year, 15/35 and 43—percent by four years. We have limited studies on FTC and the case definitions for resistance as I said, at least pharmaceutical studies so they have own particular case definition. And if you look at the dapivirine Identacs data for E-antigen, it's in positive

patients which was 4.4 and 21—percent. That's against the alamidine placebo, lidamidine treatment arm. Ten percent which we know in the real world in our case definitions are no longer 25—percent. So you can only double these values for telbivudine. And interestingly E-antigen positive patients fared much worse than the E-antigen negative patients. So really I think that's a pretty I would say depressing story.

So, why is Hepatitis B resistance a bigger issue at HIV in Hepatitis B than in HIV? Well I don't have the time today to sort of go into the last slide of the virus but we really unfortunately have only one enzyme mediate target, that's the viral polymerase which is a complex enzyme. And with that one viral target the event unfortunately got one class of drugs of nucleoside/nucleoside analogs. But the virologists and the medicinal chemists have classified those, that one class of drug into three groups. And for the purposes of understanding the resistance pathways we found this quite a useful classification so I will bore you a little bit of medicinal chemistry. That telbivudine and clevudine are all nucleosides and they just had the sugar on their base basically rotated 100 degrees against the natural D-arsmer. Adefovir, tenofovir are acyclic phosphonates and typically classified and our system of acyclic of 15 depicted sugar against the glycocin base.

Now the case definitions that we are pushing the hepatologists hard to accept which I think being in the more recent guidelines of the American Gastroenterology Society is using a viral load definition is that drug resistance, an increase in viral load from nadir is greater than one log or international units per mill, not copies. International units everywhere are pretty much a reference standard. Is that a full page definition but at the end of my talk I will show you data where we stay a little bit concern about that case definition based on some evolution of new evidence that we are seeing in the patients.

Increasing serum LAK, things of deterioration relate signs, and what is more recently being used in patient management is the use of the genetic markers of drug resistance within the viral clematitis. And if you just sequence a Hepatitis B virus assay from a patient baseline you do get a lot of variation in the genome. In the Hepatitis B virus a poly involved pathogen event which has been around a very long time.

A homo evolution so that we, it’s a very adaptive virus. To us it’s a height genotypes and an enormous amount of genetic diversity within the genotypes. So really genicity reference sampling comparisons with baseline can be a little bit flawed with danger. And really it’s often the second or

third sequence sample that really does help us in guarding out definition of primary resistance mutations. This is secondary probably favoring mutations. And we classify primary resistance mutations and we have a very strict classification event. This is that we are in the laboratory. We have established a significant antiviral effect once as a consequence of that particular mutation, i.e. a significant shift in IC50. And this is a classic infectious disease definition.

The secondary and compensating mutations do not affect the IC50 but actually have an affected replication yield or replication thickness. And unfortunately Hepatitis B we don't grow the virus very easily into a culture. We do not have useful replication yield or replication fitness assay. So we have to sort of dispense against among yield. But it is very important to accept primary resistance mutations are what we think it's all about.

I will summarize on this slide where fitting that case definition, my laboratory believe that the primary resistance mutations live and here we have a linear representative of the viral clematis and though the function remains of the enzyme and I have shown for you here the documented primary resistance mutations from a almadine and telbivudine. Basically what any one field very or into for VRI, interestingly, clevudine we

only see the I which for reasons, I'm not sure a hundred percent can explain yet, for adefovir and tenofovir, for adefovir we see the IR already on TV and the N236T is probably resistance mutations. With tenofovir we see osculating co-infection with 194 plus 190, 2R4VR in collaboration with scaphas aurone and with 181TV and in association with 204, is a primary resistance mutation. Entecavir it's not meant to say that entecavir is in general more resistance because of all the mutations we see. What we, as you will see in a minute, with entecavir that immediately three mutations to appear concurrently in naïve patients to encode absolute resistance. Typically these are at either 180 and 204 with one arm, 184, a 202, or 250, that's the Bristol Myers definition. We also see cases with 169.

Now these number of mutations though shown on the previous slide what we have been able to characterize from a database and subsequent analysis is that we have identified and distilled these primary resistance mutations down to eight important code arms shown for you here. Then we have looked at the natural history and the clinical information and the virologic information generated from that database of over 3,000 patients there and tried to sort of understand them in the HIV concept of the pathways. And what I have shown for you

here is the proposed key pathways that I would like to share with you today.

If I remember the setting in Hepatitis B land, is that we started mainly with monotherapy. The patients tended to start with l-nucleosides, lamivudine or telbivudine that typically run down the infer to a full pathway. If they start with acyclic phosphonates like a Denavir they run down what we call the 236T pathway. Now that's the majority of patients. Typically between say sort of to 60 to 90—percent.

We have identified recently a third pathway which we call a shed pathway which is cross resistant to both the l-nucleosides and to the acyclic phosphonates. This is the RTA181TV. And as I said in the city of naïve patients with entecavir you need at least three mutations, 18204 plus 184, 202, or a 250, to appear at the sight and time. In entecavir naïve patients this is, I just pulled this, entecavir naïve pathway, — interestingly enough for entecavir in previous experience patients they only need one mutation path. This is 204VI. And we are identifying more in the last 12 to 18 months multi drug resistant pathways. So I'm just showing you one for today which is actually resistant to every drug that we have in our laboratory. And we have isolated several patients in Melbourne. It's the 181T, 233V, 236T, and MT250O.

The reason why I raise this issue is because I don't think Melbourne is unique in Hepatitis B and drug resistance. I think we just have a laboratory that's interested in drug resistance and that's why we are seeing these particular mutations. 181T and 236T are critically resistance to entecavir and the l-nucleosides. Our feeling is that the 233 and the 250 are compensating mutations but you need this class step to engender multi drug resistance for reasons we don't fully understand. But I think there is a really important message here.

