NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE (RAC)

First National Gene Transfer Safety Symposium: Internally Deleted, Helper-Dependent Adenoviral Vectors

This is an excerpt of the transcript of the March 8-10 RAC Meeting. It has not been edited and the NIH makes no representation regarding its accuracy

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DR. MICKELSON: We will go on then to the mini-safety symposium today that deals with some of the issues in the use of an internally deleted, helper-dependent adenoviral vector in humans.

The review of the particular IND and submission to the NIH, FDA requested that this protocol be brought to the committee. At that time they had -- their letter and some of the safety issues that they felt that this new vector raised can be found in your notes at tab 2115 and we are going to have a fairly detailed discussion after the presentations from the investigators.

We asked the committee or OBA asked for two ad hoc advisors and reviewers for this protocol, Dr. Frank Graham from McMaster University and Dr. Jeffrey Chamberlain who is here today as well.

I think the way -- at least the way it is down on the agenda, the way this will go is that we will have a presentation by Dr. White from the University of North Carolina, who is the actual clinical investigator. He will introduce and give a short presentation about the protocol.

Dr. Wei-Wei Zhang from the GenStar Therapeutics that generated the vector will then do a short presentation.

And then Dr. White will follow-up.

Then what we have down is a short presentation and discussion by Dr. Chamberlain, who is one of the ad hoc reviewers.

And then we will go into a general discussion for the committee that is led by Dr. Ando and Dr. Aguilar-Cordova where we will deal with all of -- there will be a general presentation by them and an attempt to deal with some of the questions that were brought up both by the FDA and then some from various nonscientists, myself, who did not -- who want some things on the table.

Dr. White, if you could start, please?

Do you have slides, Dr. White?

DR. WHITE: I do but I will hold them for a minute.

Good morning, ladies and gentlemen.

My name is Gil White. I am a hematologist from the University of North Carolina at Chapel Hill and I am the lead clinical investigator on this study.

My colleagues that are here today are Dr. Wei-Wei Zhang, who is vice president and chief scientific officer of GenStar Therapeutics; Dr. Robert Sobol, who is president and CEO of GenStar; and Dr. Gordon Bray, who is medical director of the Highland Immuno-division of Baxter Health Care.

I thought I would start my presentation off with just a brief introduction of myself. I am director of the Hemophilia Treatment Center at the University of North Carolina at Chapel Hill. This is the second largest hemophilia center in the country and one of the largest in the world. We see over 600 patients with a variety of bleeding disorders.

Traditionally, this center has its roots back in the 1930's. It is one of the oldest hemophilia centers and it has a long and strong tradition of clinical research.

The partial thromboplastin time was developed by Dr. Kenneth Brinkhouse, who was really the initiator of the Hemophilia Treatment Group at the University of North Carolina.

Factor VIII was discovered by Dr. Brinkhouse.

The initial plasma concentrates of Factor VIII were developed there.

The initial clinical trials for the recombinant Factor VIII and Factor IX were performed at UNC.

The initial identification of HTLV-3, what we now know as HIV-1, was performed using patient samples from the Hemophilia Treatment Center at the University of North Carolina.

We have a very strong interest in clinical research that leads to better treatment of patients with hemophilia.

I am also chair of the Research Working Group at the National Hemophilia Foundation and in that position and in collaboration with others at NHF we have developed the research for a cure program at NHF and I have a very strong commitment to gene transfer research.

I have a strong belief that gene therapy ultimately will provide a very important treatment approach to patients with hemophilia and I am believe I am supported in that belief by the patients and the parents of the patients that I serve who have strongly encouraged our center and at the level of the NIH gene transfer studies.

So that is my background. I just want you to know where I am coming from and why I am involved in this clinical trial.

Can I have the first slide?

(Slide.)

The other clinical investigators involved in this study are Dr. Arthur Thompson from the University of Washington; Dr. Wing Wong from the Children's hospital in Los Angeles; and her colleague, Dr. Donald Kohn.

(Slide.)

Hemophilia A is a severe bleeding disorder and in its most severe form in patients who have Factor VIII levels of less than one percent of normal there is a high risk of spontaneous hemorrhage which results in joint, soft tissue and organ morbidity and due to intracranial bleeds and other serious bleeding events a fairly high rate of mortality.

Current treatments of hemophilia include concentrates that are derived from human plasma and more recently recombinant concentrates of Factor VIII and Factor IX.

These products, although the safest and most advanced products that we have ever had, there is still concern by the hemophilia community about these products and they still are not uniformly effective because they require periodic infusion.

Minimal beneficial levels of Factor VIII so normal levels of Factor VIII are 60 to 150 percent but if one can actually increase Factor VIII levels even as little as one or two percent up to a level of two to five percent one can get significant beneficial effect from even these small levels and one can change hemophilia from a

severe bleeding disorder to a moderate or a mild bleeding disorder.

Patients currently treat themselves in a manner that we call demand treatment so a patient will have a bleed. He will then treat himself. That will limit the extent of the bleed and, hopefully, limit the complications from the bleed but treatment is generally after a bleeding episode occurs.

In young patients we can use various forms of prophylactic treatment once a week, twice a week, perhaps even three times a week or even every other day. And one sees in patients who have -- who are on prophylaxis regimens that prophylaxis can, indeed, as one would expect prevent joint and soft tissue bleeds.

The problem with prophylaxis -- the good thing about prophylaxis is that for young patients it is beneficial. The problem with prophylaxis is that it becomes more expensive as the patients get older and get bigger and require more factor replacement and even prophylaxis every other day does not lead to continuous levels of Factor VIII or Factor IX and, therefore, is not completely preventive.

So the thing that is exciting to this community about gene transfer treatment is that it potentially in its optimal form provides a mechanism by which continuous levels of Factor VIII or Factor IX can be achieved and would lead to improved therapy compared to what we currently have, which is intermittent injections.

(Slide.)

So the mini-Ad vector that will be used in these studies is a minimal or gutless adenoviral vector which has no viral coding sequences. It encodes the full length cDNA for human Factor VIII. It has been optimized for gene expression and stability in the liver by the presence of a human albumin promoter and genomic fragments from the albumin gene which promote this liver specific expression and stability.

The vector is high purity and replication competent adenovirus free.

In preclinical studies over eight to twelve months it has shown sustained expression of therapeutic levels of human Factor VIII both in a hemophilic mouse model and in C57 Black mice.

And in these hemophilic mouse -- in the hemophilic mouse model this sustained expression of therapeutic levels of Factor VIII has provided correction of the phenotype in those mice.

And the thing that is most, I think, exciting and most important from the point of view -- from my perspective really is the improved safety of this gutless adenoviral vector compared to earlier generation adenoviral vectors.

This is, if you will, an introduction to Dr. Zhang who will now present some of the details in each of these areas with respect to the mini-Ad Factor VIII vector.

Wei-Wei?

DR. MICKELSON: Thank you, Dr. White.

DR. ZHANG: Thank you, Dr. White, for the introduction.

Dr. Mickelson, Dr. Patterson and Dr. Noguchi, ladies and gentlemen, I appreciate the opportunity here in the RAC we have a chance to present

our data and to have a public oversight in the development of this new vector system.

Probably, also, I would like to introduce really myself before I enter the presentation. To develop a safe, effective gene delivery vector is our dream and is also the years of our pursuit. I recall -- I remember vividly about seven years ago I also made a presentation to the RAC about the first generation adenoviral vector, which is the p53 adenovirus in the other side of this building in the similar size of this conference room.

Through the years from about seven years we continually work on a safer and better, more deliverable capacity vector system from the early generation vector and today this vector is called a gutless vector. We call it minimal adenoviral vector which has been deleted with all the viral coding sequences. We would like to demonstrate to you this vector has – we have significantly improved the safety profile of this vector for gene delivery.

(Slide.)

In comparison of early generation vector the minimal adenoviral vector does not contain any viral coding sequences and only contains less than one 1 Kd virus cis element. The comparison with early generation vector are summarized in this slide.

Look at this wild type virus. Sometimes we call it RCA replication competent adenovirus. It has -- all the red bars represent the coding sequences on the viral vector and through the development of adenoviral vectors from first the generation and later on this group who could be called second generation developed into the mini-Ad up to today.

The early generation vector all depend on cell line and it all called helper-independent replication defective adenovirus.

The cell line development is critical for any new vector development for early generation because the viral coding sequence has to be put into the cell for complementation purpose. Early first -- first generation, the 293 cells was originally developed by Dr. Frank Graham -- contained an even region of adenovirus but later on the 2A region was put into the cell and E4 region was put in the cell, then the generation develops from early generation.

But the problem and limitation with this approach is that the virus sequence coding gene, put it into the cell causes cells sick (sic) and not performing well for production. Then, also, there are some no -- currently no possibilities to put all these viral coding sequences into cell permanently.

To break the limitation, you know, we developed the vector system that is called helper-dependent from scientific sense but actually it also is called a larger capacity, gutless and minimal adenoviral vector because the coding sequence are being deleted and it is provided by a helper virus.

(Slide.)

Our vector system has three components. One is minimal adenoviral vector. The other is a helper virus. We call it ancillary Ad. Then the round circle is the production cell. The minimal adenoviral vector as I have described only contains the minimal virus sequence cis element for DNA replication and the packaging.

The ancillary Ad is a typical E1 substituted first generation adenoviral vector. Please notice there is a Lac Z gene as a reporter for us to determine later what is the contamination level of this helper. And it also has a packaging signal partial deletion. This will allow the preferential packaging of mini-Ad through this production system and the production cell line is a cell line like 293 cell but we develop our own A549E1 cell I am going to describe a little later.

For the vector design we did incorporate several features to support the high level production as well as the expression and persistence of the vector in liver.

(Slide.)

It is well-known adenovirus has a tropism to liver and based on that our next thought for the vector development is how to make high level expression and a persistence of the vector in the packaging cell.

What we designed here is incorporate genomic sequences of albumin into our vector. Please notice the gutless vector has a capacity up to 36 Kd and it is critical and it is important to fill up that space with certain amount of DNA also to be useful for the vector.

And it also been well-known genomic fragments usually can stabilize foreign DNA in the target cell. We use albumin gene promoter. Also consider that albumin gene is the major hype (sic) producer and constituitively expression at high level in liver and we would like to take this advantage as well.

So in our vector we have a 12.5 Kd human albumin promoter in the front and we also have a 7 Kd albumin genomic fragment in the downstream flank the human Factor VIII cDNA and all added together it is a 27 Kd expression cassette. This is also a remarkable feature for the vector. This kind of size of expression cassette has not ever been delivered by any other vector system before.

With this design feature we hope that the Factor VIII will be expressed at a higher level and also the vector can be -- persist in the tagged cell.

(Slide.)

On the other hand we considered the expression and the production of the vector. We all -- by the vector design time we knew that the 293 cell is prone to generate RCA because it contain extended E1 region. Actually it is all at the left end of the adenovirus.

Then we trim down the E1 region to the minimum sequence and put into A549 cell and generate the A549E1 cell line. The advantage of this cell through all our production and composition (sic) will demonstrate it generates RCA-free adenoviral vector and this is the major feature for our vector system.

(Slide.)

Also this cell line can be adapted in the serum free production with other features. For a safe clinical trial initially this -- the first priority is the safety and the clinical material specification also is so critical and we had a thorough discussion with FDA and generated this final product specifications.

There are a set of parameters to guide this material be clean (sic) and high quality to enter clinical trial. The major point here I would indicate is we did not

have RCA detection in our product. The detection limit sensitivity is less one RCA per 10^9 viral particle, and also we limit the ancillary Ad contamination in the final preparation of the clinical material, and our limit is less than .1 percent, and I would like to demonstrate how this was measured.

(Slide.)

We have two methods to determine the purity of the final product to use. In the system current -- this is our purification process. We got two fractions. The fraction one -- the top band is usually contained 99.99 percent mini-Ad product. And at the fraction two contains majority of the ancillary Ad.

When the fraction one DNA is extracted then digest (sic) with enzymes, which we have specific cuts and generally specific event, running in X-gel (sic) you can see these bands are the mini-Ad bands. But if there is ancillary Ad contamination you would see a band right indicated by this blue arrow.

Here no band is visible and it is indicating that the ancillary Ad already under the detection limit by enzyme digestion. Actually this is a very solid measurement of ancillary Ad contamination but we would like to push the detection limit up using a biological method to determine the contamination.

As I indicated earlier the ancillary Ad has Lac-Z reporter gene. We use X-gel staining to determine the contamination level because any cell infected by ancillary Ad will be stained in blue. And we determined in fraction one less than .001 percent is like one cell in 10,000 up to 100,000 non-stained non-blue cell was usually observed in our preparation. And fraction two usually contained all the majority ancillary Ad, the blue staining shown there.

(Slide.)

With the purified vector we did our preclinical studies in hemophilic mouse model as well as C57 Black mouse model.

The upper panel is the hemophilic mouse model. Each line represents one mouse with a single dose tail injection at two by 10^{11} viral particle per mouse.

After injection the human Factor VIII was detectable in plasma and several mice had long-term expression and one reached more than a year, up to 500 days, close to 500 days. And there are -- out of two we had early sacrifice done for the study purpose.