So, from our database of 3,000 patients what do we typically see? Well, the lamivudine monotherapy was MA was just sort of indicator in the first instance. We typically saw in the first instance the 18204VI pathway. As you saw from my previous summary slide which is cross resistant to telbivudine and clevudine. That appears in about 90 to 95—percent of the cases. Up to five percent of cases we have A181TV pathway which is cross resistance to adefovir and partly explains the adefovir non-response after lamivudine failure.

What we interestingly we have recognized and are about to publish on is that if you maintain clevudine in the presence of the 18204 the virus continues to pick up adaptive compensating mutations. Typically a 2-only 4S or a 202R or a N250V, and why these are important, they release of the

mutations that we also need from entecavir resistance. These patients haven't even seen entecavir yet but they are already resistant to it.

We are now seeing classes of mutations of 240 and 215 which affect the adefovir and a broad group of classes especially in the Asian genotypes. The Asian genotypes are really quite different from the European genotypes. Genotypes a and D for Europe and for B and C, the L180 is a fascinating compensating mutation but does affect sensitivity to adefovir. The A181T is very frequent in the genotype Cs and as I said we are seeing the 194 in association with saphenus areola.

Now why is this important? Well I have to say we definitely know that Hep B does archive through its CCC or many chromosomal transcription film play. And so we have learned from the HIV group that compensating mutations do fix in the genetic archive so we are very concerned about these mutations because of their ability to rescue subsequence.

What's the story with adefovir? We have a lot of patients who and spend an exercise program started with adefovir. Typically we have the major pathway resistance with the 236T pathway, typically lamivudine sensitive but does actually over time become a multi drug resistant and does actually have a blunted response in our experience to tenofovir.

We are now starting to see other mutations with more wide spread use of the adefovir that affect the N236 region, the A,V, and S. We are still characterizing those in the laboratory at the moment. We see lots of A181-TMV with and without a 204 either depending on their previous exposure to lamivudine, which is broadly cross resistance to tenofovir and broadly multi drug resistant. So we are sort of seeing lamivudine as well.

Now as I said with entecavir a major experience involving with entecavir has been in the setting of lamivudine failure and therefore rescue with entecavir and typically we started out with a 18024 lamivudine resistance genotype and then we just all made it through one more mutation and at least the 169 plus a 250 that already, that's sort of compromises entecavir but does actually also compromise low risk with lamivudine and tenofovir.

Now there has been a lot of interest in entecavir in naïve patients a lot of discussion and we are going to sort of see as entecavir is approved and hopefully prescribed more frequently in the naïve situation, that what happens to a patient who receive entecavir in this particular scenario. What I have shown is that a paper that was published, a Japanese patient that was published in *The Journal of Clinical Virology* earlier this year. And it's in the public domain

kindly given to me by Christal Hans Tumman and I sure miss that paper but you can see here the viral loads shown off to the side here, they are going down and they respond real well to entecavir. I would point out that this patient for whatever reason didn't use the recommended original dose of 0.5 milligrams. This was a naïve patient. So the therapeutics dose of 0.5 but started with 0.1 for about two or three events and then switched across to 0.5. You can see still got a good response with 0.1 which is the less than optimize dose. Very good suppression but you can see here, that here is your baseline. And then you can see here that in terms of at this point now that they were starting to sort of see not one, not two but three mutations appear and then interestingly the virus takes off. But you need three mutations to occur at once. It appears to break through on entecavir in the naïve situation.

So I think it's going to be important because we know that single and double mutations pre-exist, triple mutations do require a lot more in terms of genetic barrier to actually become resistant to. But I'm starting to be convinced by patients such as there, as case records appear in the literature that we need three mutations in order to engender entecavir resistance.

Except for telbivudine, I had a few patients in Melbourne but this is from the FDA website from the package

insert for telbivudine. Again the major mutational pathway is the 204R pathway which is obviously cross resistant with clevudine. But really what we are also surprised to read in the FDA submission was the 181T is important. This is broadly cross resistant with adefovir and tenofovir and also I think the 229 pathway which we are also characterizing at the moment will have important implications and further risk review options.

So where are we going now because I have patients in Melbourne that are a little bit longer out in terms of therapy. We have a number of patients as I said who are now multi drug resistant starting with, as you saw with the entecavir or the lamivudine as a matter of this particular patient, grouped cluster started with adefovir. 3236T rescue with lamivudine failed, flipped it to 181, the combination, these are alternating mono therapies. Then went back under our combination at this stage of their treatment. That's a typical scenario in the most Hepatitis B being managed over the last decade. You end up typically now with a 180, 181, and a 204. And then one compensating mutation Y233 with a 250 and as I said, we have no drug that I am aware of that is able to treat this particular HBV isolette. That's four mutations are on the one genome.

Being a reference laboratory, it is our job to actually make some of these mutants and they get tested in vivo, phenotypes, it's really slow labor intensive process and try and get some sort of IC50 values. This as I'm showing fall increases. The l-nucleosides easy to become resistant to. Big shifts in IC50 between a 100 and a 1000 fold shifts with a classic L204I pathway. And you can see what is interesting though is you don't need a lot with 180TV pathway, real low IC50 fold increases but unfortunately they do compromise your ability to rescue with the acyclic phosphonates with entecavir and tenofovir. They are a three to five fold increase with A181TV renders these drugs ineffective clinically. And with entecavir you get a partial blunting with the 204 pathway, with entecavir a 30 fold increase, but then when you put on one or two of the extra mutations of the 250 or the 169 or the 202 or the 184, there is a very, very, very accelerated increase in ICR50.