And also there are several groups of short-term expressors, although it is quite -- it reaches 60 days already. Those mice had an antibody -- anti-human Factor VIII antibody developed and then the expression was limited.

We also had a dose response study in C57 Black mouse model and each line represented an average of six mice. With lower dose at two by 10^6 viral particle per mouse, up to four by 10^{11} viral particle per mouse we had a clear dose response curve lines and also long-term expression was obtained.

(Slide.)

In another set of studies we studied the functional efficacy of the expressed human protein in mouse -- in hemophilic mouse model. Twelve mice each, hemophilic mice per group, were treated either 2.5 by 10^{11} viral particle per mouse or

vehicle as control.

Then capillary clotting test was correct in contrast to the C57 Black mouse.

And so tail clipping blood loss was corrected as well as the tail clipping bleeding time was corrected. The hemophilic mouse with vehicle control had no clotting and no survival through this experimentation.

(Slide.)

Based on the efficacy study we moved into safety study and also this was a protocol generated through extensive discussion with Dr. Ann Pilaro and Dr. Saragin (phonetic) in FDA. We had 240 mice all together to study the following parameters:

For example, in plasma we determined Factor VIII level, anti-Factor VIII, anti-vector -- antibodies for the blood, which have the classical hematology panel, and we also studied the tissues for biodistribution vector persistence.

For serum we studied the clinical chemistry, particularly liver enzyme profile and also we did histopathology for this tissue.

(Slide.

Two representing data I would discuss here, particularly the liver enzyme profile. You can see all -- at two high dose group, 1.6 by 10^{13} virus particle per kilogram, and the next lower one, 1.6 by 10^{12} viral particle per kilogram in contrast to the green PBS control. All enzyme level is within normal limit. All except one mouse here outlier in the high dose group which is slightly higher than upper limit but may have a contribution of hemolysis.

And I would like to indicate this profile has a significant improvement from the early generation vector because at this dose, 1.6 by 10^{13} viral particle per kilo, for early generation vector usually result in death of animal or the elevation of ALT up to 1,000 unit per liter.

(Slide.)

Actually I have a table cited published data from other laboratories as well.

For example, the gutless vector from Dr. Tom Skatsky's (phonetic) laboratory -- Tom Skatsky's laboratory -- they study in mouse. Using a very similar dose they have ten-fold less liver enzyme elevation and no abnormal liver histopathology.

From Stefan Kushniek's (phonetic) laboratory, they did also similar dose and they found decreased acute toxicity and no abnormal liver histopathology.

And from Arthur -- Dr. Arthur Boudett's (phonetic) laboratory, either in mouse or baboon they found no significant toxicity at least for eight weeks on an even -- I think here it is beyond eight weeks both in mouse and in baboon.

From our laboratory we already published this information in Blood as well as in the Hemostasis, Thrombosis and Hemostasis Journal, those four mice and a dog at a dose as indicated here and liver enzyme or liver information indication are minimal. There is no liver enzyme profile difference from the same -- from the control in dog study.

And I think Dr. Chamberlain and Dr. Aguilar-Cordova probably also had a similar experience with their study.

(Slide.)

And on the other side we looked at the hematology panel. This is a classical hematology panel. The WBC, neutrophil, lymphocytes, platelet counts are listed here just for concentrated on the transient (sic). And mainly no major difference of the highest dose at 1.6 by 10^{13} per kilogram in contrast to a control. But there is a transient platelet count decrease in this highest dose group on day three post injection. At day three post-injection but it was transient and it was reversed on day 15.

This platelet decrease was also observed and also reported by either group for early generation at a dose much lower than the dose in here.

(Slide.)

For a summary of the safety study there is a transient decrease in platelet counts at day there post injection in the highest dose group. There was a mild elevation of ALT in the highest -- at 1.6 by 10^{13} viral particle per kilo but it is still under normal limit.

And, also, minimal cell infiltration in liver was found at a dose of 1.6 by 10^{13} viral particles per kilo.

Anti-vector and anti-human Factor VIII antibodies were detected in all treated mice groups as expected because it was human antigen.

And no other significant toxicities were observed in this safety study.

(Slide.)

We also looked at the biodistribution as well as the persistence as defined in the two parameters for the safety study. As ten organs per each mouse were collected and the samples were analyzed by a semi-quantitative PCR and the PCR sensitivity goes down to one copy per nanogram DNA.

You can see liver constantly is the major organ to receive the vector as reported and confirmed by all other laboratories or investigators previously.

(Slide.)

This is the slide as an example at day 15 post-injection of biodistribution you can see liver, this is a standard curve showing our sensitive down to the one nanogram, one copy per nanogram DNA.

(Slide.)

And this -- we have another set of experiments to study the persistence of DNA in liver and the method used here is to use PCR to probe five separate regions along the vector genome. Particularly important is there are two regions at the end of the genome. If there is any integration or loss or amplification of the genome then the probing of the region, the copy number will represent differently.

The samples were collected from all three long-expressors of hemophilic mice. The longest one is over a year, which is the pink line here. And you can see for along the genome this is point A and this represents point B, this represents point C, D and point E.

And please notice for point A and E all the N's are retained at a

similar copy number which suggests that the DNA of the vector are kept in an intact form in a nucleus and in antagonist cell.

(Slide.)

So for a summary the vector biodistribution and persistence, majority, more than 80 percent are the vector localized to liver by PCR assay and a vector DNA persistent in the liver in an episomal form over one year.

And so far those are the selected slides and information to present to the RAC committee members as well as to the public for the vector clinical -- preclinical data.

And Dr. White will continue to introduce the clinical protocol to you.

Thank you.

(Slide.)

DR. WHITE: This committee has considered clinical trials in hemophilia before. I do not think I need to go through the entire rationale.

I think that for this clinical trial I think I will simply point out that we believe that this clinical trial is -- should be considered at this point in time largely based on the improved safety profile of the mini-Ad vector, which you have just heard, and partially based on its tropism for the liver. The liver is the major organ for Factor VIII production and adenovirus is tropic to the liver and has a high infectivity rate in the liver.

(Slide.)

This is a Phase I single dose, dose-escalation designed trial with two doses, 1.4 times 10^{10} viral particles per kilogram, which will be initially studied in three patients in the first cohort and then a dose of 4.3 times 10^{10} viral particles per kilogram in the second cohort of three patients.

The route of administration is by peripheral intravenous infusion and I would point out that the levels of virus that are being administered here are three orders of magnitude below the level of virus that was given in the mouse model where you saw the thrombocytopenia and is also two levels of magnitude below the level of virus that gave basically normal levels of Factor VIII in the hemophilic mouse.

Even though it is two levels of -- two orders of magnitude below the level that gave therapeutic levels in the mouse or gave normal levels in the mouse, we think there is a possibility that we could see small levels of Factor VIII in humans largely because we expect that the vector will be more efficient in humans because of the human albumin promoter and more specific expression in the human.

(Slide.)

Inclusion criteria, patients with severe hemophilia A with Factor VIII levels of less than one percent, over 18 years of age, these will all be heavily previously treated patients with hemophilia in order to reduce the risk of natural inhibitor formation.

So all of the patients will have greater than 150 days of exposure to Factor VIII concentrates of any type and in a change from what was initially distributed to you the patients will be required to have normal AST levels and

informed consent will be rigorously obtained.

I emphasize that word in particular.

(Slide.)

Exclusion criteria -- I will not go through all of these for the sake of time but again patients will elevated ALT's will be excluded, patients with a prothrombin time more than two seconds above control as another measure of liver function, total bilirubin greater than two, patients with a platelet count of less than 100,000 will be excluded from the study, patients with a history or the presence of a Factor VIII inhibitor and patients with clinically significant cardiovascular autoimmune and especially pulmonary disease because of adenoviral distribution to the lung.

(Slide.)

Study parameters are summarized here. There will be four basic study parameters, safety parameters which include hematology, chemistry, urinalysis and physical exam. We are particularly interested in immunogenecity and Factor VIII antibodies and anti-Ad antibodies will be looked for.

We will look for efficacy although this is a toxicity study but we will measure Factor VIII levels and quantify bleeding events and we will also look at clearance of vector and Factor VIII levels, i.e. the persistence of any effect that we might have.

(Slide.)

We have built into the study what we call stopping rules and there are basically two categories of stopping rules that relate to the liver. Any two patients with an ALT that increases to two times the upper limit of normal or have any grade two toxicity we will initiate these stopping rules and upon achievement of these stopping rules we will suspend the study and immediately communicate with the FDA and the RAC.

The other stopping rule would be any single patient who develops a Factor VIII inhibitor or any grade three toxicity.

(Slide.)

There will be a Data Safety and Monitoring Board that will monitor these results. This board has been organized. The board will have regular reviews of the study data and will submit reports to their IRB's or to the IRB's at the participating institutions.

Safety data review with FDA will be carried out as required by the stopping rules and of course any severe adverse events will be promptly reported to the FDA and NIH as required.

(Slide.)

So, in summary, mini-Ad is a gutless adenoviral vector which encodes the full length human Factor VIII cDNA. In preclinical studies there has been sustained high level gene expression in hemophilic and non-hemophilic mice. And in those hemophilic mice there has been restoration of normal clotting activity.

Mini-Ad Factor VIII displays liver tropism and DNA persistence. There was minimal toxicity in animal models. The clinical trial is designed as a Phase

I study with two cohorts of three patients each and patients with severe hemophilia A who are HIV negative but may be HCV positive, single i.v. dosing at the doses that I indicated, and safety and efficacy will be measured as I indicated.

And I will stop there and not answer questions or answer questions.

DR. MICKELSON: No. I think we will go on to just some of the reviews and then will be time later.

Thank you very much.

What we have next -- I would like to – even though we started early we are actually just now on schedule so if we could start with Dr. Chamberlain's comments if you would, and then we will move after the comments into the general discussion led by the other two reviewers, Dr. Ando and Dr. Aguilar-Cordova.

Dr. Chamberlain?

DR. CHAMBERLAIN: I have a couple of overheads.

Okay.

Is this working? Can you hear me?

What I would like to -- I can go ahead and just flash those up when I am ready.

What I would like to do today is make a general comment about the helper-dependent or gutless or gutted adenoviral vector system and it is interesting to note that virtually every laboratory that works on this system has developed their own name for what the system is so it can sometimes get a little confusing. I will try to remain somewhat consistent in my nomenclature here.

At the outset I would like to say that I think the available data that we have seen in the literature and presented at a variety of meetings suggests that the gutless or the gutted adenoviral system really does have great potential; that it does in most side by side comparisons appears to display significantly reduced toxicity and greatly improved safety compared to first generation and even second generation adenoviral vectors.

At the same time, however, simply putting an expression cassette into a gutted adenoviral vector system does not necessarily guarantee that you have an improved system and a safer system and so I think it is very important to consider what goes into this system, how the virus has grown, and what sort of quality control assays are put into it.

So what I would like to briefly review is some of the basic features again of the gutted adenoviral vector system so that I can highly some things that I think that both the FDA and the RAC may want to consider in reviewing not only the current proposal before us but also potential future proposals that may come before these committees in deciding the potential safety of these systems.

So I am going to just overview some general features that hopefully will lead into some discussion of the current protocol.

(Slide.)

Okay. Just a brief reminder. This is similar to a slide that Dr. Zhang showed. Basically what a gutted or helper-dependent adenoviral vector system is, is composed of a plasma DNA that contains an origin of replication of adenovirus and a

packaging signal together with a therapeutic gene.

But this -- since this is not a virus itself it can only be grown in the presence of a helper virus or an ancillary virus that provides all of the necessary proteins for replication and production of the so-called gutted virus.

There are various genetic manipulations of the gutted and the helper virus that can manipulate the ratio of the gutted and the helper virus that is produced. There is genetic selections that can be imposed on this system but then the -- what is produced from these cells is a mixture of gutted and helper virus, which can then be separated physically on chloride gradients due to a difference in the size and the density of these viruses.

(Slide.)

An important point to keep in mind is that the gutted viral system is a little more difficult to grow and produce than conventional adenoviral vectors and that is because one starts out with plasma DNA that has inefficient origin of replications on it.

So typically labs have to go through a variety of serial passages taking the lysates from earlier cell lysates and putting them on new cells, and eventually the titer of the gutted virus will rise significantly approaching that which can be obtained from a conventional virus and so this leads to the issue of how the serial passages are done and in what types so that leads to some quality control considerations.

(Slide.)

Another thing that can occur -- I would just like to point out somewhat of a negative scenario that occasionally can occur although not usually, and this is looking at the titer of a gutted virus versus the helper virus during a serial passage.

This was an experiment that was performed in my own laboratory and in this situation as you can see with repeated serial passage the titer of the gutted viruses rises considerably whereas the titer of the helper virus is generally somewhat constant although occasionally you can see a situation like this where the apparent titer of the helper virus begins to plummet considerably and upon examination of what was going on in this particular experiment --

(Slide.)

-- doing a Southern blot of viral lysates from some of these different time points revealed that after a tremendous number of serial passages, in this case 9 to 12 different serial passages, we did start to detect rearranged products from both the gutted virus and the helper virus.