So obviously we need to be doing more of the scientific analysis to be able to provide the physician with some degree of confident and their ability to rescue. So in my last three minutes I will just finish up with some more of the recent activities that we are doing and just to remind you some of the interesting anatomical considerations of Hepatitis B virus.

And Hepatitis B is a circular genome with four open

reading frames and the frames shifted. You wouldn't believe your bad luck but drug resistance in terms of evolved acclimatized arrhythmia overlaps but the frame shifted in a way, the neutralization domain and the envelope. This is an extremely important consideration. And one that I wish we had more resistance to characterize but 80-year-old—percent of the drug resistance viral changes that we see do result in the significant in the viral load. And I have listed all four drugs that are approved here in Australia. They all cause changes, here they are, on the catalytic domain of the enzyme and they all affect the neutralization domain of the envelope. The neutralization domain of the envelope is also a little bit like HIV in that it's a confirmation electrode. And I'm showing here for you now the [inaudible] of the major neutralization loop. You can see here it's a group assisting rafts and you can see here is the major classic G145R our classic vaccine. Or D144E vaccines got mutant or H [inaudible]. These are limiting resistance mutations and I will show you all these drug resistant mutations, 161, 164, 173. These are all there what we call the C-terminal end but they do actually and you might think ah, that's the wrong way of life from the neutralization domain not here, but I can reassure being a confirmation epitope in a study of the [inaudible] did some years ago. These limiting resistant

mutants were compared to the classic vaccine escape mutants and their ability to bind anti HBS.

You can see here that the vaccine escape mutants typically have a binding capability of a 55 grams per mil. And that is certainly increasing or increased bind in compromise activity with limiting resistant mutants but this 173, 182, and 184 they have the same binding capacity as the vaccine escape mutant shown for you here. That to show you where that is, it's just there.

Since then we have had a bunch more. Now why is this important? Certainly it's important in common fiction in a collaborative study with Cleo Tho in Johns Hopkins and Greg Dora and Kyle Matthews proved in the National Center and showing that improving novelty which I since deduced. We looked at a HIV HPB 150 cohort that we actually had lamivudine experience. We are looking for the selection of this particular mutant that I described in the previous paper. The 173, 18204, and with the duration of lamivudine therapy you can see it's a 173 which is driving the vaccine escape or the reduced binding. It's a compensating mutation. You can see that the longer you leave them on lamivudine the more frequently you see increase in this particular pattern of selection of that virus.

In collaboration with Professor Soriano's group we looked at a cohort in Europe and the Soriano has been able to sort of described that the classic sort of spontaneous mutants that we see were associated with vaccine escape or selected pressure vaccine in HB but also he has characterized a very large number of drug select mutants at C-terminal end of the HBSOG. That is very important in terms of their ability to act in escape.

So why are these important? Well I think to an audience as sophisticated as this group here these resistant changes in HBV selected by nucleoside, nucleoside analogs, obviously affect HBSOG heterogenicity especially in genotype A patients as well as genotype D patients from Professor Soriano's group. And really, I think that does actually pose a question that we should be doing more genotyping of HVB genotyping and primarily sequencing in our patients as they go on to nucleoside/nucleoside analog therapy.

Now why this is important is that Vincent Thorbow in Paris as well as Edward [inaudible] have got several cases of transmission of lamivudine resistant HVB in the setting of men having sex with men. And so far the cases have only been resolved in typically acute Hepatitis B but I suspect that what's really happening in higher prevalence parts of the world such as Asia and in Africa where lamivudine with drug

resistance is much more of an issue, in terms of a practical issue but it's not being properly surveillance. I think we will be seeing lots of the examples of transmission of drug resistant and multi drug resistant Hepatitis B.

In my last couple of seconds, we have been with some new data that I think is important to present here today. we have been looking a lot of in the last one year, the A181T mutant especially, with reasons you will see in a minute, and the reason why that is important is that as well as causing, it doesn't cause a change, it causes a premature transmutation of the C2 [inaudible]. It's a stop code with the phenotype 172 is. And it shows you where that is. It maps on the cyclopentane antigens as failing. This was done by 91 registered students in our laboratory. So the HVB encoding the ART181T, it's defective in very variant sequestion because it's going to try to encode it in envelope but what we found which is quite important is that it acts as a dominant negative mutant for wild type HVB excretion. And as shown in this slide very nicely it's actually retained inside the cell.

And we look at that database we found that even though only two percent of the lamivudine resistant cases, that's not important but a transfer of over 20—percent of the tenofovir resistant cases. So what we are seeing is that in the presence of the A181T the kinetics of relapse or the kinetics of

virologic rebound are compromised. And I have just shown for you here three typical patients. Typically what we see with lamivudine are definitely resistants that we get two to four logs break through over three to six months. And knowing that we are starting to see is all that no change in viral load or one logs every 12 months or barely just two logs at 12 months. So I think that the presence of the A181T does start to challenge. We are seeing more of it that the classic case definition of greater than one log increase from nadir over three to six months.

So Gail, Thank you very much for the opportunity to come here today and holler at you the current patterns of analogs of NLL drug resistance in Hepatitis B. they are getting more and more complicated, I'm sorry to say. And we are seeing probably resistant mutations across bridge now. The A181TV pathway, the third pathway that I spoke about we think we are seeing that more and more frequently now as well as the 204 and 236T pathways. But more important in terms of practical patient or physician management, in terms of your ability to rescue your patients we are seeing these broad crosses of compensating mutations during lamivudine and adefovir therapy that's sets 214 and 215, 169 173, 184, and 202 that we feel really compromise your ability to actually future rescue patients. So obviously pathway sequencing is important.

There is a need for interactive database programs on a global level. And we really do highlight the concern that the virologists have on the preliminary link in the public health risk associated with that. Thank you very much.