So as opposed to a conventional first generation adenovirus it goes through one round of growth and replication. The gutted viral system requires multiple rounds of replication that has some potential to lead to rearrangements of these viruses and that is something that needs to be monitored quite closely.

(Slide.)

I think another serious issue that needs to be taken into account is how much helper virus is present in these preparations and not only how much helper

virus but what is the nature of that helper virus and how is the integrity of the helper virus and the quantity of the helper virus determined.

And here is another fairly simple experiment again that we performed in our laboratory where we did serial passaging of a gutted and helper virus and at the end we wanted to determine how much contamination of helper virus we had in our preparations.

I have only shown two assays here. We actually have now done three assays but if we assay the amount of helper virus by looking at plaque forming units you get a particular number. In this case, four times 10^7.

On the other hand, if we assay transduction of cells with a reporter gene that was present in the helper virus we get a significantly greater number and, in fact, now if we come back and use a quantitative PCR to assay how much helper virus it turns out that we would be about five-fold higher than this number here.

So it is important when people talk about determining how much contamination of helper virus they have that we pay particular close attention to which assay is used and what the true level of contamination is.

(Slide.)

So I think some of the issues that we need to consider when discussing the safety profile of these vectors is briefly outlined on this overhead.

First of all, the gutted virus itself has no viral genes whatsoever and it is incapable of viral gene expression and that seems to be a critical feature of this system to give it safety and extended persistence.

However, not every single gutted virus is going to display long-term expression and some of the factors that seem to be important in this, which Dr. Zhang alluded to also, is it appears to be very important to have tissue specific expression of the transgene within your gutted virus and I think all the published data with extended persistence have used tissue specific promoters and we have some data from our own laboratory showing that if we insert an immunogenic transgene into a gutted virus we do not get extended persistence particularly when that transgene is expressed from a ubiquitously active or viral promoter.

However, if we switch expression of this foreign transgene to a tissue specific promoter we get improved safety and improved persistence, and if we remove that completely and only express a natural gene product under a tissue specific promoter then we see the extended persistence and improved safety of these vector systems so I think it is important to consider what genes you have in your gutted virus because that can make all the difference in how safe the system is.

Equally important perhaps is the nature of the helper virus. Some issues I have alluded to are the percent contamination of the helper virus. We need to consider how much and what type of gene expression is coming from that helper virus, both viral genes and potential transgenes or reporter genes. What is the safety profile of that helper virus itself and what is the stability, relative stability of both the gutted and the helper virus?

(Slide.)

And this is my final overhead defining the questions slightly

differently that, in general, it is important to assess the toxicity of different types of gutted vectors, that they are not all going to be the same. Do they really display tissue specific expression? And do they display greatly improved safety in side by side comparisons with first generation vectors?

One other point I want to point out is that even though the gutted virus itself or the gutted vector -- I should not call it a virus because it is not actually a virus -- but the gutted vector even though it has no viral genes it is still encapsidated in an adenoviral coat and we need to consider what are the potential toxicities of high dosage administration of the adenovirus capsid even in the absence of adenoviral genes.

How much helper contamination is there and how is that measured? What is the actual structure of the helper virus? Is it stable upon repeated passages? How much transgenes does it express and is that helper virus a first or a second generation adenoviral vector? What types of quality controls are we going to require for these trials?

And I think a critical issue here is remember that a variety of serial passages are needed to obtain high enough titers of these vectors to make them useful and we need to seriously consider the issue of what is a seed stock defined in this situation.

What kind of quality control is required to verify the integrity of that seed stock and then how many additional rounds of serial passage are needed to go from a seed stock to a clinical grade vector preparation and can we be sure that there have not been subsequent rearrangements of the vector in those final round or rounds of growth?

What titers are needed and what kind of level of expression per particle is going to be a critical parameter and it is -- various laboratories have had somewhat greater difficulties growing these vectors compared to conventional adenoviral vectors and can they be scaled up and we should consider is it possible for an individual laboratory to prepare enough of this vector to achieve a therapeutic dose?

So I think I will stop right there and, hopefully, this will set up some further discussion.

DR. MICKELSON: Thank you.

Thank you. I was just reminded actually that people might want a coffee break. Would you? Fifteen minutes and be back at 20 of 11:00 and we will start the general discussion.

Thank you very much.

(Whereupon, a break was taken from 10:25 a.m. to 10:44 a.m.)

DR. MICKELSON: We would like to restart then with the presentation of the questions to -- that came from the committee reviewers and the FDA and other RAC members.

Dr. Ando is going to start presenting that with an overview of the protocol and some of the issues that are embedded in this particular protocol.

We have had a request, Dale, that if you could stand at the podium and then someone handle the overheads for you then the recording mike gets better

sound. Thanks.

DR. ANDO: What I want to do is go over some of the general questions about the helper dependent virus and this is really what we consider more of a mini-symposium on this latest generation of adenovirus.

(Slide.)

And some of the generic issues that are brought up when we come in with sort of a newer version of a virus and also clinically when we have some data with respect to previous versions of this adenovirus in both preclinical safety models and in humans.

(Slide.)

And just to reiterate the idea of the mini-symposium is to use this particular case and the experience of these investigators who have worked very hard to work out all the details of this kind of protocol to engage in dialogue and discuss and bring out some basically generic issues with respect to this kind of virus.

And this case study should focus on the minimal -- that this is a new version of a minimal adenovirus vector and some of the specific issues in the protocol is that it is intravenous administration. You can see that the current protocol is two dosing cohorts and the setting is patients with severe hemophilia A.

(Slide.)

Again key general issues would be the preclinical toxicology, preclinical efficacy, the pharmacologic issues of route of administration and dose, again in the context of a new version of the adenovirus.

(Slide.)

In addition we received several questions from the FDA that they would like to be considered by RAC and by the investigators in general discussion. I just want to go through those specific questions.

First is that the vector is encoding a -- encodes human Factor VIII deleted of all viral gene sequences and this will be delivered systemically. Given the known toxicities of previous versions of adenovirus, a discussion with respect to the route of administration and the proposed patient population, this should be greater than 18 years and is there an alternative route. So again in context of a new viral system.

(Slide.)

The second question is given what is known about the whole field of adenovirus, all generations and what we have seen with respect to toxicities related to liver toxicities and platelet abnormalities in all the generations, and the specific safety issues or safety data presented here, is this an acceptable toxicity profile for this patient population.

(Slide.)

The dosing issue relates to the specific proposed dose range of six times 10^{11} vector particles per kilogram and other viruses have demonstrated -- other adenoviruses have demonstrated toxicity at this level.

The second issue is what is the therapeutic ratio with respect to the toxic dose and the predicted therapeutic dose and this will actually be discussed a little bit more later.

And how should the preclinical and prior clinical findings be addressed in the consent form with respect to the total field of adenovirus and then specifically with this new type of adenovirus.

(Slide.)

And with respect to the lack of toxic threshold seen with this virus in these animal studies, is a starting dose of 1.4 times 10^{10} vector particles appropriate?

(Slide.)

And the second is, is a three-fold dose escalation also appropriate given the data presented so far?

(Slide.)

This issue is with respect to the current trial. If these two doses are safe and appear to be -- but maybe perhaps not effective or not achieving therapeutic levels, in the future what should be the approach to dose escalations? For example, should it be mathematic or logarithmic? And what adjustments would be appropriate given various degrees of toxicity with respect to this question?

(Slide.)

And, finally, is there -- given all the data that is known with adenovirus, all generations, and what was seen so far with this new type of virus, is there an absolute limit that should be set? You know, what factors would play into this? You know, the issue of an absolute limit should be discussed and the issues that would impact on setting this type of limit or not should be discussed.

(Slide.)

And, in summary, with respect to some of the key points of these would be selecting patients for helper adenovirus clinical trials, what is the match, the route of administration, criteria for dose escalation, informed consent given all that we know about adenoviruses at this time, and clinical monitoring.

These questions have been submitted. I think they are on Tab 2115 and I think the investigators are aware of these questions and will comment on that.

The next section, Dr. Aguilar-Cordova has thematically grouped basically four fundamental types of questions and will sort of present those questions one by one to lead the discussion.

(Slide.)

DR. AGUILAR-CORDOVA: So this is more of a generic follow-up on what is different about this helper dependent vector and when there are these advances in technology such as those of my overheads here, how should we address those advances and what to do on a step-wise fashion.

As Dale mentioned a little earlier there is a -- the whole issue of vector and disease match, how does the vector that one uses match the disease? All vectors are not like to be useful for all diseases.

Product specific issues, and we will go through some of the specifics of the helper dependent vector that actually Dr. Chamberlain outlined very well, what kind of safety packages and toxicity studies, pharmacodistribution studies do we think might be required for these type of vectors? How should the dose escalation design of the clinical studies and where does one analyze these? And then these are basically all

the generic issues.

Now I think something that is important that we have discussed at length with Dr. Ando is that we do have a significant database on the first generation adenoviral vectors and that what we are looking at here is something that looks on the outside identical to those and so whatever gets delivered to the patient is identical to what would have been in the first generation vectors from which we have a tremendous amount of data.

What is different is what comes inside and although there are some very small shared pieces there are significant differences.

(Slide.)

So the first issues are vector disease match and for that we need to take the viral features or vector features in this case, the potential for integration, the immunogenecity, what tissues are targeted and their expression or duration, is that really different.

Now in the case of the helper dependent vectors there is a much larger content of -- in the case of the vector that is being described here a much larger content of genomic sequences. Will this significantly affect the potentially for homologous recombination and thus integration into the cellular genome? And if it is not with that would -- even without the homologous recombination potential can integration occur at random and is there any increased potential for long-term duration due to that rather than other issues?

The immunogenecity, there would be two levels of immunogenecity that have been described with adenoviruses and that is the acute reaction and then the chronic reaction and it is a biphasic response in the cotton rats and in mice as well.

And we would expect possibly that with these vectors since they look identical on the outside the acute reaction would be probably very similar to those of either wild type or first generation vectors. However, if the chronic reactions are mostly due to viral gene expression in the cells these may have a much improved chronic response.

The target tissues since the outside again is exactly the same as those of the first generation, we probably would expect that the target tissue would be the same with these helper dependent vectors in as far as the vector itself is concerned.

Clearly the expression would be regulated by what is inside and thus it may be improved or directed by the promoter sequences chosen and the expression duration is probably a function of all of the above and it is likely to be different from these vectors than from the others.

Based on that one, one also has to match the disease features. Is it an acute versus chronic disease and based on what we had seen in the OTC deficiency trial from the University of Pennsylvania there was a general consensus by the participants in the meeting in December that adenoviruses generically speaking are probably not well suited for chronic diseases. However, it was particularly relating to adenoviruses of the type that had been used for vectors up to that point, that is first or so-called second or third generation vectors.

Are these vectors sufficiently different and is their expression

sufficiently persistent without increased immunogenecity that it would warrant their acceptance as potential vectors for chronic diseases such as hemophilia?

And clearly whether the disease is fatal or nonfatal would weigh in, in the evaluation of the risk/benefit ratio of any vector.

What are the required levels of expression? We just heard that in order to have an effect in the hemophilia patient one only needs two to six percent of the normal levels of Factor VIII. Well, is that achievable with the doses that can be given with a high degree of safety confidence?

Is the disease monogenic or polygenic? And clearly many of the vectors that we have to date do not have the capacity to carry more than one or two genes and thus would not be applicable to polygenic disease. Would these helper dependent vectors with an increased capacity allow such potential?

What are the required location of expression? If it is just a secreted gene maybe the liver is a perfectly good target although we did hear also in December that in humans maybe the liver is not quite as predominant of a target as it is in mice.

And what are the disease affected tissues? Therefore, where would one need to deliver the gene to or the vector to and how does these particular type of vectors differ from any other in as far as that?

(Slide.)

The second issue that we had in the generic issues to discuss is the helper-dependent specific product issues and again I would like to reiterate that we are building on an existing adenovector database. That is we do have plenty of – or maybe not plenty but we have a lot of data with regard to the first generation vectors and distribution, as I mentioned earlier, is unlikely to be significantly different given that the code (sic) is identical.

The duration of expression or the presence of the vector now -- that will likely differ and it should be clearly evaluated for each protocol and that might differ not only with regard to – because it is a helper dependent vector but also with regard to what is the content of the vector as we have heard the group of GenStar speak about the genomic versus cDNA and the levels of duration in that case. But also would that increase integration potential and such? So it is not just expression of the gene but also the presence of the vector.

The genome stability, Dr. Chamberlain spoke at length at this, we have -- in other laboratories and ourselves, we have had experience showing that with these helper-dependent vectors because of such increased size and because there may not be a selected pressure to maintain integrity of the genome there may be more propensity towards recombination and thus genome rearrangements. How to set up the assays and what levels of genome rearrangements would be acceptable?

(Slide.)

The percentage of first generation contamination is .1 percent acceptable and how do we measure those levels and how does that affect all the other criterion that we have been speaking of? If we talk about one of the greatest advantages of these vectors being nonimmunogenic and also being long duration what percentage of first generation vector contamination can there be present in the

preparation to where it sends it back to the characteristics of the first generation vector? Is one percent too much? 0.1 percent, .01 percent? These type of studies to define and quantify such things would be very useful.

And, of course, as with any other adenoviral vector we are also concerned about the potential for RCA contaminations, replication competent adenovirus.

(Slide.)

So given that preamble of what we want from the vector and what are some of the specific characteristics of the vector, how do we build the safety package that would be acceptable for continuation on to the clinical trial? The safety package, otherwise a toxicity study or pharmacodistribution studies.

Distributions: We probably have a lot of data on how an adenoviral vector distributes within both animal models and some within humans.

Duration: We have already spoken about that. It is a greater -- especially, though, when we are thinking about going towards a clinical trial we must take into consideration that preexistence of antiadenovirus antibodies since the majority of the population has been exposed to adenoviruses, will this greatly affect the advantages that might be attributed to this type of vector? And, also, once the vector has been administered to a patient we could expect that that patient will immediately form or form an even stronger response upon second administration of the vectors. So those patients would definitely have a high degree of antibodies.

Again one must integrate what is the target tissue and what is the delivery site.

We know that when -- from the December meeting we know that the majority of toxicity that has been observed with adenovirus is either liver toxicity or thrombocytopenias and we must take that into consideration when deciding the disease match. If the disease already has significant liver toxicity or a bleeding disorder then one has to wonder whether these vectors will enhance that disease status and how will it interact under those circumstances.

We have spoken about stable integration of the vector and whether there will be rearrangements *in vivo* not just of the vector but if it has multiple stable integrations will it ever lead to rearrangements of the genome itself.

And, of course, the site of injection and that will mean that the response from the patient and the dose that one might use will be dependent on where the vector is delivered, i.v. versus intramuscular or intraperitoneal. All of these have very different characteristics and responses from -- in animal models one could expect that it would be the same in patients.

And, lastly, it is the potential for threshold effect and is there variability between studies and also variability between patients.

So what we have had heard presented at various meetings and again at the December meeting what we saw is that there seems to be a threshold effect in the toxicity levels tolerated by adenoviruses so where there was very little toxicity observed at -- I think it was three times 10^{12} viral particles per kilo in a baboon study.

At three times 10^{13} it was almost fatal.

Thus there seems to be a threshold effect that should significantly impact on how do we do with the escalation at those levels. I may be a log off on those numbers.

Also, we have seen significant variability of those numbers between studies. Is that a characteristic of the vectors themselves or is it an effect of the methodology that we have in place to quantitate and characterize these vectors? And there has been quite a bit of discussion in this as to how do we do the quantification and characterization of these vectors.

Now the helper-dependent vectors add increased complexity of characterizing the content of a mixed biological product. Nobody at this point is claiming that they will have no first generation contamination. We will all have some first generation contamination. How do we quantify that and then also quantify specifically the functional titer of the vector that we want.

(Slide.)

So based on that we wanted to discuss the dose escalation schemes and should there be different levels of dose escalation. Can one ramp up at a much faster rate at what we can consider in quotation marks known safe doses and is what we have seen that has no effect in first generation vectors, can we expect that would have no effect on these helper-dependent vectors as well and thus can ramp up a lot faster?

But what about at higher levels when we start approaching the type of toxicities that we saw on the first generation vectors?

And can animals adequately predict what will happen in humans?

And a correlate of that is what is the value of human experience and, of course, that addresses again the risk/benefit issue of clinical trials in this level although at that point the benefit will be more societal than individual.

And within the context of thinking about these issues and the dose escalation one must consider that a patient who has received that first dose may be prevented from future doses. This is of critical importance if we know we are starting at doses that are well below what is predicted to e of therapeutic value in cases, for example, as the protocol that is being used here as an example.

If these patients are then prevented from receiving what might be a therapeutic dose of these vectors at a higher dose, is it valuable to all the same begin at a much lower dose that would then basically vaccinate them against the higher dose.

The threshold effect we have discussed. And are there predictors of susceptibility? We mentioned that there may be differences between patients in which -- at a given dose are there predictors within those patients that we can utilize to separate those that may be susceptible to toxic effects and those that may not?

(Slide.)

So, lastly, these are -- the generic type of vectors can be -- or issues can be described in these four points that Dale mentioned. That is what are we injecting with the quantity, the quality, the structure and the genetic content of what we are injecting.

Where are we giving it, and to evaluate that we need to know what

species and the site. Is it oral, intravenous, intratumor, intraperitoneal, et cetera?

When is it given? The age of the patient may have a significant effect and certainly health status might also influence that.

And how is it given? What time? The duration of injection? Is it the bolus injection or a slow injection? What volume and the carrier?

And those are some of the generic questions that we would like to present.

As a follow-up now bringing it back to a little bit more of the specifics of the protocol that we are using here to exemplify (sic), I think Dr. Ann Pilaro has some specific questions or issues to raise so if she could come up.

DR. PILARO: Thank you. I would like to thank the RAC and especially GenStar for giving me the opportunity to present some information to you.

(Slide.)

I do want to say that the GenStar package really had a lot more safety data than they presented to you today. I think in the interest of time they focused on the most crucial findings that they have but they have given me permission to discuss some of the other information that was contained in the package that may support our decision from the FDA of the doses that were chosen for the clinical trial.

When we talk about safety data and we talk about defining a no observable adverse effect level or an NOAEL we are looking at the highest dose that was given to the animals in the absence of any detectable toxicities.

In the Black 6 mouse study that you saw that Dr. Zhang presented and in another study that they had included that was in your package and that we reviewed, we saw that the difference between the starting dose for the clinic and the dose at which -- the highest dose at which no toxicity was seen in the mice was about 114 to 285-fold difference, and that is when it is actually scaled by vector particles per kilo. The same route of administration. The same dose scaling comparing apples and apples in this case.

Another study that was included in the package that we reviewed was a cotton rat systemic administration and in this study the highest dose that the animals received in the absence of toxicity was 10^{13} vector particles per kilo. Again scaling this to the human dose -- starting dose for the clinic gives you a safety factor of about 700-fold greater than the starting dose for the clinic.

So we believe that the safety of these vectors -- of the helper-dependent vectors are supported by the toxicity data that have been done in animals.

And in an additional study the pharmacologic activity of these vectors was titered in hemophilic dogs and in this study they also collected safety information. In the dogs that were treated there was no evidence of toxicity. There was a transient elevation in transaminases in one dog but it was still within the normal limits similar to what was seen in the mice. There was no evidence of platelet consumption.

However, the pharmacologic activity in those dogs really did not show the same effect that was seen in the mouse. In this case it was an issue of not being able to give enough vector to the animals. They gave the maximum dose that

they were able to give to these dogs which was 7.6 times 10^{11} vector particles per kilo and saw no evidence of pharmacologic transfer, which is what we call an NOEL or a no effect level dose.

I do want to point out that this no effect level dose of 7.6 times 10^{11} vectors per kilo is actually about 50-fold higher than the starting dose that you want to see for the clinic.

So we believe from the data that we have seen and reviewed that the safety of this novel vector system is supported.

The next point that I want to bring up, though, is what are we worried about here. Should we be worried? The mouse and the dog pharmacology studies do not show effect of gene transfer until you are up to doses of 10^{12} per kilo or higher.

However, the no effect doses from the animal studies show no toxicities at doses of up to four times 10^{12} per kilo. So this brings up what Dr. Aguilar was saying about how do we define where the effective dose range should be and how our escalations should proceed.

(Slide.)

When we look at the two doses that are proposed for the present trial we see that both doses are approximately 100 to 200-fold lower than the no effect level dose in the mouse and 50-fold lower than the pharmacologic activity. Again where should we go from that second dose if no toxicities are seen and how are we going to decide what escalation scheme we should be going for?

We do want to point out also that the dose at which the effective gene transfer is seen in the animal studies in this case is approximately two to ten-fold higher than the dose that Jesse Gelsinger received of the first -- actually the E1/E4 deleted adenoviral vector into the liver. However, the safety data in this case do support the use of the higher doses and we want to bring that point up for discussion.

We do not want to set a threshold dose at which we are going to stop everybody based on just a fear factor. We really want to look at the scientific evidence and evaluate what is going on before we really decide to go forward.

And at this point I will turn it back to Dr. Ando and Dr. Aguilar for further discussion.

Thank you.

DR. AGUILAR-CORDOVA: Would the investigators like to take five minutes to respond to some of the issues that were brought up that were specific to the protocol?

DR. ZHANG: Thank you for those questions.

Actually those questions also we have considered at all times to make sure we have a good answer and the data to support a clinical trial.

As far as Dr. Ann Pilaro indicated, the dose, the safety margin which we already measured for our initial dose, we have also -- particularly the thoughts, those are the -- at least the two logs lower than the dose which has no observed any toxicity in the mouse model.

And for the dose we also -- we also hope or we predict that we may

have some different outcomes for the efficacy level for the expression of human Factor VIII because the vector contained full length human albumin promoter and it may expect to function better in mouse and in dog. Therefore, we had a discussion with FDA and we felt that the entry dose at 1.4 by 10^{10} is adequate.

And there are also a group of product related questions Dr. Aguilar-Cordova mentioned. Shall I answer one by one of your questions? I need to get the questions. Please correct me if I am missing any points or questions.

For the vector features, integration as I presented in the slide, the -- our PCR analysis suggests the vector kept intact in antagonist cell because the N's are not lost for the vector in the PCR analysis.

Dr. Aguilar-Cordova pointed out the length of the genomic fragment that may facilitate homologous recombination and this also is an area we would like to understand better but generally from a scientific point of view of the early studies we understand homologous recombination frequency is generally low, is at a level of one in a million or less.

So if anything happens we also have that type area to -- human albumin, which is probably a site that could facilitate that expression but generally our data suggests the vector kept in cell -- antagonist cell as an episomal form.

For the immunogenecity we also 100 percent agree with Dr. Aguilar-Cordova's point that the capsid is kept the same so the induction of neutralizing antibody to the vector will be solicited -- will be there and this has been confirmed by the animal study.

For the cellular immune response so far from our long-term expression we did not see that same come up as showing in the early generation vector. The long-term expression also support us for the minimized immunogenecity of the vector.

For the --

DR. AGUILAR-CORDOVA: Wei-Wei?

DR. ZHANG: Yes, sir.

DR. AGUILAR-CORDOVA: Perhaps since you -- rather than just accumulating all of this perhaps Dr. Chamberlain can address some of his experience with recombination and genome stability of these helper-dependent vectors just as a generic term?

DR. CHAMBERLAIN: Well, I think the experience from our lab and several others suggests that every vector is a little different in terms of their stability. Some even grow repeatedly without seeing any rearrangements. Others rearrange quite a lot and so I assume that this is something that you have looked at already and I think it would be important to hear maybe some comments from you on how stable is your vector system and what -- how often have you seen rearrangements in either the ancillary virus or your virus?

DR. AGUILAR-CORDOVA: How do you detect rearrangements? I think I saw that you were detecting them by restriction enzyme digest and then running them on a gel. I believe that is -- you would have to have a fairly significant --

DR. PILARO: Percentage.

DR. AGUILAR-CORDOVA: -- percentage of a specific rearrangement in order to see it with that kind of technology. I am not sure what the detection level is on the ethidium bromide but it would have to be a specific band of a specific rearrangement, at least five percent of the content I would think.

How are you detecting your rearrangement?

DR. CHAMBERLAIN: Well, we have done it in a couple of ways, including some of the ways that Wei showed. Initially if you have a reporter gene in there you can compare the expression of that reporter gene to the particle count that you have for the helper virus but the most sensitive way, I think, is by Southern Blot analysis and that is generally what we do rather than looking at an ethidium bromide stained agarose gel. We will actually do a Southern Blot to try to bring up the sensitivity several-hundred-fold.

DR. ZHANG: Dr. Aguilar-Cordova, actually we -- that is another area about vector stability and rearrangement so in that area so far our vector has been shown by the enzyme digestion Southern Blot as well as some fragments PCR demonstrated integrity of the vector, and we did not see the vector rearrangement so far.

DR. AGUILAR-CORDOVA: It is always an interesting question because it would be difficult to distinguish multiple different types of rearrangements with a PCR which is the same for homologous recombination. You are asking for a negative result because you have specific primers and you are asking to see something that no longer has those specific primers.

DR. ZHANG: The fragments -- that region probed --

DR. AGUILAR-CORDOVA: Right, but that region is -- if that primer is no longer there or whatever then you will not see it.

DR. ZHANG: Right.

DR. AGUILAR-CORDOVA: So it is a negative result.

DR. ZHANG: Okay.

DR. AGUILAR-CORDOVA: It is just a difficult assay to perform specifically.

DR. CHAMBERLAIN: If I could make another comment. It is also difficult in terms of, as you mentioned, the sensitivity, and one of the problems that we have run into and maybe you, also, is that sometimes to really go through a prep and convince yourself that you do not have any rearrangement you end up using almost all of the prep with the assays that you are performing and so I think maybe something that we may need to consider here is to go through and do multiple preps and demonstrate you are consistently not seeing rearrangements and then rely on less sensitive methods when you actually have a prep that you want to save and be able to utilize in the clinic.