[Applause]

GAIL MATHEWS M.B., C.H., M.R.C.P.: Stephen thank you as usual for an illuminating talk about Hepatitis B resistance which is only going to become more complex I think as time goes on.

So question from the microphone there.

RICHMOND: Alright. Richmond, San Diego. Stephen that was terrific as usual but you have been telling us this story for many years and nobody seems to be doing anything about it. So sort of analogous to global warming do you think the problem is physicians and drug companies don't believe it or they don't want to do anything about it.

STEPHEN LOCARININ, B.SC.(HONS), PH.D., M.B.B.S., F.R.C.(PATH): Well, it's, I think in our world it's a huge issue and I believe this, there is not enough commitment to really defining the problem. If you don't take a temperature you can diagnosis a fever. So the extent of resistance is really its epidemic in Asia because of the inappropriate use of lamivudine. Lamivudine is widely available in Asia and the way it's treated is, the way it's used and prescribed is completely

inappropriate and it's a mess. But no one seems to care. It's only Hepatitis B so why should you worry. It's only the insulating chorus of death row one. Limit disease but we can't seem to getting anyone really interested anymore. I guess we got HRV and Hep C so Hepatitis B will fall off the white house ground.

GAIL MATHEWS M.B., CH.B., M.R.C.P.: I think Marion has got a question.

MARION PETERS, M.D.: The DHSS guidelines in the US for coinfection recommend dual therapy. The ALDS guidelines recommend dual therapy for transplant and cirrhosis so we are slowing getting there. I think that's an important point it's a change of over the last six months.

STEPHEN LOCARININ, B.SC.(HONS), PH.D., M.B.B.S., F.R.C.(PATH): If I might response to that, Marion, my reading of the guidelines was that combination can be considered in cirrhosis. Even the American gastroenterology guidelines were can be considered. There was not a straight out you use combinations. It was can be.

MARION PETERS, M.D.: That is correct.

STEPHEN LOCARININ, B.SC.(HONS), PH.D., M.B.B.S., F.R.C.(PATH): Which is not enough? I don't think it's enough, not in 2007. I was really disappointed when I read those guidelines last night.

GAIL MATHEWS M.B., Ch.B., M.R.C.P.: Another question just on the left there.

STEVE O'BRIEN: Hi Steve O'Brien, Oakland. For those of us who treat co-infected patients and have access to the tenofovir FDC fixed dosed combination, our strategy in naive patients is often just too empirically start that fixed dose combination. Can you comment on that?

STEPHEN LOCARININ, B.SC.(HONS), PH.D., M.B.B.S., F.R.C.(PATH): Well, I think that's a great combination. And I think that when you, in a golden world of infectious diseases your first chance is your best chance. I have presented you with scenario that was basically become, because of the way drugs were approved in Hepatitis B and the way, so you know the practitioners to be fair, only had lamivudine for a very long period of time so they tend to just kind of kept pace with lamivudine but you know if we could go wind the clock back and say we had Prezista straight up or lamivudine plus a adefovir straight up then or well, I mean, I mean entecavir if entecavir was a three mutation profile, is equivalent to a combination. You would have to argue that for a mutation of virologic standpoint so if you wind the clock in years those sort of drugs with a high genetic barrier either a combination of FTC plus tenofovir or lamivudine plus adefovir or entecavir 1030 with that current understanding and I'm a committed combination

therapy person but I have to concede that yes with entecavir in naïve patients you need three mutations to generate resistance then yes, that's actually a good way to go. But and that has to be the way to go and I think the responses we see when we do treat naïve patients are great. And you get very good suppression with Hepatitis B virus DNA with those sorts of approaches if you start out in the initial naïve patients with those three various approaches.

GAIL MATHEWS M.B., CH.B., M.R.C.P.: Thanks Stephen once again. [Applause]

JURGEN ROCKSTROH, M.D., M.P.H.: Okay we are going to move on to our last presentation which will be given by Professor Vincent Soriano. Vincent works in Madrid Spain at the Hospital De Saludo Carlos and he is going to give a talk on HVB evolving treatment paradigms in HIV-HBV co-infected patients.

VINCENT SORIANA, M.D., PH.D.: I realize to say [inaudible] a little difficult for [inaudible]. So I will focus on something more clinical and involving the co-infection of the Hepatitis and HIV. I will not try to cover every thing and in fact I just decided to move what on the use of the new diagnostic laboratory methods and to make the appropriate [inaudible] of these patients when to treat and which [inaudible] to use. I will comment on at the end about failure

but I do speak on sometimes but not on everyone and also about the co-infection of Hepatitis B and C in this operation.

So on the first lesson is that in the old times we just make attention in the [inaudible] with clinical Hepatitis B to define with people with [inaudible] longer than six months. This is the definition of the clinical HVB. Just looking at the liter, with our patient and demonstrating that was Liber and Lifeman looking at the amino transferases and looking at the serology without patient were not with the old [inaudible] pathway, antigen E or in doing clinical biopsy. The treatment until just a few years was limited to a very few options. And in fact in the past we recommended that they start drinking, avoiding spicy meals and relaxing [inaudible].

But now in 2007, really and we have learned many things from the HIV field and we give respects to my colleagues in serology with me that probably all the advances that the research in HIV has with in the room, has done a lot to change the mentality to a broad ship of HBV and HEP C. in fact looking at the new methods to monitoring this disease we look at the viral load. We look at the genotypes, and we have learned and in the previous presentation about the use and importance of monitoring resistance and for little biopsy we have now [inaudible].

We have [inaudible] that's right for the market but now in addition to the all interferon we have lamivudine and clevudine, adefovir, entecavir to really and probably within a few years or months we will have also tenofovir that you know very well. All these drugs, the important new drugs, entecavir or FTC or any of them are used by many HIV clinicians because they get approved for HIV but not yet for the people with HVB.