DR. ZHANG: That is a good point, Jeffrey.

Shall I move on?

For the targeted tissue to answer the question, you know, we also demonstrate the vector has a tropism to liver and we do have a design for high level of expression and stability of the vector in liver cells with albumin promoter and albumin

gene fragment as far as the expression and the duration of the vector, which has already been demonstrated in both hemophilic mouse model as well as C57 Black mouse model.

For the disease features I think I would like to have Dr. White to answer after I answer the other questions.

For the product issue -- shall I move forward?

DR. MICKELSON: Well, I had something on the toxicity. Just a general question. I -- many of -- even the first generation Ad vectors had reasonably good safety profiles in mouse studies and yet when they went into humans that was not replicated in humans in terms of -- and it was not until -- and I am just making a statement.

I would like to hear some discussion that some of the toxicities at high doses at least for first generation adenoviral vectors was not seen until they went into primates with them.

And I am wondering if maybe we should be asking -- and this was brought up but how predictive are murine studies particularly for a brand new vector and -- because some of the safety issues that were seen at least in the Gelsinger case the toxicities arose so quickly it really -- not to -- it was not -- it could not be anything that was inside the vector or at least the initiation of the adverse events and that some of the strong toxicities and adverse events that were seen in humans were seen in primates.

And I am just wondering if maybe we should talk about the appropriateness of animal models looking at -- and I -- the distribution for receptors is different in humans. The hemophiliac dog also is different than the murine studies and I am just wondering how would you -- what would you consider about -- and how does the FDA feel about primate studies for particularly new vectors and vectors where we have had some indications of toxicities?

DR. NOGUCHI: Yes. I will have Ann answer the question of primates but I think it may be a little bit misleading to say that the early animal studies did not show toxicity.

DR. MICKELSON: Yes.

DR. NOGUCHI: We were also not looking at the same number of particles back then. In fact, the ability to produce such enormous numbers of vectors per ml just to get up to these very high doses is a fairly recent development. So I think you are correct in that the earlier animal studies may not have shown as much toxicity but we did not have as much vector really put in animals at that time.

DR. MICKELSON: So you are saying that those high doses that went into humans were not done in any animal studies?

DR. NOGUCHI: Actually I think Ann would be --

DR. PILARO: Actually I will comment on that. When they got -- when the field had advanced to a point where they actually were able to make high enough vector titers that they could treat animals with doses that were feasible to get that high they actually did see similar toxicities.

There were very marked elevations in transaminases with the i.v.

administration of the OTC vector no matter which generation that it was in and the data that I actually presented at the RAC in December showed there was really no difference in the level of toxicity between the mouse and the baboons.

The only reason they got to the failed doses in the baboons and in the rhesus monkeys was they went another dose higher than what they saw in the mouse but that was again a case of -- it was volume and they could give that much more.

The one point I did want to bring up about the predictability of the animal models, we heard at the December meeting that the number of receptors in the mouse liver for adenovirus is much higher than it is in the human or in other animal models.

Our feeling is that the mouse model tends to over predict the pharmacology but that also gives you a worst case scenario for getting the maximal amount of adenovirus into those liver cells and it may actually over predict some of the toxicities.

Of course what we have seen so far in the humans it really has sort of been more parallel in terms of the toxicity that is seen in the animals versus the patients.

DR. MICKELSON: So to predict target tissue, though, as specificities?

DR. PILARO: It would appear from what we have seen so far, yes, that it does predict.

DR. BREAKEFIELD: Yes. I was -- actually your second point was what I was referring to or thinking about, which was it seemed from that last meeting that some human hepatocytes were not as infectable with adenovirus.

Now I do not know what level that is or whether -- do you think it is possible that they might -- you might have to hit them with more virion particles and there might be some toxic effects due to that if we actually got a successful transducing unit into them or do you think that they just bounce off them?

DR. PILARO: Yes. One question that we have continually discussed before the RAC and that keeps coming up is do you have to have some sort of inflammation set up by the virus before you can get an effective transduction. That is something that we really do not know. I mean, I think the only way that we are going to get that information is from the clinical trials.

DR. BREAKEFIELD: Well, I guess that -- if I can just throw in one other question here, that is sort of what I was -- when you made your comment, Jeff, about the sort of relative transducing units to particle and then how much that can vary for the gutted vectors, I am just wondering how much -- you know, given that range of variation compared to what you see in say a first generation adeno, are they packaged much less efficiently so are we talking about very large numbers of viral particles in general compared to the first generation to get a transduced amount?

DR. CHAMBERLAIN: No, I do not think so. Actually the data that I presented showing different levels of transduction depending on which assay you use, that was actually looking at the helper virus which is a conventional adenovirus.

So that is -- I think that is just an inherent feature of adenoviruses that has been published on by several groups that when you throw a certain dose of virus on to a plate of cells not every single virus particle is going to be taken up so depending on what type of assay you are using you are going to get a different titer.

From the limited data we have of trying to look at particle counts versus transducing units, we have not seen any obvious difference between a gutted virus and a conventional adenovirus.

DR. AGUILAR-CORDOVA: Dr. Gordon?

DR. GORDON: I just wanted to ask a question about this toxicity. It seems that your discussion would imply that hepatotoxicity is what you are looking at in the sense that the mouse would be a provocative test because it has higher receptor density in the liver for toxicity.

But one of the things that came to mind in the December RAC meeting is -- to me at least -- is suppose you do not have enough receptor in the liver to take the virus out of the bloodstream and you distribute it widely throughout the person through the hepatic vein and it is enough to overwhelm neutralizing antibodies. Could this be another type of toxicity which might be unique to humans?

DR. PILARO: Well, the point that we have brought up before when you are designing the toxicity studies is we do ask that you look at a systemic panel of markers so you are really looking at not only liver toxicity but you are looking at the BUN creatinines, you are looking for Kd, you are looking at CPK's, you are looking for vessel and heart cardiac damage. You really are looking at a very broad panel.

It just seems that with the adenoviruses the two major toxicities that keep coming up are the transaminitis in the liver and the platelet consumption and that seems very consistent for the first generation, the second generation, the E1/E4 and now the gutless vectors.

The mouse picks those toxicities up. The nonhuman primate picks them up. The dog actually picked up the transaminitis in the case of this gutless vector. Again it was a slight elevation still within normal limits but it did pick it up. So it is telling us that we are looking at what we are able to look at and we are picking up the major factors and these are the warning flags that we should be looking for in the clinical trial.

DR. ZHANG: I also mentioned that the hepatocytes does in -- is a susceptible to the adenoviral infection because the -- our own data demonstrate the hepatoma cell infection was quite good and also primary human hepatocytes culture infection with adenoviral vector was very effective and then dose also demonstrate the liver is susceptible for the virus gene transfer.

DR. AGUILAR-CORDOVA: Dr. Friedmann?

DR. FRIEDMANN: Could I ask two technical questions? One is a follow-up on what we have just been talking about and that is could I hear a discussion about the use of the phrase "tropism of adenoviruses" to the liver?

Operationally if you inject the adenoviruses systemically much of it winds up in the liver so operationally there is a hepatotropism but that -- sometimes the implication of that is that there is some biological reason for that rather than

structural reason and we know that at least part of the reason why adenoviruses like to go to the liver is because they happen to be able to get through the fenestrations in the endothelial layer of the biliary circulation.

Is that as true in the human as it is in other animals? What is known about the biodistribution of adenovirus in human and can we expect any greater or less accumulation in the human liver as opposed to other mammal liver?

And then the second question I would like to hear some discussion on is the physical chemical explanation of the different bouillon densities of the adenoviruses. Particularly in relation to the capacity of the vector.

If you are loading the same amount of DNA into the particle then the protein DNA ratio should be roughly the same and then I do not quite understand the reason for the differences in bouillon density and the true efficacy of the purification procedure. So maybe those two questions to start with.

DR. ZHANG: To answer the first question, the "tropism", I think that this has been a term used for adenoviral vector field and everybody understand this -- there is a reception mediated virus of phagocytosis to the cell as long as you have a CAR (phonetic) receptor and you have a the integrate -- the 35 receptors then adenovirus will have good entry of the cell.

But for the tropism so far we can -- we experienced or demonstrated by publication probably resulted from the liver structure which has a first pass effect for all different particles and drugs in the bloodstream so probably that is explained why the vector accumulated to the liver and the liver structure also probably allowed the vector to slow down its circulation and have a chance to the infection.

DR. FRIEDMANN: Are the fenestrations in the human liver the same size as permissive for adenovirus particles as --

DR. ZHANG: I believe so. This is the histopathology -- histology. Structurally they have the similar structures of these hepatocyte structures.

DR. FRIEDMANN: That is a known fact?

DR. ZHANG: Right. And to answer your second question about bouillon density, our vector was designed to be separate for this system. The ancillary Ad has 1 -- now 105 percent of the wild type adenoviral vector genome size. So it is a 38 Kd. It has already the maximum the size. Then we also have our mini-Ad at about 80 -- no, 90 percent of the genome size of the wild type genome size at a 31 Kd. So there is a six Kd DNA difference between the two vectors and that results in the bouillon density difference for the system and the efficacy efficiency of purification has been demonstrated.

DR. AGUILAR-CORDOVA: From Dr. Pilaro's description there was a great variability on the efficacious dose depending on the studies and, therefore, the factor of difference between what you are proposing as your starting dose in clinical trials versus that what was done in the preclinical studies.

As we mentioned, that may be very important since the patients that might receive the first dose will likely not be receptive to additional dosing.

Could you comment on what kind of characterization or equivalence or preclinical and clinical preparations are you doing? In particular, the ones that you

used in the studies presented here. Do you know the ratio of helper and nonhelper vector? You all are using a PBS-based buffer which others have reported to have some stochastic precipitation of vector especially after transport.

What is the stability over the vector that you have used during those studies and the potency of that vector that was used in the dog studies or the mouse studies?

DR. ZHANG: Okay. To answer the first question about the variabilities of expression of Factor VIII at different animal models, we all have a clear, clear indication there may be a threshold which we reach the level of one by 10^{10} viral particles per mouse and also that is the level we can see all expression.

But for dog we did not reach that threshold so we did not see but we also have some prediction that human albumen promoter will work better in human so that could potentially support it.

DR. AGUILAR-CORDOVA: I guess my question was more related to your preparations.

DR. ZHANG: Okay.

DR. AGUILAR-CORDOVA: So the preparations: Do you have variability in the percentage of helper vector and are they stable during transport and at the time that you are giving them to the dogs or to the mice or to when you would propose to be giving them to the humans?

DR. ZHANG: Right. To answer this question is the quality control of the vector and also the contamination level of ancillary Ad. We have standard methods to determine the -- we use that X-gel staining as well as the enzyme digestion to release all for animal studies and we do have all the raw data. To also support the parameters of the specification we have discussed and also presented here, which is less than 0.1 percent. Actually it is -- sometimes we reach 0.001 percent of the ancillary Ad.

And then as far as the particle and unit ratio that is also a very good question. We have been systematically studying our vector based on the particle number, the transduction unit can be determined indirectly in our study. We use the amount of viral particle input into $10^{6\ \text{million}}$ cells for infection in a culture for 24 hours and then we check the product level in the medium because the Factor VIII is secreted into cell culture medium and also the medium volume is certain then because the Factor VIII is in the secreting format. It is hard to do this *in situ* immunostaining.

Therefore, our current technology did not quantitatively determine that transducing unit as *in situ* per cell unit but we have a constant value to determine the secretor proteins such as 50 to 100 nanograms per ml per million cells per 24 hours and based on 1,000 viral particle input. Those are constantly in the laboratory and those also will be the measurements for the clinical lots.

DR. FRIEDMANN: You have said a couple of times that you hope and expect that the human albumen promoter will work better in the human, is there any evidence to that to justify that hope? I mean, there is no reason to expect that it would not but do you have experimental data to indicate that it does work better in the human?

DR. ZHANG: We have -- we have tested the human cell lines such as FG2 cell and 293 cells, those are human origin, and the expression of the vector transducer cell was quite satisfactory.

DR. FRIEDMANN: It was better?

DR. ZHANG: It is -- the level is quite high.

DR. FRIEDMAN: But better in murine cells because you will not have had a chance to test in non -- in human cells that are not hepatoma cells.

DR. ZHANG: We did not -- Dr. Friedmann, we did not have a direct comparison of primary murine hepatocytes and the primary human hepatocytes. We did not do this.

DR. FRIEDMANN: One other quick question. In Dr. White's presentation he showed us a side by side representation of transduction with the gutless vector and with the traditional vector in Lac Z stained cells and there were a fair number of blue cells in the control section that only a few years ago would have been interpreted by some of us to show some degree of effective transduction.

DR. ZHANG: Right. Those are the --

DR. FRIEDMANN: Other than that and the fact that the Lac Z staining under estimates the number of transduced cells, some cells are expressing too little Lac Z to really be seen easily histologically depending on how long you stain the cells, could you again tell us what your best estimate is of the number of -- the amount of contamination, the extent of contamination and the percent of cells transduced with the vector?