The importance of the viral load in the diagnosis of HVB has been clearly demonstrated in papers last year but the message here is that the science, the [inaudible] right now will allow on viral load to dictate what will happen in the near future. And in fact if using something, [inaudible] we put these patients with below 100,000 copies per ml which means dividing by five, 20,000 in international units per ml. these patients will show less inclination unless [inaudible]. If we get these patients below ten to the fourth it will be more dutiful response of antigen E loss. It would be [inaudible] low on function if [inaudible] very difficulty to this [inaudible] like in HIV if there is no known application the selection of resistant mutations we can be very, very difficult for the patients. And as long as you have complete [inaudible] it's just a matter of time. You will lose this [inaudible] antigen.

As I mentioned before that are critical and for those interested to learn more about the back and the critical role of Hepatitis B [inaudible] in prognosis in Hepatitis B were here are the reference. In the first viral load this definitely correlated possibly with the resource developing [inaudible] and in the second the [inaudible] is also correlated with the risk of developing basal cell carcinoma. The geno types like in HIV would have several ultra types in HIV and some of them have clinical inclinations. HIV2 for example, is not transmitted with nucleocytes [misspelled?]. Well, we don't have a lot of information but we have a starting to learn that some of the genotypes, for example genotype A, is more sensitive to interferon and at least times is usually associated with higher types of beremia [misspelled?] and may develop faster resistance to 3TC.

Hepatitis and genotypes B and C are more common in Asia, and B looks like a little better in the sense that it is more [inaudible] and more sensitive to interferon. Genotype D is the other prevalent genotype in Europe, especially with genotype A, and this genotype usually provides a lot of trouble to our patients usually they are antigen E negative and the prognosis tend to be worse.

For the assessment of neural fibrosis, as I mentioned before, now we are starting to collect more and more replacing

liver biopsy. But we need to justify the treatment of Hepatitis B, is to demonstrate some damage to the liver. Some fibrosis may just predict the risk of developing cirrhosis, the end stage of the disease. And now we found seven fibrosis markers and the fibrous gun, a new image technique, we can clearly distinct those persons with very advanced fibrosis, significant fibrosis would justify therapy and thus with no evidence of lesions in the liver, we can wait to start therapy. We can repeat the fibrous gun every six months for these patients and collecting information longitudinally and in one moment decide this is the moment to start therapy.

In HIV, Hepatitis B is a priority and there is a lot of information collected over the last years to justify that. The infection is common, as we will see later on, it's a dilatory influence of HIV and clinical Hepatitis B in terms of more rapid progression to end stage liver disease, increased risk of liver related mortality, and poor [inaudible] to anti-viral drugs when you have underlying Hepatitis B.

There are several guidelines to treat contracted patients and we will discuss a little bit on that. And the advantage here, and this is something new, some of the agents that we are using in treating HIV are at the same time useful to treat Hepatitis B, so we can just budget, we can fight two

different agents with the same compound. And this is the case, as you know very well, for tenofovir.

Well, the numbers in the world of patients who are infected. We estimated that we are at around 40 million people living with HIV. There's 400 million more with chronic Hepatitis B, and the intersection, does the HIV having chronic Hepatitis B is around let's say 10 percent overall. So that means four million people in the world is having both HIV and Hepatitis B.

Everything that happens in the natural history of Hepatitis B just including naturally infection, this is 95 percent global, 63 percent HIV, develop the higher rapid state and for those with high beremia develop a stage 3 [inaudible], everything as I say is worse in the HIV sitting. The rate of clinicity [misspelled?] is higher when you are infected with HIV, the proportion of people with antigen B, the rapidity form is higher, the [inaudible] tends also to be higher and the risk to develop end-stage liver complications of course faster. And in the fact, the data for example, from Eroshida [misspelled?] and also by John Hopkins by Cloey Fio is that looking at the new age events there is not much difference compared to people with antigen with clinical Hepatitis B, but looking at the global mortality and especially the liver related mortality in the multi- [inaudible] analysis, these patients die more.

So when the treat Hepatitis B? We need to treat everyone with the [inaudible] antigen? No. Some of these patients may have a tendency. They may start with no liver inflammation at the moment, but later on maybe that situation may change. And in fact all European guidelines published in 2003, recommended that 3 month probably was probably justified in the presence of elevated LP levels, antigen E or high levels of beremia. And why? Because doing a liver biopsy in these patients almost all of them have significant fibrosis to justify therapy because these patients progress faster. In this situation, was a justification to initiate therapy. The new guidelines from the American Association for the Study of the Liver in summary, just focus on the same message more or less. When you have elevated LDL levels, despite whether or not with antigen E, there is the justification for therapy.

In the specific situation that LP levels are normal and you have antigen E negative, but you have high beremia, also in that specific situation these are probably the patients who have the mutant that precludes the production of the antigen E, these patients progress faster to end stage liver disease and also there is a justification for therapy.

What drugs to use? I mentioned before and we heard before from Professor Locarnini, we have to plenty of new drugs. And with the concern for resistance and drug

resistance, but now register we have interferon alpha, [inaudible] interferon to help to glaze the classical interferon alpha. The treatment is for one year, but for all the others we need to provide therapy forever, let's say until the moment we will reach the serum production for antigen. Sometimes being more conservative, the antigen E serum conversion and we have lonidamine [misspelled?], alephovir [misspelled?], decovir [misspelled?], [inaudible] just approved a few months ago. And just because we are treating infected patients, we have the chance to use FTC and fenalfovir [misspelled?]. The new molecule that will probably be approved soon, will be clovadine [misspelled?], which is very potent.