DR. ZHANG: Okay. That is also a very good question. For the ancillary Ad we have a separate control with the particle and the that would indicate the transduction efficiency of the helper along and also --

(Simultaneous discussion.)

DR. ZHANG: Right. -- and also we have the mixture --

(Simultaneous discussion.)

DR. FRIEDMAN: This is like the Lac Z marker?

DR. ZHANG: Right. Exactly. And this -- for our fair estimation we actually already reduced the contamination number because the true testing we already obtained the results. We usually get one out of 10,000 or one out of 100,000, which is 0.001 percent. And then we see if there is any margins of variations in assaying and then it will not reach back to 0.1 percent.

DR. FRIEDMAN: In Dr. White's slide looked as if I could see maybe five or six or ten blue cells in that section – in that plate and I would presume that that is an under representation of the number of transduced cells so it looked a little bit more extensive than 0.001 percent.

DR. AGUILAR-CORDOVA: I think what that -- what that is addressing, and I agree with Dr. Friedmann, it is addressing an issue of the assays developed to actually quantify the level of the helper contamination and perhaps a better understanding of what that means in as far as the product effect.

Perhaps we can follow up a little bit with Dr. White and Dr. Ando so that we can look a little bit more about the disease vector.

DR. ANDO: Actually for the RAC time is getting sort of short but what I wanted to do is ask Dr. White basically some of the key issues addressed by the FDA but fundamentally the first issue is that it appears that the current new vector and the safety studies supports very well the current dosing but you have a previous history of other adenoviral toxicities and that is associated at a certain level so you will be approaching in the clinic with a better vector -- a better vector at least in animal studies but you have a previous history of other problems so how do you approach what you feel clinically about his and also how do you approach the informed consent issues with your patients? So that is the first part.

The second part is once you -- as you begin dose escalating, say you do not see much therapeutic effect but you -- the study shows that it is very safe and you begin to move ahead, what are your thoughts about the kind of dose escalation and could there be, you know, some surrogate markers like cytokines or something that could help guide you as -- you know, as you move up towards or push ahead into the therapeutic dose ranging and what are your thoughts about that?

DR. WHITE: Well, I think the first question regarding the perceived improved safety of the gutless adenovirus obviously to my way of thinking and discussions among the investigators that is a very attractive feature of this study.

I think we have tried to think of it more in terms of a benefit toxicity ratio and our perception is that the benefit toxicity ratio of this gutless adenovirus is, indeed, improved. What that means is that we can go to lower levels of virus relative to nongutted adenovirus and we get, we hope, similar clinical effects. That is obviously an advantage for a patient. If you are giving less vector and getting the same clinical effect you hope that you are going to see less toxicity in the patient.

I think in terms of the informed consent I believe very strongly that one has to go through in great detail what you think you are not going to see in that study, that is what you are afraid is going to happen in the study, what your fears are, and we spend a lot of time doing that with our patients and the investigators have spent time talking about that as an approach to this clinical trial that we do have to emphasize the liver toxicity that has been seen with adenovirus, with nongutted adenovirus, or with second generation and third generation adenovirus.

We do need to talk about pulmonary toxicity. We do need to talk about hematological toxicities. That will not change with a gutted adenovirus. We will do the same thing. Those issues are still there. We only hope that the ratio of those toxicities relative to the benefit is going to be improved.

Your second question was what?

DR. ANDO: The second question is approaching the dose escalation. There is probably two pieces of that.

DR. WHITE: Yes.

DR. ANDO: When are you in a safe level? The second as you begin to sort of march upwards to try to find therapeutic dose.

DR. WHITE: Well, we were in favor of a low dose. I am very sensitive to the point that the FDA has raised that we are excluding these patients from additional clinical trials and from repeat dosing but I think that is part of participating

in this study. I mean, this is one of the things that we will emphasize to them and make sure that they understand that by participating in this study they are not going to be candidates for another dose later in the study.

They are not going to be candidates for a certain period of time for other gene therapy trials. This is their contribution to the development of gene transfer technology in the treatment of hemophilia.

I think we were also -- but despite that we felt that safety was very important and that we needed to start at a dose that was a low dose. We are very comfortable with the dose. The investigators are very comfortable with the doses that are suggested and we are very comfortable with a small increment in dose in this Phase IA trial.

I think where we would go from there I think depends, as you suggest, on what we saw but I would urge the committee and we as investigators would like to remain very cautious in our approach to dose escalation. We do not want to do any harm here. We do want to see if this vector is an effective and safe vector.

DR. ANDO: One final point being addressed in the second FDA question was given the profile of toxicity with respect to liver abnormalities and platelet abnormalities, how do you feel as a hemophilia clinician and patients feel with the use of these types of vectors in general, is this a major issue or something that could be dealt with, with respect to appropriate monitoring?

DR. WHITE: You mean with respect to the platelet and the liver tox -- the liver changes?

DR. ANDO: Right. In setting up patients with hemophilia.

DR. WHITE: Well, so a hemophilia model. Can I address it that way? Yes, I think it should be considered as a possible model and I use that term in quotes for this particular vector.

I think the advantage of looking at this vector in patients with hemophilia is that one can use small doses of vector and potentially get a benefit if you only get two, five, ten percent levels of factor. So you do not have to go to high levels to get the benefit that you are looking for. That is the nice thing about hemophilia with respect to this clinical trial.

The bad thing is that the vast majority of our patients who are candidates for this trial are hepatitis C virus positive and do have abnormal liver function tests. They do bleed from their hemophilia and the addition of thrombocytopenia on top of their hemophilia is a problem.

Those are things that we are going to have to look at very carefully.

The good thing about the liver function changes that we have seen, the AST changes that we have seen, is that they are very small and well within normal limits. That is why we would like to start out with patients who have normal AST levels. We do not want to start out with somebody that has got AST levels that are five times elevated.

We agree with the FDA and the RAC that that is something that should not be done and felt that way from the beginning.

The thrombocytopenia is transient. We can cover patients with Factor VIII during the period that they have thrombocytopenia so that is a potential offset to the thrombocytopenia.

If it were long-lasting thrombocytopenia that would be a different issue but since it does appear to be very short in its time frame I think we can cover patients adequately with Factor as we normally do and I think that would pose no increased risks for those patients.

DR. MICKELSON: I just had one question to follow-up. It was something that we had alluded to earlier but had not been followed up. The vector to disease match, this vector stays episomal pretty nicely. You have got good data on that and you know that these patients would probably be excluded from subsequent participation in other trials. Is that a good match? A monogenic disease with a vector that does not integrate and stays episomal. One tends to think of possible therapeutic vectors as wanting to go for integration, and just your comments.

DR. WHITE: Well, we have had some of the same discussions about integration. If I can -- with a caveat that I do not know whether it integrates or not. I think there is some data that it is not integrating but with that as a caveat I will try to answer the question.

I think one of the things that RAC has talked about and we have talked about in our gene therapy meetings that we have had through the NHF over the last few years is the desirability of having a nonintegrating vector (a) because of the potential consequences of integrating and (2) because how do you reverse an integrating vector.

The nice thing about this is if it does remain episomal it is potentially reversible at some point in time presumably. That could be looked at as a positive feature.

From a hemophilia point of view you would like to see something that down the road is going to permanently cure a patient. I do not think we are at that step. I mean, I do not think that is even an issue to think about in considering this as a clinical trial. We know we are not permanently curing patients with what we are doing at this stage in gene transfer of hemophilia.

So having something that remains episomal could be looked upon potentially as a benefit, that it is not a permanent change in the genome of the patient.

DR. ANDO: Dr. Friedmann?

DR. FRIEDMANN: You may have discussed this before and, if so, I apologize for raising it again. What -- tell me what is wrong with the following thinking: That you said in your discussion earlier that you see immune response not only to the vector but to the transgene product.

Is it possible that you would convert some of your noninhibitor patients to inhibitor patients by exposing them to an adenovirus vector and thereby making them less responsive actually to traditional therapy? Is that feasible?

DR. WHITE: Oh, more than feasible. It is absolutely a concern and again --

DR. FRIEDMANN: I guess the evidence is against it happening in

mice.

DR. WHITE: Well, I think -- so let me discuss that in two ways. One, all of these patients get Factor VIII through an intravenous route so they are processing Factor VIII protein through Class 2 mechanisms.

We are now going to be putting Factor VIII inside their cells and they are going to process that Factor VIII through a Class 1 mechanism. We might anticipate that through no effect of the vector that is being used but simply by now expressing Factor VIII in a way that it has never been expressed in that patient before, we are likely to get some patients who may develop antibodies.

There is no way currently of predicting patients who are likely to be at risk for that. I think the technology is potentially at hand to begin to think about ways that one could predict that but I do not think we are there yet.

So there is that risk and I think that if a patient does develop an inhibitor the question is going to be was it adenovirus or a gutted adenovirus vector that did it by some way that was dependent upon the vector itself or is it by some mechanism that is totally independent of the vector and something that is inherent in the nature of a genetic disease that when you provide the antigen that they are tolerant to in one form in another form are you going to get antibody formation?

DR. FRIEDMANN: So is the presence of material an exclusion for -- criterion for inclusion or exclusion in these patients?

DR. WHITE: No.

(Simultaneous discussion.)

DR. WHITE: All of the molecular defects -- we have talked about trying to define the molecular defects but we have not generated entry or exclusion criteria based on the molecular defect in the patient.

Is there evidence that this will or will not happen? Well, all I can say is that in the clinical trials that are ongoing there has been no reports of inhibitor formation in the patients that have been treated so far. I think that tells us that this is something that is not going to be terribly common but I do not think it answers the question.

DR. FRIEDMANN: I am sorry. So tell me again. How many patients have been treated so far and by what means?

DR. WHITE: Well, it is upwards of around 20 patients in the three trials that are ongoing currently.

DR. FRIEDMANN: With which vector?

DR. WHITE: With retroviral vector, with AAV vector, and with an *ex vivo* nonviral vector technique.

DR. BREAKEFIELD: Yes, I have a -- my question was related to your's also, Ted, and maybe there is other people who could answer this. I tend to think of the -- even the adenovirus virion as somewhat more antigenic than let's say the AAV virion and we have another protocol where they want to do a vascular delivery of another clotting factor with an AAV vector so I just wonder if anyone here had any idea is that kind of a conception I have or is there any data to that?

Let's say if you took a mouse and you put Lac Z in an AAV vector

compared to a gutted adeno, would you get a faster immune response to the Lac Z in one or the other or is it kind of the same?

DR. CHAMBERLAIN: Yes. I think I can answer that. You would still get a much more robust immune response in the gutted adenovector than in the AAV vector but it depends on the promoter that you are using to drive Lac Z expression and data that we have in our lab is if you use a tissue specific promoter that is not active in dendritic cells the immune response against Lac Z even in an adenovirus is severely attenuated and it is thought that the reason AAV is not very immunogenic is that it has no natural tropism for dendritic cells.

So it sort of gets back to one of the points I was trying to make in my presentation that a gutted virus in and of itself is not guaranteed to always be safe. The tissues that express your transgene need to be taken into account and that is going to be dependent on what promoters you have in your construct.

DR. BREAKEFIELD: So the actual virion itself -- there is no difference in antigenicity of a virus virion versus an AAV --

DR. CHAMBERLAIN: There may be but that does not -- it looks like that is not the primary difference. I think the major difference in the immunogenecity of the two vectors is their tropism for antigen presenting cells.

DR. WHITE: That was the next point I was going to make to Dr. Friedmann's question. I appreciate you making it.

I do think since we do have a liver promoter in front of this that although this vector may get into antigen processing cells that the anticipation is that it will not be expressed in those cells and, therefore, would not be likely to lead to the same changes that have been seen with nongutted and nontissue directed adenoviral vectors.

DR. AGUILAR-CORDOVA: However, there is -- you do not have a direct comparison of that data yet and so you really do not know what level of expression you will get in an episomal -- even though it has the albumin promoter it will get into the dendritic cells and the level of expression has not been determined as far as I know. Is that correct?

DR. ZHANG: We did not check the expression in dendritic cells but probably it is --can be predicted albumin promoter is mainly functional in hepatocytes.

DR. FRIEDMANN: I think I agree with your comment you made earlier about the theoretical advantage of keeping something episomal as opposed to integrated but we have to remember, of course, that herpes virus is the virus -- onset of an episomal agent that loves to stay around forever and it is certainly difficult to get rid of, not that we have tried all the tricks to get rid of it that are possible but being episomal does not guarantee that it would be easier to get rid of it when you need to.

DR. AGUILAR-CORDOVA: Dr. Noguchi and then maybe after that we will try to wrap this up.

DR. MICKELSON: There were some comments from the public.

DR. AGUILAR-CORDOVA: Oh, I am sorry.

DR. MICKELSON: But after Dr. Noguchi.

DR. NOGUCHI: Yes. I do want to broach the question that I hinted at in the original request to review this, which is all the data in animals and *in vitro* aside, it is my impression that *in vivo* the actual evidence for receptor mediated endocytosis leading to transduction is not really always supported by what we think. It is certainly true in the mouse that you get the maximal transduction when you have trauma to the tissue itself.

And the question I am really asking is all these other things aside if, in fact, the basic mechanism of transduction to the liver is one of more mass action, that is the more -- you have to get a certain total number in order to get transduction and if that is being mediated to some degree by damage to the cell membrane then all the advantages that you are talking about still -- if you still need, let's say, 10^{14}, whatever, to get the cell to be transduced, even if it is more efficiently expressed in terms of Factor VIII, you still have the cell -- you could still have the cellular damage.

So that is the question for the committee as a whole is really what evidence do we have in humans that receptor mediated endocytosis actually occurs with adenoviral vectors and that is a requirement for transduction or is it just plain old mass action?

DR. ANDO: That gets back to some of the discussions we had in December. What is the mechanism of the toxicity? Is it the actual particle or is it receptor mediated endocytosis?

I think Inder Verma has published some data showing that you can just boil adenovirus and get a certain level of toxicity.

My understanding is that the answer to this is not clear and maybe Dr. Chamberlain could comment.

And the other question, I guess, for the ethicists would be if that question is not clear then -- and we have to approach this in humans then the safety factors are basically the preclinical studies, the informed consent, and the very careful dose escalation, and is that adequate?

So if Dr. Chamberlain and then maybe the ethicists can comment.

DR. CHAMBERLAIN: Well, I mean, I think it is true that there are still some unknowns here that have not been fully addressed as to how adenovirus really gets into cells.

Certainly if you add enough virus to a plate of cells you see an obvious cytopathic effect which you do not get at low doses if you are still transducing but nonetheless there is also a threshold of transduction even with injecting adenovirus in the mice so at lower doses you tend to not see any expression and then you hit a certain level and suddenly you have quite a lot of expression which argues against a very simple receptor mediated uptake but I am not aware of much data at all out there that directly addresses this issue.

DR. ANDO: The other corollary is do -- I guess Dr. Noguchi, I think, is trying to get at --do you have to have damage in order to see expression and how linked are those two features? Is there any scientific data?

DR. NOGUCHI: There is one study done by the North Carolina group a number of years ago where in a cystic fibrosis mouse they were actually

looking at the trachea and they were not able to transduce the trachea at all except for a small band and when they looked at that small band that was actually where the technician had crushed the epithelial and so you could see viable epithelium, crushed epithelium that was very blue and then viable epithelium. That was published, I believe, in Nature maybe -- I do not know -- six or seven years ago so that is the only data that I really know about but it really begs the question of what is -- are we really seeing any receptor mediated endocytosis at all.

DR. GORDON: Can I just comment very quickly on this?

First of all, I should think that with the cell damage approach that you would expect then particles to be a good correlate of toxicity because they can damage the cell membrane just as well. That is the first thing.

The second thing is that we have been doing some experiments in our lab which are very preliminary and I do not want to talk at all in detail about them but we have been able to get adenoviruses into cells while completely bypassing the endocytic mechanism and when you do that you cannot see any Lac Z expression.

DR. CHAMBERLAIN: Could I give a quick follow-up to Dr. Noguchi's comment?

You know, a possible explanation for that that we should keep in mind is that, you know, it has recently been shown that the epithelial cells of the lung and the trachea do not actually express the CAR receptor for adenovirus but it is expressed on the basal side of the cells and perhaps what we are seeing -- the experiment you described is the damage exposed the portions of the cell that express the receptor for adenovirus and that may not be a correlation that holds true for all tissue types.

DR. AGUILAR-CORDOVA: And also as a follow-up to that to follow up Dr. Gordon's comment, there is the biology of the adenovirus that has to go through the endosome mediated release for a pH change to release its genome into the cell and then go on through the expression so that would not follow very well with just the cell damage going through or into the cell but what Dr. Chamberlain said is very true. The distribution of the CAR receptor may not be uniform prior or post-trauma.

DR. ZHANG: Can I respond to Dr. Noguchi's point?

I think this is really an area we need to do further studies but certainly there are already publications particularly with the adenovirus entering cells through this CAR receptor and into receptor for the two steps and also there are evidence from Dr. -- from GenVax (sic) and also from Debbie Currieux's (phonetic) group.

They do the -- they -- you know, we also -- in our own experience if we have a panel of cells either different cancer cell line and infect with either first generation of our minimal adenoviral vector it has a different infectivity due to the distribution of the CAR receptor.

And when Dr. Currieux and GenZyme's -- GenVax's study demonstrate they can retarget the vector to those transferring heparin receptor just to modify the surface of the adenoviral vector which to the cell line previously is not so susceptible and becomes very infectable.

Then in terms of what type of CPE (sic) cytopathic effect can be caused by the viral particle loading per cell, from our vector we demonstrate we usually can obtain more than 100,000 viral particle or 10,000 particles with no cytotoxic effect in contrast to the early generation vector when you reach 1,000 viral particle per cell in CPE.

So those are all the laboratory information I think we can help to understanding all the safety and entry of the viral particles to cell.

DR. ANDO: Thank you.

Ms. King?

MS. KING: Since Dr. Ando specifically asked for some comments from ethicists I want to just go back to in so far as I understand the scientific issues what Dr. Aguilar talked about as the value of the human experience, it seems as though the discussion that we are having in general about the use of this particular vector has to do with some safety concerns as to whether the gutless vector is really improved in safety and the projections about efficacy and when that is connected with the issue of preclusion, that is that subjects will be precluded from participation in -- from getting potentially effective doses should those be developed and from participating in other gene transfer, that is quite a lot to ask of subjects. Not to say that people cannot be altruistic but that is -- but the question arises whether it is fair to ask that of subjects.

So for me the ethical issue really then focuses on crystallizing the value of moving into clinical trials with humans and really being able to say that the information that you are likely to get from moving to humans given all these factors makes that a fair thing to ask of subjects.

And I would like to hear some -- you know, what some of the science -- how some or all of the scientific information that has been put out sort of addresses that because a lot of people have said a lot of things that seem for me to coalesce on that point.

DR. ANDO: Dr. White, you have a comment?

DR. WHITE: It is a good question. It is a tough question.

I mean, I personally believe you have to tell a patient that they cannot participate in other gene transfer clinical trials otherwise you do not get information from the clinical trial you are doing so it has to be a criteria of the study in my opinion.

I do think that that is going to be and should be a discussion point. I think that if -- and to some extent I think that that is going to define, if you will, patients who are looking at this the wrong way and perhaps patients who are looking at it the right way.

If a patient is looking at this clinical trial the right way, that is as a Phase I toxicity study, I do not think that that is going to be as much an issue for them as if they are looking at as well I know you are saying it is a toxicity study but in my mind I am hearing benefit.

And I think if they are looking at it from that point of view that they are probably less likely -- that that is going to be a more important point for them.

So it is conceivable that this may -- that -- you know, that this may affect whether or not a patient is going to participate in a trial. I am sure it will but I do think that it is something that one has to do in order to interpret the data that one is generating.

MS. KING: I agree completely but I want to go one step back. I understand you are definitely raising an important -- a really important point about the discussion and about what potential subjects understand going into the trial.

And, like I said, I am not saying that people should not be allowed -- I am not necessarily saying people should not be allowed to make that choice but I think there is a prior question which is, is it a fair thing to ask of subjects? That is, you know, are -- have you gotten to the point where it is actually a reasonable thing to ask subjects to participate altruistically with such considerable consequences to themselves, that is human data so necessary that that is a fair question to ask, which is not an informed consent question but a prior question about the responsibility of the investigator in moving from preclinical to clinical trials.

And that I think is one of the things that the FDA really wanted us to focus on in this discussion which is not even about your particular trial but about the use of this vector in general.

DR. WHITE: Well, I think that is a very individual answer. I mean, I can give you my answer but I do not know that my answer means anything for the rest of the committee except that I am an investigator on the study and I am the interface between the patient and the -- and the study and the RAC in general so my answer is, yes, I think that that is something that you can ask a patient but you have to ask a patient and make sure that the patient understands what you are saying.

MS. KING: Yes. I think I must not be making myself clear. I mean, what I am really asking is that I think what we need to ask about the use of this vector in moving into human trials with this particular vector is, is this the right time?

DR. WHITE: I took the question that way and answered it that way.

MS. KING: Yes.

DR. WHITE: I think it is -- as an investigator knowing my patients and knowing as much as I know about the vector, which is a lot, my answer to myself is, yes, this is the right time. But I said this is a very individual answer. I do not know how to answer the question for -- I still look like I am not answering the question you are asking.

DR. SOBOL: I do not think it is a foregone conclusion and perhaps this perspective can help you with the question that you are posing that patients could not receive repeat administration or that patients would necessarily always be precluded from any other gene therapy trial just because they would participate in this study.

I think discussions we have had with FDA that before repeat administration would be contemplated that additional preclinical studies showing the effects of repeat administration, assessing the safety profiles of repeat administration would be necessary.

But I think to say that just participating in this study would preclude

someone from ever receiving any other gene therapy approach, I do not think that that is correct.

DR. WHITE: Yes. I did not -- if that is the --

DR. AGUILAR-CORDOVA: That was Dr. Sobol.

DR. WHITE: If that was inherent in your question I misled you. I did not mean to imply that patients would be precluded from ever participating in any other gene therapy studies in hemophilia. Only as long as this study was going on.

DR. AGUILAR-CORDOVA: Dr. Juengst, you had a comment?

DR. MICKELSON: Then Dr. Noguchi had a question.

DR. JUENGST: Yes. Just on this point I heard two things about the cost to the patient. One was that they would be precluded from enrolling in subsequent gene therapy studies, maybe not forever but to some extent, and the other was that they might be put at risk for being resistant or inhibitors to conventional treatment.

And if that is true I guess my response is that this is a remarkable group of volunteers that will take the altruistic point of view and do this for their community and the breakpoint, the judgment point about whether it is time to go into that group of volunteers really does depend on the absence of any other way to get a firmer grip on the risks that they might be facing.

So if there are other interesting strategies for doing more preliminary research that would reduce the risk then we should do that before going to the clinical trial. If nothing is left except the human experience then it is time to look for these volunteers.

DR. AGUILAR-CORDOVA: Dr. Noguchi?

DR. NOGUCHI: I think those two points are exactly the point that we at FDA are trying to get to, which is -- and I have heard here broaching the question about *in vivo* delivery of an adenovirus, is it really receptor mediated or is there a mass action effect to this.

I have heard -- the answer that I have heard is that we do not have enough information to make that judgment and the question does arise are there scientific studies that could be done to better understand whether this, in fact, truly represents a demonstrable increase in safety or are we, in effect, going to repeat the same experiment where some preliminary preclinical evidence suggests that it is safer but that when you use it in humans it really is not any safer.

That is the question that we are posing and when I hear things like it is unlikely to happen that means it has not been studied. And when we hear that it has not been studied it is always our point of view, well, maybe we should study it so that we have more data to make a rational decision on whether to go forward or not.

As an example, damage to a cell does not necessarily mean that that cell cannot be transduced. I think that is a clear statement. I do not think there is very much data that really looks at that because that is not the normal way people look at trying to get a vector into a gene. They are looking for the positive effect.

We are looking for studies and information that can help guide us to the very question that Eric is posing, you know, have we exhausted the reasonable

things that we can do in order to move forward with what we hope is a safer vector. That is do we -- are we confident enough from a scientific basis and the available clinical data that this, in fact, may represent an increment in safety? And if the answer is yes then we deal with how you work the informed consent.

DR. BREAKEFIELD: Yes. I just want to say one thing about that, which is I think that, you know, one issue is have you done all the preclinical data and I think that there is some more information we would like to have. It is always hard to have everything.

The other question which Claudia raised is if you were going to go with a gutless adenovirus vector into people, you know, is this the disease to pick if they have some liver damage already and if they -- there is a real risk of them forming inhibitors. Are there other diseases that might be more appropriate to gain the human experience in where we would not be as concerned about this particular patient population.

DR. BRAY: May I just --

DR. AGUILAR-CORDOVA: Yes.

DR. BRAY: -- I am Gordon Bray by the way.

Just a little bit of background information for the members of the committee who are not aware with respect to the whole inhibitor issue. Inhibitors are a well recognized complication of conventional hemophilia replacement therapy and, in fact, in this particular cohort of patients, patients with the most severe phenotype, the risk of developing an inhibitor, one that would preclude replacement therapy, subsequent replacement therapy with exogenous Factor VIII occurs in between 20 and 30 percent of patients.

Now the study was designed, in effect, to select patients who are inherently low risk of inhibitor development by virtue of the fact that they have had extensive prior exposure to exogenous Factor VIII infusions. In other words, the risk of inhibitor development is greatest at the very earliest stages of substitution therapy.

Once you become even beyond the age of four or five when you have had beyond 150 days of exposure to Factor VIII your risk of inhibitor development declines dramatically and so, in essence, what we have chosen here is a population of patients who would be, in theory, at inherently low risk for inhibitor development by virtue of the fact that they have already been exposed to an antigen, mainly Factor VIII, which they could, in theory, develop a humoral immune response.

Now --

DR. FRIEDMANN: What is the risk to exogenous Factor VIII?

DR. BRAY: Yes. Now there are differences fundamentally, and I think Dr. White, I think, I think discussed those very well, about potentially the way a coagulation protein could, in theory, be presented to a host immune system in a gene therapy model versus a conventional infusion of coagulation factor concentrate.