Two years ago in fact, the manage of the drugs on infected patients was very dependent and this is still true right now. The need to treat HIV. We don't need to treat HIV with anti-retroviral drugs, we have to defend our positions. One is when the patient has antigen E. In that situation, the use of PG rated interferon for one year, may provide sustained reduction in Hepatitis B perennia in around 20 percent of the patients. In co-infected patients, probably this rate maybe lower, but the chance is so high that probably with very high CD4 counts, the chance to use PG rated interferon can be defended for some patients. When you don't have antigen E, the response to interferon is quite poor. Let's say very poor.

And this why. Nucleus cinereus, which are very over rated, they are oral drugs once-a-day, we can discuss the use of decovir [misspelled?], or ethovir [misspelled?], or deldovine [misspelled?]. And you are very much aware that just in November, a few months ago, there was claim that decovir may select some resistance in HIV. The same has been of some concern of ethovir. We have been using 10mg approved for Hepatitis B and with deldovine we have learned that it has not been approved against HIV, but the selection of resistance is between ethovir and [inaudible]. So really using monotherapy with deldovine in this situation, probably no one would put the senior cure there. This becomes much easier when you need to treat HIV, because we have [inaudible]. We just have a question a few minutes ago. We thought this was something magic. I can tell you that. So you have experience a lot about that. In co-infected patients there is a justification in initiate HIV therapy or risk using trebata. This is the way to reach undetectable beremia in Hepatitis B.

What would happen if the patient has some kidney troubles or disturbances? What would we do? The data using combination therapy in Hepatitis B as discussed, has not demonstrated a more pronounced production in beremia and so for the benefit is just limited to the immunization to the reduction of the selection of resistance for recommending use.

Well, this data has already been discussed by Professor Locarnini, but this is the rate of selection of resistance mutations using alimodine [misspelled?] or efovir. With the new drug the alimodine we are using the needle. But to deliver the message here, is that I think no one will put the fingers and say that probably deldovine will be a monotherapy drug to be used in people who don't need anti-retroviral therapy because selecting HIV, there is no evidence of activity against HIV. In deldovine you will lose microsity [misspelled?] stamps, many options in the future, including tenaclovir [misspelled?].

Already has been mentioned by Professor Locarnini and what about the risk of transmission of this resistance variance? There is not much information on that, but I'm sure as all these drugs, especially in the HIV co-infected field are widely used by our patients, infection is not clear. And these patients continue to be in risky behavior, we will see more and more patients with resistance to amevodin [misspelled?] in Hepatitis B. Ethovir is effective in the long-term with substantial cell conversion in antigen E and S, there is selection of resistance but this is a low rate and less than amevodin. There are a specific mutation that don't cross in general. Cross resistance to amevodin is at some positions like 181 like mentioned before, but for some reason is still

unclear. Let's say that five percent to 10 percent of our patients taking all their medications and are compliant, don't respond. And this has been a matter of debate and controversy. Another difficult question is if we use efovir, 10mg, we won't need anti-retroviral therapy, we will select in HIV the 65 R mutation that may confer drug resistance to tenofovir. Very few studies have looked at that and just Joey Sheldon and our lab and a small group of our patients from Europe in cooperation with Eurgan [misspelled?] and the study inhouse in Germany, there was no evidence of selection of AK65 in HIV people exposed other interferon therapy for a while. The analysis extended to endpoint [inaudible] to look for amino species present in these patients. At that moment, it looks like it is relatively safe to use delefovir when using the treatment with Hepatitis B without needing the anti-retroviral therapy.

The reasons to fail delefovir [misspelled?], as I mentioned before, one of the reasons is that we are using 10mg which is a low dose and because higher doses, as you know very well, may produce necrotic toxicity. Low antiviral activity intrinsic by the drug, [inaudible] some compartments in the liver, some [inaudible] to more resistance than has already been mentioned before. Drug resistance to amevodin, especially when the mutations appear at disposition, and in geno type A2,

which is the predominant in Europe and the U.S., [inaudible], this poly-morphes is in the pathway with another one to produce natural resistance to ethovir. Like we see, for example, in HIV 2 there is natural resistance to non nuclide [inaudible]. In decovir, the decline in infected patients previously exposed to amevodin show that the drug was potent and produced a reduction of [inaudible] at one year. As time passes by and these patients continue to be on entecavir [misspelled?], it's just a matter of time. Those are who still with detectable beremia will accumulate mutations and they will fail because the mutations that produce resistance to lamidodine [misspelled?] are in the pathway for the resistance to entecavir.

Just one month there was intriguing information that was already reported [inaudible] at the CLOE [misspelled?], that entecavir when is used in patients without anti-retroviral therapy may select for resistance in patients with HIV contribute antiviral activity against HIV [inaudible]. The data came from three patients and followup at the John Hopkins and other [inaudible], the reduction in HIV was in one lump. One of the patients doing clinical analysis of six months was a lowly accumulation of the 184B in HIV. But there was another patient, number 3, that despite being exposed to decovir amino therapy for 7 months, there was no evidence of selection of the

184 mutation. Just in that one analysis, out of I think 50 clones had this 184B after seven months of exposure to decovir. This is something a little strange. In the in vitro analysis done by a very expert group, they were able to demonstrate some activity in vitro viral culture of entecavir against HIV. And if the FDA make a warning and recommend it to avoid the use of endecovir amino therapy when there is no [inaudible] anti-retroviral therapy use. However, I do experience, some in the audience maybe using endecovir as amino therapy in co-infected patients, but we have the opportunity to study one of our patients that was a patient with Dilda [misspelled?]. Dilda, as you know very well, may super infect Hepatitis B patients, and because there is viral inference when you have Dilda, many of the patients have undetectable beremia for Hepatitis B. We look at this patient with Dilda with entecavir with more than 500 CD4 cells because he was asiropic [misspelled?]. He was progressing very fast. And we provided something that we thought was the only opportunity to [inaudible].

And this is the data. So at the beginning there was undetectable beremia for Hepatitis B, was high levels of beremia for Dilda. And putting this patient on entecavir, there was nothing significant changing beremia in HIV using 1 mg of entecavir. But there was more. On the followup, and this is six month at the moment, there was no selection of the

184B. So for some reason, and still my view, this topic is still open and for some reason still unclear, this potential activity of entecavir against HIV merits further investigation.

There is more than that. We recommend using entecavir in people who need anti-retroviral therapy. We will need to have more information about interactions and we have learned about that, for example, the story of riboviden [misspelled?] and abacavir to link [inaudible] with anti-retroviral therapy. Both are [inaudible] and they may compete and in fact there is data from the Riboviden Trial and from our group that show that people treated for Hepatitis B, C taking abacavir may respond less because of the competition between abacavir and riboviden. Entecavir is also [inaudible], so we decide to treat a co-infection between HIV and Hepatitis B in people who [inaudible] in abacavir, what will be the effect of using entecavir? There will be some interference and in amino competition. We don't use AZT and D4T because they omit each other. We need this information before recommending to use entecavir in people using anti-retroviral therapy, especially taking abacavir.

Tenofovir. Well, tenofovir not yet approved for Hepatitis B but clearly the data from the clinical practice from all of you probably, is that there is a significant reduction in people with already a resistance to amibodin and the interesting thing here is that sometimes the response rate

tends to be low to tenofovir. Very potent drug, but sometimes it takes a long for it to reach undetectable beremia. Sometimes more than one year.

Marion Peters, one of the speakers, she was the leader of one of the ACGs comparing non-interferon trial, the activity of tenofovir versus alefovir [misspelled?]. And really the message here, the study was discontinued prematurely because the endpoint was reached. Was the non-inferiority of tenofovir versus alefovir. But the message here is that we ended the study. Probably we would conclude that there was more activity of tenofovir versus alefovir. So tenofovir is very potent against Hepatitis B.

As many drugs, as many will say, if something is active will select for resistance, there is not something magical in all these antiviral drugs. That's true. And in fact with the collaboration with Professor Locarnini and Julie Sheldon studying patients from Europe, they check that people with detectable beremia and on tenofovir for longer than six months and she was able to recognize a mutation that in combination with the mutations for clomidodine [misspelled?] produced in vitro were [inaudible] for the drug. The interesting thing here, and we have now three of these patients, is that these patients with undetectable beremia after one year even despite the presence of this mutation. So really the exact role and

the clinical relevance of the mutation is still merits further pursuit.

Just to make things simple, but they are more complex, at the moment we can just put in NEXUS [misspelled?] the potency and the genetic barrier, we are familiar with using this drug HIV. But for Hepatitis B we may say that the drugs with low genetic barrier are mainly clomidodine and FTC with more potency of tenofovir and decovir and eldidodine [misspelled?] and with less potency than elfovir. For interferon there is no evidence of resistance in the virals. There is something different, a different mechanics. So let's say totally separate. The message here, also is that when we think about combination therapy, all the pathways Dr. Locarnini has mentioned before, need to be restricted because there is only one passage to resistance rate of Hepatitis B to the potential of combination of the drugs in red with the drugs in green. The nucleocytes [misspelled?] with the nucleotides. This is not a good idea to try to combine amidodine with LDT or entecavir with LDP because all of them are in the same pathway of resistance. Now Fabuengulen [misspelled?] in a more conservative approach than combination therapy, that as I mentioned before, the data should both be more antiviral [inaudible] are still lacking, no evidence for that, just protection of selecting resistance has made this point anything

is attractive in the sense that he defend their probably using very potent drugs, not a Lamodomin, because it's too fragile. But like Indecadeal or Ideforel [misspelled?] or Danafodel we kind of start with mono-therapy and in six months we don't reach undetectable viremia. This is a threshold for starting to put this drugs in place for resistance. We need to want something more. And they start the combination therapy, not from the beginning, but at six months. But if you reach undetectable viremia with only one compound, you can spare the costs and the tolerance of new drugs added from the beginning to this approach.

Well the benefit of putting these patients with undetectable viremia for a while, just two points, and this has been reproduced also by the French group of investigators. As long as you stay with undetectable viremia, it's just a matter of time. The rate of circumvention of the antigen E [misspelled?], the rate of circumvention of the [inaudible] antigen is increasing. So as long as you have undetectable viremia, and this is the goal, to put these patients with undetectable viremia for a while. And in this [inaudible] for example, this is five years with Anthrax active against Hepatitis B and HIV co-infected patients. The proportion rich in clearance of the surface antigen is growing and growing and growing up. And this is certain those who are not benefiting

[inaudible] and using for example the fiber scan. You can see that a proportion of patients, co-infected patient with six years of average of undetectable viremia for Hepatitis B is really low. And this explains that in a country like my country that we have transplanted more than 100 patients right now. The proportion with chronic Hepatitis B is very low, almost all the liver transplants in co-infected patients have been with [inaudible] and few Delta. But for B you see manibobine [misspelled?] and for the last five years danafobile [misspelled?], all these patients are going off the list of transplants because [inaudible] stop and then sometimes there is evidence of digression.

Just what it's like on Delta and the dual infections, Delta, this a strange, very small virus is actual [inaudible] satelic [misspelled?] of Hepatitis B [inaudible] that used this as percentages for genotype encapsulation, is a circular iranea [misspelled?] molecule, very small, it half Hepatitis B. Five-percent overall in the world of Hepatitis B carriers are infected with Delta. But that represents 50 million people. Half of patients progress through [inaudible] services within 20 years. This is the more aggressive Hepatitis virus. Delta [inaudible] use not puliceramus [misspelled?] from virus on the hospiramus [misspelled?]. And that is critical because we will not develop a specific drugs against Delta. And intensive

interferon therapy is the only available treatment option. The response is quite poor in any case.