My feeling is that to a greater or lesser extent these risks are going to be inherent to all gene therapy protocols involving hemophilia patients where there is theoretically a transduced cell that is manufacturing a coagulation protein that may be expressed on the surface of that cell and that may, indeed, cause the antigen presenting

cell to detect -- to process that antigen in -- as it would in association with Class 1.

So I think that at least with respect to the inhibitor issue the concerns that have been expressed are somewhat -- are somewhat -- are going to exist with just about all gene therapy protocols in patients with --

DR. FRIEDMANN: That puts you in a wonderful position with this model and with this vector system to provide an answer to that generic question. In the mouse, for instance, maybe -- on one hand we are saying that the mouse is a good model and if it is a good model you are on the brink of being able to show yes or no that a transduction -- a transgene product produced in the hepatocyte will or will not lead to the production of inhibitory immune responses in the animal.

Is that a question that can now be studied in your mouse model prior to the human experiment?

DR. WHITE: Actually the mouse model will not answer the question for us I am afraid because the mouse has a homogeneous HLA background. It is a clonal mouse and so you do not get the HLA diversity in the mouse model that you have in humans.

The only way -- you can do all the studies of adenovirus that you want. The only way you are going to answer the question about immunogenecity of gene therapy in patients with hemophilia is in the patients with hemophilia I am afraid.

DR. BREAKEFIELD: May I just -- you can cross these mice in this background, though, can't you get, you know, different congenic strain --

DR. WHITE: You can do that but you still have a very limited HLA background. I mean, another way to do this is to put a hemophilia transgene into the mouse and use a human hemophilia transgene into the mouse and those -- that is being done but it is still going to be a very limited HLA background in the mouse.

You are not going to get the HLA diversity that you have in the hemophilic population in humans in a mouse model unfortunately.

(Simultaneous discussion.)

DR. AGUILAR-CORDOVA: What we have said here is now that in general it would be desirable to know whether the risks are increased, especially the immunological risks are increased when the liver via an adenoviral vector expressed from within the cell rather than exogenously the adjuvant factors of the cell. It is impossible that with the particular gene that is being used. Perhaps one should consider an animal model that would at least address the differences of similar molecules.

I think this is the type of issue that you are addressing. Is this right, Phil?

DR. NOGUCHI: I actually think that our previous experience with the other three gene therapy trials in the decade's long experience by hemophiliacs renders the question of inhibitors as a constant one and we are satisfied that as much as can be done is being done, including development of very extraordinarily sensitive assays to really distinguish between reactive versus actual inhibitory antibodies. It is a very complex issue but it does not seem in that sense to be something new to a hemophiliac. That is a big difference in another disease where there is no replacement

therapy.

I think we are still getting back -- as far as FDA is concerned and my colleagues at the end here can certainly have their other questions as well but it really is the question of do we have cumulatively enough data to reasonably or at least to some degree expect that this vector delivery in this gene is going to have a better safety profile than previous generations. I think that is the question.

We are not making an argument for go/no go. We are asking the question in public and cumulative experience do we have enough to go forward vis-a-vis the actual safety of the vector?

DR. AGUILAR-CORDOVA: From the comments from the ethicists in the panel I think what we have heard is that this increases -- since this may potentiate the patient's nonresponse to traditional therapy, it increases the risk factors of the equation thus the potential benefits need to be crystallized at a higher level.

DR. SOBOL: Perhaps I could comment on that.

DR. AGUILAR-CORDOVA: Dr. Sobol?

DR. SOBOL: Yes. I think if you look at the data from the mouse studies, this is with a human protein that is supposed to be more antigenic than a mouse protein, we see long-term sustained expression in large numbers of animals that are infected with this mini-Ad vector.

So already in animal studies you can see that expression of the Factor VIII in liver, in hepatocytes, does not necessarily induce an inhibitor so that data already exists in the animal studies. So I think that that data is there to support that you can get sustained long-term expression.

And our clinical protocol is designed that if such inhibitors are seen -- if a single patient develops an inhibitor the trial is stopped for us to evaluate and assess that.

And again, as Dr. Bray mentioned, we have chosen patients who are unlikely to develop inhibitors because they have had the exposure for many, many years. So I think that combination of data, I think, should find comfort and again other gene therapy protocols where the same issues might be seen are already approved and in clinical trials so I think it is not proper to hold back this approach from going forward.

And then one final comment for things that Dr. Noguchi had mentioned, I think the data is there and I think the data is compelling. I think you have data from several laboratories showing that in preclinical models this gutless Ad and mini-Ad has a much better safety profile. And I think it is ready for testing in human subjects and I think that that data is very strong.

DR. ZHANG: May I add one point?

DR. AGUILAR-CORDOVA: Well, actually I would like to draw --

DR. ZHANG: Very quickly. I would like to also emphasize that gene trans -- the transgene product of this human Factor VIII produced by this Factor VIII is identical to the recombinant protein which is in market in a protein level.

DR. ANDO: I would like to thank the investigators for presenting to us and I think it is very important to have a public discussion on these issues and that

you are bringing a new adenoviral system with improved safety into humans and in the context of hemophilia. I think airing these issues publicly is very important.

Next we have a comment from the public.

DR. MICKELSON: There is a microphone at the end of the table.

DR. AGUILAR-CORDOVA: This is Dr. Richard Schneider from Harvard.

DR. SCHNEIDER: I was just going to bring up actually a very technical issue since we are talking about inhibitors. When looking a the purity of these vectors I would hope that people are looking at contaminating factor in the vector prep so when you make an adenovector or a retro or an AAV vector expressing a clotting factor those cells that are producing a vector will also produce the clotting factor and during the purification of the vector these clotting factors could become denatured, fragmented or undergo some other orientation, which when injected – you know, if they are not actually separated from the vector for the gene therapy protocol, could be administered to these patients and could be a source of inhibitor forming protein.

DR. AGUILAR-CORDOVA: Thank you.

DR. MICKELSON: Any other questions?

DR. AGUILAR-CORDOVA: Another question from the public?

DR. DELENICK: This is a scientific question for Dr. Zhang. In your biodiversity --

DR. AGUILAR-CORDOVA: If you could introduce yourself?

DR. DELENICK: Joann Delenick. I am a member of the public.

In the biodiversity experiments in mice did you say you had to pull ten animals to do the PCR?

DR. ZHANG: No. Ten organs per each mouse. Ten different.

DR. DELENICK: Samples.

DR. MICKELSON: Combined?

DR. DELENICK: No. You did ten separate organs from one animal?

DR. ZHANG: Per mouse.

DR. AGUILAR-CORDOVA: Are there any other comments from the members of the committee?

DR. MICKELSON: I just think that one of the main issues that we were trying to grapple with here is whether the deletion of the adenoviral structure proteins and the toxicity associated with synthesis of those proteins intracellularly is the main component of adenoviral toxicity and destruction of transduced cells versus whether the amount of toxicity that remains in the envelope, and whether that provides a high enough barrier to preclude or to ask for either more preclinical data.

Or whether the data that is there now is sufficient in our opinion and that it is a discrimination between those two particular modes is what we have been trying to grapple with and whether the safety profile that has been presented to us is adequate to address those issues and not -- and it leaves one -- possibly one avenue of potential toxicity unaddressed.

Is that a summary of what we are looking at? Okay.

DR. AGUILAR-CORDOVA: And perhaps the answer to that is that the data is not at that level because the quantities of the -- even first generation vector that need to be administered in order to get to that toxicity are fairly large and those quantities to produced of this helper vector or helper-dependent vector seem to be more difficult to acquire.

DR. MICKELSON: Right. I think one of the things that is -- and it is only my opinion -- is that at least one of the issues about contribution to toxicity or one of these -- contribution by one of these two arms has been or can be looked at and has been looked at in some of these models so that if one of the questions is then is this an incremental step in increased safety for these vectors I do not see that we could say that the data would not support that that is, in fact, the case.

DR. AGUILAR-CORDOVA: And actually Dr. Ando mentioned the study by Dr. Verma.

DR. MICKELSON: Yes.

DR. AGUILAR-CORDOVA: Where there was some evidence of just viral coat toxicity.

DR. MICKELSON: Absolutely but to quantitate it and then to -- that is -- and that gets to the question of how infinitely mutable is a vector and when does it actually lose becoming an adenovirus and that is a different question.

And other questions that we left unresolved I think still are the questions of the vector characteristics to disease model and whether things that might remain persistent versus integrated and then use of a type of vector that may have residual toxicity associated in use in patients that probably have at least some liver damage where liver is, in fact, probably --

(Simultaneous discussion.)

DR. MICKELSON: Okay.

DR. AGUILAR-CORDOVA: Clearly there are a lot of questions still left and we tried to just give a sample of some of the issues that were brought up here and that may be pertinent to this particular vector type.

(Slide.)

Of the issues that we discussed with endproduct is genetic stability and it was discussed by Dr. Chamberlain that perhaps multiple -- it is a question of sequential preparations and by sequential he meant the continuous amplification with -- it should be tested with a very high sensitivity assay particularly because one has to use so much of the preparation that this will not be practical on all preparations.

And establishing a measure of genetic stability of the particular construct prior to making a large vector preparation that would then be suitable for clinical use in which a perhaps less sensitive assay would serve as the surrogate marker.

Helper vector contamination was discussed at length and I think there was some consensus that the sensitivity of the assay must be determined and that it would be desirable to have some indication of how much is too much and what kind of effect might that contamination have on study design, expression, duration and

immunogenecity.

There was some discussion on the stability and equivalence of preclinical or clinical lots and that would also address the potential for the equivalence of multiple batches of clinical product.

There was some discussion of stability and potency after the transport to the study site and that would be both the preclinical sample -- preclinical toxicity studies as well as obviously the clinical samples.

(Slide.)

Those were things having to do with product primarily and then we discussed at length the dose escalation scheme and it was stated that safety should be the first concern but that a critical issue would also be the potential for future escalation exclusion of the patient that is preclusion as Ms. King mentioned.

And that this should be considered perhaps as we progress and specifically in diseases such as this where preclusion from even standard of care might be a consequence that the initiation dose should be crystallized as much as possible in the preclinical models prior to beginning the clinical dose.

And there was a lot of discussion about vector disease match, whether this vector is, in fact, significantly improved in toxicity profile and duration profile for -- to warrant further clinical studies.

I think it was mentioned that a perfect match by Dr. White would be difficult to achieve at this point but it should be considered in the context of the current alternatives.

Risk/benefit ratio, of course, is always considered and within that in this particular case and perhaps in most monogenic diseases is the potential for preclusion from further studies.

So those are a list of some of the things that we discussed here and with minor recommendations at this point.

Does anybody -- any of the members have further comments that we could add?

Yes, Dr. Friedmann?

DR. FRIEDMANN: Just a quick point. I think I want to agree very much with Dr. White and his comments that there are times when one has to study human disease in the human.

I also want to agree with Dr. Noguchi that one of the questions in front of us is this system better than previous adeno systems and I think that it clearly is.

But what I am still a little bit unpersuaded by is the notion that we have pushed the preclinical animal models to the limit of information in them that would help us to decide when to go to the human study.

I think still the thing that is most worrisome to me is the issue of doing real harm to patients by inducing a state of nonresponsiveness when we can ask those questions at least partially in the animal models that are available to us.

I think that it is a very complicated issue and I recognize the difference between Class 1 or Class 2 responses. I understand the difference -- the

difficulty in knowing when -- and predicting which humans are going to respond with inhibitors but I would like to feel more comfortable myself with the idea that we have derived all the information that is inherent in the animal model before we move to the human. I still have a level of some unease and concern about that.

DR. AGUILAR-CORDOVA: Dr. Gordon?

DR. GORDON: I just wanted to say that I felt one of the not definitively answered issues was whether a tissue specific promoter was really going to behave in a tissue specific manner but then in the productive phase which would allude to the public comment about elicit production of Factor VIII and to the *in vivo* phase.

I tend to agree that there is a very tissue specific enhancer in albumin but we do not really know for sure yet what is going to happen with an unintegrated vector which may be acting sort of like a transient transfection.

DR. AGUILAR-CORDOVA: And that addresses then the issue that -- the questions of whether it is expressed in dendritic cells and perhaps also in the production cells which are A549's or others and these are certainly addressable questions.

Any other comments?

Okay. Well, thank you very much. Thank you to the investigators for participating.

And I will transfer it back to you.

DR. MICKELSON: Yes. We thank the FDA for bringing this protocol to us and --

DR. NOGUCHI: Likewise, we appreciate very much this discussion because it mirrors and extends the discussions that we have had and it gives us more confidence in being able to address these issues.

DR. MICKELSON: Again, thank you, Dr. Pilaro and Dr. Kaiser. We appreciate you coming and helping us with the discussion.

What do you think about coming back from lunch at 1:35?

DR. _____: 1:40, sold.

DR. MICKELSON: 1:40?

DR. _____: Sold.

DR. MICKELSON: Sold. We will try for 1:40.

(Whereupon, a lunch break was taken from 12:48 p.m. until 1:50 p.m.)

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