Well we have the opportunity to establish 15 Delta patients co-infected with HIV. So this is HIV, Hepatitis B, and Delta. And we provide them [inaudible] FDC plus minus denafobere [misspelled?]. And we selected these patients because they have undetectable viremia for Hepatitis B because most of them because the interferons, the have undetectable viremia for Hepatitis B from the beginning. And Delta viremia was very high. And what we saw extending the duration of follow-up for six years was a significant reduction in Delta viremia. And in our knowledge, this is one of the first evidence that nucleosides [misspelled?] for extended period of therapy and being successful at making complete suppression of Hepatitis B replication maybe somewhat active against Delta. And in fact, in this series this was presented in Paris a few months ago by Julie Sheldon [misspelled?]. There was a normalization of LD levels in a significant proportion of these patients. And in two of them that discontinued therapy for some reason there was a flare up in Delta viremia and in transaminases [misspelled?]. So it looks like prolonged successful therapy for Hepatitis B may benefit also Delta virus.

And for those who are infected with Hepatitis C and chronic Hepatitis B, what to do? These patients, and there are several papers have shown that. And they progress faster to [inaudible]. So their prognosis is much poorer. We in this study in treating patients who have Hepatitis C replicating virus or Hepatitis B, the message was that treating Hepatitis B was no evidential at any single rebound in Hepatitis B viremia. And just the contrary also was true in the majority of patients treating Hepatitis C was no evidence of significant virexium [misspelled?] B viremia when the treatment was provided and they stop it later on, interferon plus [inaudible]. So that means that treating these patients, think with the replicating virus in these patients, there is not much release, at least in a significant proportion of these patients to a rebound or escape of the other virus because Walsh [misspelled?] presumed viral interference at the beginning.

So in summary, chronic Hepatitis B progressed faster then HIV. It increased the risk of [inaudible] using antiretroviral agents. The treatment of Hepatitis B should be considered as a priority for these reasons. All co-infected patients should be tested for viral load, the genotype, and liver fibrosis as is by either non-invasive [inaudible] biopsy. Then you stick the C or FDC as only anti-viremias be aging as from 2007. This is very clear. And only economic restrictions

may justify to do the country. Hepatitis B therapy plan should be individualized based on the need for HIV treatment. This is the first condition and pre-[inaudible] therapy. To stop integration of advanced [inaudible] can be seen with prolonged [inaudible] of viremia. It's good always, [inaudible] percentage in most of these patients and treat the active replicating virus in dueling type of patients.

And just to finish, this was present [inaudible] one year and a half ago at the Denver conference. And I think this is the message probably would be the best thing to take home from this lecture is that this happens in the US. This is not Tanzania or very poor country. No, no, no, it's the US. So they presented this place. So this is a group of 362 co-infected patients. It's a large number of co-infected patients. Only 18-percent of them have been tested for the E-antigen [misspelled?] or half make the biolog [misspelled?] test for Hepatitis B. And only one-third have this [inaudible] starting HAAR [misspelled?]. Among 162 patients will be on HAAR, the median number of tests for HIV viremia at one year was three in contrast for Hepatitis B viremia was zero. The frequency of hepatic bullosum [misspelled?] was only one-third of the patients. Only 43-percent of cytotic [misspelled?] half of [inaudible] there is for [inaudible] carcinoma. And the conclusion of the other [inaudible] was that they progressed

much better in HIV than in Hepatitis B cure, at least in this co-infected population.

And just for those more interested in co-infection, just announced that next year in June there will be the fourth co-infection meeting and will be held in Madrid and I think 21 of the next year. And thanks for your attention [applause].

MALE SPEAKER: Alright. Thank you very much for this very nice and comprehensive overview. I think Gail has a question.

GAIL MATTHEWS, M.B., CH.B., M.R.C.P.: Yes, Vincent, I was just going to ask about hepatic [misspelled?] flare after initiation of Hepatitis B active heart and whether you see this often in your population and whether you have a specific strategy for those patients that you can't treat through, so people with sclerosis maybe or baseline?

VINCENT SORIANO, M.D., PH.D.: This is a good question. Sometimes with start with Turbata [misspelled?] in a patient with Hepatitis B and another compound [inaudible] nucleoside and after a few weeks you see an elevation in transaminases [misspelled?] and always they develop these whether there is a [inaudible] or the antiviral drugs you are using or this is a just a circumvention for the Hepatitis B occurrence that is associated with an elevation in transaminases. You need to check that repeating the serologies [misspelled?] because I

would wait to do that and in some cases if the transaminases are not going very high, let's say more than three, four I will recommend to stay on therapy and not putting the patient at risk. But if the transaminases are going very high, I will stop the therapy at the moment. Yes.

GAIL MATTHEWS, M.B., CH.B., M.R.C.P.: Do you ever use an induction strategy to bring down Hepatitis B DNA first before you start?

VINCENT SORIANO, M.D., PH.D.: That's also with western, but becomes very difficult because you will need to use same drugs that are active against HIV pulling the resource electing HIV resistance. So you'll will it do something like with averrable [misspelled?] at the beginning and later on switch to denaferber [misspelled?] and FPC. And I feel that many of the practitioners will not do that.

FEMALE SPEAKER: Okay. There are no further questions and we're a bit out of time, so I'd like to bring this session to a close and thank once again all the presenters for really very excellent talks today and a really nice summary of the data. [Applause]

[END RECORDING]